# APPLICATION OF GAMMA INDUCED MUTATION IN BREEDING FOR BACTERIAL WILT (*Ralstonia solanacearum*) DISEASE RESISTANCE IN POTATO (*Solanum tuberosum* L.)

**BY**

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# DECLARATION

**Declaration by the candidate**

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without prior permission of the author or University of Eldoret.

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# DEDICATION

To God Almighty, I praise you; your works are wonderful I know that full well because you created me in your own Image, I am fearfully and wonderfully made.

To my precious family, relatives and friends; thank you for your prayers, support and encouragement.

This thesis is dedicated to you all, who set and supported my roots to school life now the fruits are ripe.

## ABSTRACT

Potato (*Solanum tuberosum* L.) is the second most important staple food crop in Kenya after maize and fourth in the world, therefore, plays a vital role in food and nutrition security, and sustainable development. Despite its importance, potato production in Kenya is still low due to biotic and abiotic constraints. Of the biotic factors, bacterial wilt in potato is regarded an important disease causing significant yield decline of about 50 to 100 %. It has been reported to affect 77 % of the potato farms in Kenya. Breeding for resistant varieties can play an important role in managing the disease. However, improvement of potato through conventional breeding has been difficult due to the narrow genetic diversity of the crop. Desired genetic variations could be generated through the application of induced mutations from which putative mutants can be selected. The objective of this study was to induce mutation on potato varieties to create variation and identify desirable allelic variants of genes underlying important quantitative traits. The study involved irradiation of three commercially grown high yielding Kenyan potato varieties: Asante, Kenya Mpya and Kenya Sherekea. A total of 570 mutant microtubers were developed using gamma rays from $Co^{60}$ source under different dose rates (0 – 30 Gray) for the three varieties. The microtubers were then established at M1V1 and developed to M1V2, M1V3, and M1V4 generations at the University of Eldoret. The mutant populations were assessed for morphological, ploidy and genetic diversity. Bacterial wilt resistance screening was carried out at M1V4 generation at KALRO-Kabete station using alpha lattice design. The results showed that the total number of irradiated potato mutants that survived to produce tubers at the M1V1 stage was less than half for each genotype that was initially irradiated in all dosage rates across the three genotypes used. The highest tuber weight was at dosage rates 9 Gy in Asante (22.0 and 57.0 tons/ha), 15 Gy in Kenya Mpya (31.0 and 46.8) and 10 Gy in Kenya Sherekea (48.4 and 49.0) at M1V2 and M1V3 generations respectively. The number of ploidy level distribution was decreasing in diploids and triploids and were increasing in tetraploids from M1V1, M1V2 to M1V3 in all the three potato mutant populations. The reactions of potato mutants to bacterial wilt were varied and there was significant difference in selected agronomic traits and bacterial wilt resistance among varieties and between families of individual varieties. The days to onset of wilting, area under the disease progress curve and percentage of symptomatic tubers of total tuber number per ha was significantly different in all the three potato mutant populations. The genetic variability of the potato mutants showed that 20 SSR primers were polymorphic with 211 alleles (average eleven), Asante, Kenya Mpya and, Kenya Sherekea generating 69, 75 and 67 alleles respectively. The dendrogram and PCoA analyses showed that the 160 potato mutants and three parents were clustered into three groups, though the STRUCTURE analysis supported by the dendrogram confirm that each sub-population affiliate gave six clusters. Success in the use of gamma-induced mutation in the development of new varieties was observed and will lead to improved potato production, which will respond to enhanced food and nutrition security. The information from this study will inform potato variety release for commercial production and sustainable development and for future potato breeding programme.

# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PLATES

## LIST OF APPENDICES

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

The cultivated potato (*Solanum tuberosum* L.) is the second most important staple food in Kenya after maize and the world's fourth major food crop after wheat, rice and maize (Ministry of Agriculture (MoA), 2008); de Haan, & Rodriguez, 2016; Food and Agricultural Organization Statistics (FAOSTAT), 2017). Globally, the total estimated area under potato production is 19 million hectares with a total production of 381.7 million tonnes in 2014 (FAOSTAT, 2017). Potato is grown in more than 150 countries worldwide from latitudes 65 °N to 50 °S (Acquaah, 2007; FAOSTAT, 2017) and can grow from sea level up to 4700 metres above sea level (International Potato Center (CIP), 2014). The major potato producing regions are Asia and Europe, accounting for more than 80 % of world production, while Africa produces the least, accounting for about 5 %. Currently, China is the biggest potato producer, and a third of almost all potatoes in the world are harvested in China and India (FAOSTAT, 2017). In sub-Saharan Africa (SSA), the East and Central Africa region accounts for over 45 % of potato production and 52 % of area harvested. Kenya is the fifth biggest producer of potato in SSA after Malawi, Rwanda, Ethiopia and South Africa (FAOSTAT, 2017).

In Kenya, potato is mostly grown by small scale farmers as source of food, employment and cash crop, therefore plays an important role in food security and provides high energy, protein as well as substantial amount of vitamins and minerals (FAOSTAT, 2017). Potato farming in Kenya employs 3.3 million people at all levels of the value chain. Potato is grown by about 800 000 farmers on about 158 000 ha per season, with an annual production of about 1.6 million tonnes in two growing seasons. The annual

potato crop is valued at KES 13 billion (USD 150 million) at farm gate level, and KES 40 billion (USD 362 million) at the consumer level (Ann., 2009; National potato council of Kenya (NPCK), 2014; FAOSTAT, 2017).

Despite the importance of potato in Kenya, its production has not been achieved to its full potential with the national average yields of 18.5 tonnes per hectare (ton/ha) against an average crop potential of 40-60 ton/ha (FAOSTAT, 2017). The low yields are due to the following constraints; inadequate supply and untimely availability of high quality certified seeds, low soil fertility, low yielding varieties, diseases and insect pests among others Kaguongo *et al.,* 2008; FAO, 2009; FAOSTAT, 2017). About 1-2 % of the national certified seed potato requirement in Kenya is being met which negatively impacts on the potato value chain (Ayieko & Tschirley, 2006; The Organic Farmer, 2013). Many growers often opt to use informal seed systems which contribute towards use of poor quality seeds leading to sub-optimal yields as well as spread of diseases and pests (Kaguongo *et al.,* 2008). Of the diseases, bacterial wilt (Yabuuchi *et al.*, 1995) is a major disease found in all the potato growing areas of the country (Kenya) (Muthoni *et al.,* 2013; The Organic Farmer, 2013) affecting 77 % of potato farms (Kaguongo *et al*., 2010).

Bacterial wilt, caused by *Ralstonia solanacearum* strains of race 3 biovar 2A in potato is regarded as an important disease contributing to significant yield reduction of between 50 to 100 % (Kaguongo *et al.,* 2008; Muthoni *et al.,* 2012). The disease is considered more difficult to control in field crop production owing to pathogen's properties as a soil-borne bacterium, their broad host range and the genetic variation level within the strains which makes it difficult to employ a universal control measure

(Champoiseau *et al.,* 2009). However, effective and long term control or management strategy could be feasible by using a combination of diverse control methods such as the use of resistant/tolerance varieties, chemical, biological and cultural practices (Champoiseau *et al.,* 2010; Riungu, 2011).

Bacterial wilt resistance in potato is very complex in nature; it is probably a function of genetic and environmental adaptation (Schmiediche, 1985; Tung *et al.,* 1990; 1992). Studies indicate that inheritance of resistance to bacterial wilt is dominant, polygenic and quantitative in nature, and entail genes with major and minor effects (Tung *et al.,* 1993; Cook & Sequeira, 1994). Interaction between genes for resistance and those for adaptation is an essential combining ability which appears to be a substantial attribute for expression of resistance (Tung *et al.,* 1990; 1992a; 1992b).

The cultivated potato (*Solanum tuberosum* L.) is a tetraploid ($2n=4x=48$, 4 EBN (Endosperm Balance Number)), that exhibits complex tetrasomic inheritance patterns (Bradshaw, 2006). The crop is highly heterozygous in nature and upon selfing suffers inbreeding depression (Bradshaw, 2006). The level of heterozygosity is influenced by the four different alleles within a locus; the more diverse they are, the higher the heterozygosity and the greater the number of interlocus interactions, hence greater heterosis (Ross, 1986; Bradshaw & Mackay, 1994; Sleper & Poehlman 2006; Machida-Hirano, 2015). Understanding the implications and complexities of tetrasomic inheritance in the cultivated potato breeding is vital in enhancing efficiency in a breeding programme. Conventional potato breeding is cumbersome, takes long time and less successful in crops like potato because of the heterozygous nature, making it an excellent crop to be improved by mutation.

Plant breeding requires genetic variation of useful traits for crop improvement. In potato, most often the desired variation is lacking due to preferences of few elite local traditional cultivars for potato improvement in most parts of the world and hence narrow genetic variability due to common parentage (Cheng *et al.,* 2010). The narrow genetic diversity in cultivars has led to an increased vulnerability to new abiotic and biotic stressors, especially those directly and indirectly arising from changing climatic conditions making it difficult for yield improvement to be achieved (Gopal & Oyama, 2005). Knowledge of genetic traits such as heritabilities and genetic correlations are also required to help guide an effective potato breeding strategy (Li *et al.,* 2018).

Potato breeding for bacterial wilt resistance is very demanding with limited success owing to the pathogen variability, lack of resistance sources in the species, genetic complexity involved in resistance and the tetraploid background nature of the crop making the long road even longer, complex and rather vague (Sequeira & Rowe, 1969; Hong *et al.,* 2005; Jansky & Hamernik, 2009; Narancio *et al.,* 2013; Lopes *et al.,* 2018). Currently, selection of bacterial wilt resistant potato clones or varieties is being done traditionally using phenotypic selection. Field selection has been effective in identifying stable resistance in progenies derived from crosses involving resistant wild relatives. Though field selection efficiency is reduced by pathogen variability, infection and disease development variability is laborious and requires uniformly infested fields.

Mutation breeding is an essential tool for crop improvement based on the probability of altering desired genes (Shu *et al.,* 2012). It involves exposing the vegetative parts, cells, tissues, gametes, pollen or seeds of potatoes to physical or chemical mutagens to develop new varieties and generate genetic variations from which desired mutants may

be selected. Mutation breeding has been effective in inducing many traits in vegetative and seed propagated crops thus improving yield, growth habit, plant stature (semi-dwarf), disease and pest resistance, physiological traits, nutritional value in cereals, tubers (potato and sweetpotato), ornamental crops fruits trees and other crops (van Harten & Broertjies, 1989; Mba, 2013).

Characterization of induced mutants is important to understand their genetic diversity and distinctness for further use in breeding and germplasm conservation (Liao & Guo, 2014). Molecular markers are useful tools for genetic diversity assessment of alleles of interests and classification of genetic materials (Spooner *et al.,* 2007; Nováková *et al.,* 2010; Tiwari *et al.,* 2013). Several types of molecular markers have been applied extensively for potato genetic characterization (Nováková *et al.,* 2010). Simple sequence repeat (SSR) markers have been extensively used in potato and other crops for various breeding and diversity studies (Muthoni *et al.,* 2014; Tesfamichael *et al.,* 2014; Biniam *et al.,* 2016; Kassa, 2017; Berdugo-Cely *et al.,* 2017). The SSRs are highly polymorphic, co-dominant markers (Ghislain *et al.,* 2009; Tiwari *et al.,* 2013), low operational costs, and are highly reproducible (Favoretto *et al.,* 2011). The study therefore explored the use of induced mutations to create new genetic variations within selected potato varieties, characterize, evaluate for bacterial wilt resistance and any unexpected useful traits.

**1.2 Statement of the problem**

Potato breeding requires genetic variation of useful traits for crop improvement. In potato, most often the desired variation is lacking due to preferences of few elite local traditional cultivars for potato improvement in most parts of the world. Cultivated

potato cultivars have a narrow genetic base due to common pedigrees of breeding materials (Cheng *et al.,* 2010). This presents a serious limitation to potato crop improvement, especially with the emergence of new diseases, pests and climatic changes making it difficult for yield improvement to be realized (Gopal & Oyama, 2005). Irradiation of planting material with suitable doses, though genetic differences could exist, can produce small effects with several important biosynthetic processes and morphological traits (Mba, 2013).

In Kenya, *Ralstonia solanacearum* is an important pathogen found in all potato growing areas affecting 77 % of potato farms and contributes significant yield reduction of between 50 to 100 % (Kaguongo *et al.,* 2008; 2010; Muthoni *et al.,* 2012). The pathogen has a long survival period in the soil, complex in nature, wide biological variation and host range which makes it difficult to control, however, integrated combination of diverse measures is an approach for management of the disease (Martin & French, 1985; EPPO, 2004; Champoiseau *et al.,* 2010). New improved varieties are urgently needed to counter the demands of food security, poverty alleviation, and emerging trends of pests and diseases. The use of mutation breeding could widen the genetic base for selection of specific traits of interest.

**1.3 Justification**

Potato crop plays a major role in nutritional and economic values and serves as a poverty alleviation and food security in Kenya (MoA, 2008). It has a high energy content and ease of production has made it an important component of urban agriculture which provides jobs and food security to more than 800 million people globally (Hoffler & Ochieng, 2008; FAO, 2008; FAOSTAT, 2017) and employs 2.5 million people in

Kenya at all levels of the value chain (Ann, 2009). Hundreds of millions of people in the developing countries including Kenya are faced with food crisis as the cost of their staple foods continues to rise.

The potato biological seeds are genetically diverse due to segregation and recombination from sexual reproduction. The success of introducing improved cultivars in potato has been hindered by low seed production. This is due to: failure of plants to flower, failure to produce viable pollen, low pollen production, dropping of buds and flowers either before or after fertilization, male sterility and self-incompatibility (Sleper & Poehlman, 2006). This therefore, necessitates the use of induced mutations to create new genetic variations within crop varieties where natural genetic variation is limited and insufficient (Ahloowalia *et al.,* 2004). Induced mutations have been used to generate variations to obtain desired mutants with agronomically important traits (Jain, 2005). Induced mutation has been postulated by current researchers as a valuable alternative tool to conventional breeding because of the benefit of creating variation in crops (Mba, 2013; Jankowicz-cieslak *et al.,* 2017). Mutation induction in potato has produced mutants with diverse characters (Al-Safadi *et al.,* 2000; Cieśla & Eliasson, 2002; Li *et al.,* 2005; Muth *et al.,* 2008; Albiski *et al.,* 2012).

Induced mutations have played a major role in increasing world food security, since mutants released as food crops have contributed significantly to an increase in crop production especially in marginal areas (Kharkwal & Shu, 2009). Mutation breeding has been used in Kenya as a method of breeding and various crops (MVD, 2016) have been developed through mutation such as cowpea (Pathak, 1996) and wheat (Kinyua *et al.,* 2000; 2008; 2014).

Assessment of the genetic diversity of potato mutants is very critical for utilization in breeding programmes. Simple sequence repeat markers are considered as the markers of choice as they are the most efficient markers for assessing genetic diversity studies in several crop plants such as sweet potato (Gwandu *et al.,* 2012), sorghum (Tesfamichael *et al.,* 2014) soybean (*Glycine max*) including potato (Muthoni *et al.,* 2014; Biniam *et al.,* 2016; Kassa, 2017; Berdugo-Cely *et al.,* 2017) . The SSR markers are able to detect variation in allele frequency at many unlinked loci, abundant in plants, have high level of polymorphism and are adaptable to automation.

## 1.4 Objectives:

### 1.4.1 Main objective:

The main objective of this study was to improve potato production through mutation breeding to create desirable allelic variants for bacterial wilt disease resistance.

### 1.4.2 Specific objectives:

1. To evaluate the effects of induced mutation on selected morphological and agronomic traits of potato
2. To evaluate the ploidy level diversity by chloroplast counts in stomatal guard cells of potato mutants
3. To assess the potato mutants populations for bacterial wilt disease resistance
4. To determine the genetic variability of potato mutants using SSR markers

## 1.5 Alternate Hypotheses

1. Induced mutations significantly affected selected morphological and agronomic traits in the potato
2. Induced mutant potatoes differed significantly in ploidy level by chloroplast counts in stomatal guard cells
3. Potato mutant's populations differed significantly in terms of bacterial wilt resistance screening
4. Potato mutants differed significantly in genetic variation using SSR markers

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Potato crop

### 2.1.1 Origin and distribution

The potato (*Solanum tuberosum* L.), a tuber-bearing *Solanum* species is assumed to have been the first domesticated crop approximately 8,000 years ago and eaten by man in the region of Lake Titicaca in South America (Hawkes, 1978). It is believed to be the primary center of origin and diversity of the potato crop and its wild relatives (Machida-Hirano, 2015; de Haan & Rodriguez, 2016). The Spanish were the first Europeans to discover this tuber crop when they invaded the Inca Empire in 1535. Around 1570 the crop was introduced to Spain and then to Ireland and Scotland in 1590. Later, potato introductions to North America were by immigrants from Scotland and Ireland to the American colonies. The wild species are widely distributed through Mexico, Guatemala, Honduras, Costa Rica and Panama. The cultivated potato species are restricted to the high altitudes of Andes of South America stretching from central Peru to central Bolivia (Hawkes, 1990).

### 2.1.2 Economic importance

Potato is second most important food crop in Kenya after maize (MoA, 2008) and is the fourth globally after wheat, maize and rice (FAO, 2008). Being a multi-facetted crop, it is used globally not only for human and animal consumption, but also in the food industry for starch and other industries for textile, paper and alcohol production. The crop is one of the most important sources of income and employment in the rural areas. The annual potato acreage in Kenya is about 158,000 ha per season, which is distributed among approximately 800,000 smallholder farmers (FAO, 2014).

### 2.1.3 Taxonomy

The cultivated potato, *S. tuberosum,* belongs to the family Solanaceae, comprising over 2,000 species (PBI Solanum Project, 2014). The tuber-bearing *Solanum* species have reported 7 cultivated and 228 wild species (Hawkes, 1990), 196 species (Spooner & Hijmans, 2001) and approximately 110 species (Spooner, 2009). Lately, combinations of molecular and morphological studies have reduced the number of species to 107 wild and 4 cultivated (Spooner *et al.,* 2014). The potato is called *"batata" by* South Americans while Spaniards later called it *"patata*", from which the English name, potato probably originated (Grun, 1990; Hawkes, 1990).

### 2.1.4 Genetics and cytology – ploidy levels

The *Solanum* basic number of chromosome set consist of 12 chromosomes, x= 12 established in wild potatoes. The cultivated potato species range from the diploid 2n=2x=24 to pentaploid level 2n=5x=60 while the wild species include all the ploidy levels in addition to hexaploid 2n=6x=72 (Huaman, 1986; Hawkes, 1990; Douches & Jastrzebski, 1993; Carputo & Barone, 2005). The even-numbered polyploids (2x, 4x and 6x) are sexually fertile, while the odd-numbered polyploids are male sterile (Douches & Jastrzebski, 1993). There are four cultivated diploid species, *S. stenotomum, S. phureja, S. goniocalyx,* and *S. ajanhuiri,* two cultivated triploid species, *S. chaucha,* and *S. juzepczukii,* one tetraploid species *S. tuberosum* with its two subspecies, *tuberosum* and *andigena* and one cultivated pentaploid species *S. curtilobum.* A common wild hexaploid, *S. demissum*, is the source of the major R genes that confer resistance to late blight of potatoes (Hawkes, 1990; Carputo *et al.,* 2003; Sleper & Poehlman, 2006).

The cultivated potato is an autotetraploid (4 EBN) with probably four interchangeable alleles at a given locus with the possibility of intralocus interactions (heterozygozity) and interlocus interactions (epistasis) occurring (Ross, 1986) which are important in breeding to improve certain traits (Bradshaw & Mackay, 1994; Sleper & Poehlman, 2006). Potato crossability is affected by ploidy species type and EBN which ranges from 1 to 4 with diploid and tetraploid having 2 and 4 EBN respectively (Jansky & Hamernik, 2009). The level of heterozygozity is influenced by how different the four alleles are within a locus; the more diverse they are, the higher the heterozygozity and the greater the number of interlocus interactions, the greater the heterosis (Ross, 1986; Bradshaw & Mackay, 1994; Sleper & Poehlman, 2006). This makes conventional potato breeding cumbersome and time consuming effort and hence less successful when compared to other important crops, making it an excellent crop plant to improve by mutation or genetic transformation.

### 2.1.5 Botany

Potato is herbaceous, annual crop plant, about 0.3-1 meter tall. It is an insect pollinated; out crossing species and self-incompatibility reported in diploid cultivated and wild species. The leaves are irregular and alternately pinnately compound. Inflorescence are terminal consisting of several flowers (1 to 30) of about 3-4 cm in diameter. Flowers are pentamerous, actinomorphic, perfect and sympetalous colored corollas giving the flower a star shape depending on cultivar. The stigma is bi-lobed and protrudes above a cluster of large, bright yellow anthers while anthers that are produced on male sterile plants are light yellow or yellow-green in colour. The petals vary in size and colour varies from white to a complex range of blue, red, and purple (Caligari, 1992; Sleper & Poehlman, 2006; Acquaah, 2007).

Potato fruits are absent in many cultivars and bicarpellate. Fruits (berries) contain about 50 to 500 seeds with an average of 200 seeds (CIP, 1984). Tubers form underground from rhizomes, from which adventitious roots are developed to become a fibrous mass (Burton, 1969). The tuber bears the buds, commonly known as "eyes", which sprout on germination and grow into plants and tuber size differs with age and cultivar. They are grown in fields in ridges to maintain developing tubers under soil. The tubers start developing when the plant flowers, and their formation ceases when fruit formation begins.

### 2.1.6 Genetic diversity

The *Solanum tuberosum* species has a monophyletic origin, and hence has a narrow genetic diversity. There are two major subspecies of *Solanum tuberosum*; *andigena* or Andean, and *tuberosum* or Chilean with unresolved genetic relationship (Raker & Spooner, 2002). The Andean potato is indigenous to Andean region from Venezuela to northern Chile and Argentina (Hawkes, 1990). It originated from equatorial and tropical regions where it is adapted to the prevalent short-day conditions (Raker & Spooner, 2002). The Chilean potato is thought to have originated from the higher latitude region of southern Chile, especially Chiloé Island and Chonos Archipelago, and is adapted to the prevalent long-day conditions (Hawkes, 1990; Hijmans, 2001). Grun, (1990) suggested that *tuberosum* was distinct from *andigenum* based on cytoplasmic sterility factors, geographical isolation, and ecological differences while Hawkes, (1990) distinguished subspecies *tuberosum* that it has fewer stems, more horizontal foliage, less-dissected leaves, wider leaflets, and thicker pedicels than *andigenum*. In addition, *andigenum* has five chloroplast genotypes (A, C, S, T, and W) while *tuberosum* has only three (A, T, and W) (Hosaka & Hanneman, 1998).

## 2.2 Breeding techniques in potato improvement

### 2.2.1 Conventional or classical breeding

Conventional potato breeding involves initial crossing of parents possessing complementary traits based on phenotype in order to generate genetical variation followed by phenotypic selection in the subsequent clonal generations (Sleper & Poehlman, 2006; Bradshaw & Bonierbale, 2010). The aim is to identify clones with as many desirable characteristics as possible for release as new cultivars. The choice of parents is an important principle for their crossability and unrelatedness (Wolfgang *et al.,* 2009) performance *per se* and largely depends on the aims and objectives of the breeder (Caligari, 1992). Potatoes are highly heterozygous and heterosis can be exploited so that dominance and epistatic effects contribute significantly to clone performance. The crop is also generally vegetatively propagated and favourable traits are fixed in the F1 generation although progenies produced by selfing of clones reveal strong inbreeding depression (Arndt & Peloquin, 1990).

### 2.2.2 Induced mutation breeding

Mutation is defined as "heritable change to the genetic make-up of an individual that occurs naturally in plants" (Mba *et al.,* 2009). Plant mutation breeding involves several processes of mutation induction, mutation detection, mutation fixation, mutant line development and release of new mutant cultivars. Induced mutations are highly effective in enhancing natural genetic variations in traits that appear to be recessive compared to the wild type plant (Shu *et al.,* 2012). The length of the process depends mainly on the nature of propagation of the crop, the targeted trait, the ability to recognize and select individuals carrying traits of interest in segregating populations and developing selected lines.

Mutation is said to be permanent when variation occurs at DNA and chromosome levels. At the molecular level, mutations could be due to base pair changes, recombination or transposition which are independent on the mutagen used (Walbot, 1988). Cytologically, mutations can be unnoticeable gene alterations, insertions or deletions (INDEL) or various forms of translocation. Variation at DNA and chromosome levels is the basis of permanent mutation. Varieties differ in their mutability and the kinds of mutant alleles that may be obtained because the gene(s) controlling the desired trait may not mutate, or the mutated gene may not be expressed, or transmitted or may have a lethal effect on the plant and therefore cannot be selected (Mba *et al.,* 2010). All genes are induced at the same time but cannot mutate at the same rate because of differences in gene sizes, gene location and chromosome structure. House-keeping genes occur in multiple copies and may mutate but the mutation cannot be recognized phenotypically.

**a) Gamma irradiation**

Gamma rays are electromagnetic radiation emitted by radioactive decay and having energies in a range from ten thousand ($10^4$) to ten million ($10^7$) electron volts (EV). They are ionizing radiations that result principally to lethal and mutagenic effects from incompletely or incorrectly repaired DNA lesions or multiple damaged sites (Ward *et al.,* 1994). Gamma irradiation (from a $^{60}$Co source) accounts for 80 % of mutant plant varieties released in China (Nagatomi, 1992) and 61 % of more than 200 direct-use mutant varieties released in Japan (Hitoshi, 2008). Gamma rays have proven to be useful in inducing variability and increasing mutation frequencies coupled with high and uniform penetration of the multicellular system (Jain, 2005).

**b) Plant material and Radio Sensitivity Test**

The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants, and the mutation efficiency. The mutation rate is affected by the total dose of the mutagen employed, and can be modified by physical and biological factors. To induce mutations and recover useful mutant plants, radio sensitivity tests of different cultivars and plant material must be conducted (Mishra *et al.,* 2007). Dosage tests or radio sensitivity tests must be conducted so as to determine the lethal dosage which causes 30 or 50 % reduction in plant height ($LD_{30}$ / $LD_{50}$) when compared to the non-irradiated control for each experimental plant or variety (Brunner, 1995; Owoseni *et al.,* 2006; Babaei *et al.,* 2010; Jain & Suprasanna, 2011). This is done by exposing the specific plant material from each variety to different dosages of mutagen treatment and calculating the damage in comparison to the non-treated control. The lethality rate depends on the breeder, as some breeders use $LD_{30}$ while others use $LD_{50}$ (Owoseni *et al.,* 2006; Mishra *et al.,* 2007; Harding & Mohammad, 2009; Taher *et al.,* 2011; Mejri *et al.,* 2012; Bado *et al.,* 2016). The $LD_{50}$ values vary with the plant parts and reference doses could be sourced from literature or obtained from the international Atomic Energy Agency (IAEA). This is helpful for researchers who lack the facility to conduct their own tests before the actual mass irradiation. The radio sensitive test or $LD_{50}$ dose of the similar test material must be known before mass irradiation of the test sample follows. The greater the number of test planting material to be irradiated, the higher the chances of finding a useful mutant.

**c) Induced mutation nomenclature**

The modes of propagation of the plant species, either seed or vegetatively propagated have different population nomenclature. Parental lines are designated as P1, P2, P3, and

so on, while offspring generations are called as F1, F2, F3, and so on, and backcross lines are called as BC1, BC2, BC3, and so on. In induced mutation, seed prior to mutation treatment is called the M0 population (also known as generation zero); once irradiated the seed(s) is known as the M1 generation. This M1 is chimeric which produce a plant with different tissues anchoring different alleles, making the measurement of heritable phenotypic differences complex to unfeasible. The offspring of the M1 is the M2 representing the earliest generation, in which screening for mutations is viable. In self-fertile species, the M2 represents the first chance for the generation of homozygous mutant alleles, which allows the expression of recessive traits. Single plants may therefore be phenotypically screened and selected in the M2. Selection may be carried out in subsequent M3 families with an advantage that segregation of the mutant traits within the family can be observed in determining if the family is homozygous for the mutant allele. Selection may also be extended to M4 and other subsequent generations. Later generations are useful in detecting rare phenotypes that are the result of specific mutant combinations. This selection system is usually adopted in self-pollinating species such as barley, rice and wheat (Shu *et al.,* 2012).

In vegetatively propagated crops such as cassava, bananas and potatoes, the terminology is a little different. The starting material is still referred to as M0, but after mutagenic treatment is termed the M1V1 and subsequent generations are M1V2, M1V3 and so on. Like in the M1 of seed crops, the population carries chimeras hampering the capacity to score heritable phenotypes accurately hence usually resolved through multiple rounds of vegetative propagation (Ahloowalia & Maluszynski, 2001; Hewawasam *et al.,* 2004).

The generation's of mutant individuals with the desired characteristics are easily detected via various stability tests (Saif-ur-Rasheed *et al.,* 2001; Zhen *et al.,* 2001; Gulsen *et al.,* 2007). Salt-tolerant mutants of Marfona potato node explants were treated with various dosages of gamma irradiation and the M1V2 and M1V3 clonal generations were developed in which 47 % difference was detected in mutant plants produced by 20 or 30 gray (Gy) (Yaycili & Alikamanoğlu, 2012). In Kenya, mutation breeding has not been widely adopted as a method of breeding, however, few crops have been developed through mutation such as cowpea (Pathak, 1996) and wheat (Kinyua *et al.,* 2000; 2008; 2014; MVD, 2016).

**d) Mutant population development and handling of mutagenic populations**

Successful mutant isolation relies on efficient mutagenesis as well as the population size, the handling of the first generation and screening methods. The ideal population size for mutation breeding experiments can be estimated based on the frequency of a mutation even for a specific locus of interest and from experience. A large population size from efficient mutagenesis raises the likelihood of discovering mutants. Since induced mutation is a random process, large mutant populations need to be developed for screening, and this calls for consideration of available logistics (human power and infrastructure). One approach to maximize the chance of recovering a trait of interest, especially a mutant trait that is rare is to undergo several rounds of mutation. The observed frequency of mutants can then be used to adjust the mutagenesis treatment to provide desired frequencies.

The first mutant population M1 or M1V1 should be sown and grown up under optimal growth conditions to maximize plant survival and success in delivering mutants into

subsequent generations where they can be screened. Optimal growth conditions include sowing time, moist seed beds, optimal irrigation and nutrition. Additionally, it is important to prevent biotic and abiotic stresses. M1 plants have a higher tendency to out-cross particularly in sub-optimal conditions (Ukai & Nakagawa, 2012) and it should be isolated either by appropriate distance or by bagging floral parts to prevent out-crossing. The M2 seeds can be harvested from individual plants, either from single floral structures on M1 plants (such as spikes of wheat), by one-plant-one seed (Ukai & Nakagawa, 2012), or by bulking all seed produced from the M1 plants. The strategy will depend on subsequent screening and handling capacities, targets and costs (Forster *et al.,* 2012). The different procedures have consequences in the management of the M2 population (Donini & Sonnino, 1998): population bulk system, ear to row, single seed or multiple-seed bulk, plant to row and ear, branch, pod or fruit to row. Protocols for mutant population development have been reviewed in seed crops by (Ukai & Nakagawa, 2012) and in vegetatively propagated crops by (Suprasanna *et al.,* 2012).

**e) Mutation induction in potato improvement**

Potato mutation breeding started in 1931 with the ground-breaking effort of Asseyeva, (1931). Since 1931 to 2014 merely 6 potato mutants have been registered in the FAO/IAEA Mutants Variety Database, however, only 1 of these varieties was created via gamma irradiation (MVD, 2016). Mutation induction in potato has produced mutants in various characters such as modified starch biosynthesis increased yield (Al-Safadi *et al.,* 2000; Li *et al.,* 2005; Muth *et al.,* 2008), long shelf-life (Baskaran *et al.,* 2007), modified histological and texture properties (Nayak *et al.,* 2007) and increased tolerance to abiotic and biotic stresses (Al-Safadi & Arabi, 2003; 2007; Albiski *et al.,* 2012).

Gamma irradiation is the most widely used mutagenic treatment in crop improvement for vegetatively propagated crops, it enhances the microtuber production with minimal genetic change (Mahfouze *et al.,* 2012; Matijevic *et al.,* 2013). Prior to mutation induction, radio-sensitivity tests require to be performed to find out the optimal dose treatment for mutation induction. This consideration is even more fundamental for vegetatively propagated crops, because of the impossibility to restore the genetic background by back crossing.

**f) Use of induced mutations**

One of the main limitations of induced mutation is lack of reproducibility in induced mutagenesis. Repetition and adherence to published irradiation conditions may not result in the same mutation (Mba *et al.,* 2010). Mutation induction is a random process with generally low mutation frequencies and results are unpredictable without any specific selection pressure (Jain, 2005; Ceballos *et al.,* 2008). In a mutation breeding programme, large number of irradiated plant materials are required to increase the chance of finding a useful mutant. Handling and screening of large mutant populations is important in creating a fair chance of detecting desirable mutations though it is costly, laborious and time consuming (Brunner, 1995; Mba *et al.,* 2009). It is therefore important to develop easy and quick screening methods to identify and select promising mutants from the large mutant populations generated.

### 2.2. 3 Value of induced mutation in crop improvement

**a) Heritability**

Progress in crop improvement programs does not only depend on the amount of genetic variation present in the population but also the knowledge of heritability and extent to which the desirable characters can be transmitted from one generation to the other (Waqar-Ul-Haq *et al.,* 2008; Wang *et al.,* 2011; Hussain *et al.,* 2011).

Heritability estimates the relative contribution of genetic factors to the phenotypic variability observed in a population and is subject to prediction of gain from selection and determination of the relative importance of genetic effects (Kashiani *et al.,* 2010; Laghari *et al.,* 2010). Heritability values can be used as a measuring scale to determine genetic relationships between parents and progeny (Memon *et al.,* 2007). Better heritability values recorded points to the possibility of improvement in the parameters so that attention may be focused on important traits while synthesizing genotypes (Ahmed *et al.,* 2007). Heritability indicates the proportion of the total phenotypic variance (variation in a trait after accounting for variance attributable to known fixed effects and it is composed of both genetic and environmental parts) attributable to genetic effects, both of which are subject to change both within and between populations. Phenotypic variation observed among plants or varieties is due to differences in their genetic makeup, environmental influences on each genotype and interaction of the genotype and the environment.

$$\boldsymbol{\delta_P^2 = \delta_g^2 + \delta_e^2}$$

Where $\delta$ denotes variance, and *p, g* and *e* refer to phenotype, genotype and environmental parts, respectively (Falconer & Mackay, 1996).

Genotypic variance encompasses the additive effects of genes, as well as dominance and epitasis, i.e. the effects of genes at the same locus, and interactions between genes

at different loci. Environmental variance in this sense means all variation not due to genetic influences as well as measurement error and individual stochastic effects.

**b) Genetic advance**

Genetic advance indicates the magnitude of the expected genetic gain from selection cycle (Hamdi *et al.,* 2003). It is the measure of the expected genetic progress that would result from selecting the best performing genotypes for a character being evaluated (Allard, 1999). Selection is a major important process in breeding for improvement of one or more plant attributes which involves the retention of the desired genotypes and elimination of the undesirable ones.

High value of heritability and predicted genetic advance clarifies that the selection among genotypes would be effective for yield and yield components (Ghandorah & EI-Shawaf, 1993). Studies have shown that high heritability with considerable amount of genetic advance is very vital for selection in advanced generations (Memon *et al.,* 2007; Mangi *et al.,* 2008). Haydar *et al.,* (2009) reported high estimates of heritability and genetic gain for plant height, number of branches, tubers number and yield indicating that these traits are largely controlled by additive gene action and that strong selection for them would be effective. Haydar, (2007) recorded comparatively high heritability in potato for fresh weight and tuber weight/plant. Ara *et al.,* (2009) reported high heritability and genetic gain percentage for fresh weight/plant, number of main stem and tuber fresh weight; indicating these characters are largely controlled by additive gene action and that straight selection for them would be effective.

## 2.3 Application of Marker techniques

The characterization of genetic diversity is also important for cultivar identification, cultivar protection as well as to ensure the trademark and intellectual property rights (Coombs *et al.,* 2004). Information on genetic diversity is used in co-ancestry/ pedigree studies to avoid closely related parents and hence inbreeding depression in a crop like potato (Tarn *et al.,* 1992). There are three major types of genetic markers: (1) morphological ('classical', 'phenotypic' or 'visible') markers are phenotypic traits or characters; (2) biochemical markers include allelic variants of enzymes called isozymes; (3) DNA (or molecular) markers, which reveal sites of variation in DNA sequence (Winter & Kahl, 1995; Jones *et al.,* 1997).

### 2.3.1 Morphological markers in potato

Phenotypic identification of plants has been used as a powerful tool in the classification of genotypes and to study taxonomic status, based on morphological traits recorded in the field. Most important agronomic characteristics are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions. Morphological characterization has been used for various purposes including studies of genetic variation patterns, identification of duplicates and correlation with characteristics of agronomic importance (CIP, 2007).

### 2.3.2 Biochemical markers

Biochemical markers are routinely used to detect differences between individuals. These markers only sample actively expressed regions of the genome hence limits their use in certain aspects of plant biology and genetics as co-dominant neutral genetic markers due to lack of adequate polymorphism (Tanksley & Orton, 1983).

### 2.3.3 Molecular or DNA markers

Molecular markers are the most widely used mainly because they are much more numerous, they do not disturb the physiology of the organism. They reveal more sites of variation at the DNA sequence level which might be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA (Jones *et al.,* 1997). Because polymorphisms are DNA sequence variations, these markers are applicable at any plant stage and tissue and are independent of growing conditions (Hahn & Grifo, 1996). They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996).

The most widely used molecular markers are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) or microsatellites (Collard *et al.,* 2005) and Single Nucleotide Polymorphisms (SNPs) (Hamilton *et al.,* 2011). The SSR or microsatellites (sometimes referred to as a variable number of tandem repeats or VNTRs) are short segments of DNA that have a repeated nucleotide sequences. The SSR markers have been confirmed to be the most informative and appropriate for most potato because of their valuable attributes (Coombs *et al.,* 2004). These attributes of all SSR markers are robust, polymorphic, technical simplicity, sensitivity, co-dominance (Wang *et al.,* 1994), analytical simplicity (unambiguously scoring of data), high reproducibility (Dayanandan *et al.,* 1998) and are high abundance.

### 2.3.4 Genotyping by sequencing for potato improvement

The potato genome sequencing has provided a major boost to a better understanding of potato biology and strengthens genomic breeding (Potato Genome Sequencing Consortium, 2011). The next generation breeding techniques that can be applied in potato, includes the genotyping by sequencing (GBS), genomic selection (GS), genome wide association studies (GWAS), genome editing and next generation phenotyping among others. These techniques have become credible tool for the studies on genetic diversity and crop improvement (Lin *et al.,* 2014; Fu *et al.,* 2014). Next generation sequencing reduces the cost and time required for sequencing the whole genome and the convenient approach for genomic improvement in potato (Butler & Douches, 2016; Barabaschi *et al.,* 2016; Zia *et al.,* 2017).

## 2.4 Bacterial wilt (*Ralstonia solanacearum)*

### 2.4.1 Origin

The origin and subsequent dissemination of the disease remain undetermined. The earliest reports were published about the same time, towards the end of the 19$^{th}$ century, in diverse parts of Asia, South America, USA and Australia, where it was already apparently well-established (Kelman, 1953).

### 2.4.2 Taxonomy

The pathogen causing wilt disease was first described by E.F. Smith from potato, tomato and eggplant in 1896 called *Bacillus solanacearum* since it seemed to have peritrichous flagella (Smith, 1896). Later, he suggested the pathogen as *Pseudomonas solanacearum* (Smith, 1914) since it was found to have a single polar flagellum (Smith, 1914; Kelman, 1953). In 1992 it was proposed to be placed in the new genus

*Burkholderia* on the grounds of 16S rRNA sequences, DNA-DNA homologies, fatty acid analyses, and other phenotypic characteristics (Yabuuchi *et al.,* 1992). Later phylogenetic and polyphasic phenotypic analyses allowed to accommodate the bacterium in a new established genus of *Ralstonia*, in 1995 and hence the bacterium is named *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995).

### 2.4.3 Morphological and physiological characteristics

*Ralstonia solanacearum* is a gram negative strictly aerobic rod-shaped bacterium with an average size varying from 0.5 to 0.7 by 1.5 to 2.5 μm (Denny & Hayward, 2001). The bacterium is commonly cultured on both liquid and solid (agar) growth media. On solid agar medium, individual colonies are usually visible after 36 to 48 hours of growth at 28 °C and Kelman's tetrazolium chloride (TZC) agar is regularly used for its isolation (Kelman, 1954). After two days on TZC medium, virulent wild-type colonies are large, elevated, fluidal, and either entirely white or with a pale red center.

### 2.4.4 Host range

Globally, the disease has been estimated to affect about 1.7 million hectares of potatoes in approximately 80 countries, with global damage estimates of over USD 950 million per annum (Champoiseau, *et al.,* 2009). The disease affects over 200 plant species from more than 50 families (Hayward, 1991) especially tropical and subtropical crops are most susceptible to one or other races of *R. solanacearum*. Worldwide, the most important plants susceptible to *R. solanacearum* are: tomatoes, Musa spp., tobacco (*Nicotiana tabacum*) and potatoes. Some minor host crops are: *Anthurium* spp., artichoke, groundnuts (*Arachis hypogaea*), *Capsicum annuum*, cotton (*Gossypium hirsutum*), rubber (*Hevea brasiliensis*), cassava (*Manihot esculenta*), castor beans

(*Ricinus communis*), egglant (*Solanum melongena*), ginger (*Zingiber officinalis*), *Eucalyptus, Pelargonium, Piper hispidinervium, Pogostemon patchouli*, pumpkin, sesame, turmeric and most weeds (Remenant *et al.,* 2010). These therefore increase the possibility of *R. solanacearum* to build up inoculum (Kelman, 1953; Hayward, 1994). *Ralstonia solanacearum* is pathogenic also on model plants such as *Arabidopsis thaliana* and *Medicago truncatula* therefore facilitating analysis of basic molecular mechanisms governing pathogenicity. The *R. solanacearum*'s host range is not restricted to *solanaceous* plants but encompasses many other botanical families among dicots and monocots (Hayward, 1991; Boschi *et al.,* 2017).

### 2.4.5 Infectious cycle of *R. solanacearum*

*Ralstonia solanacearum* is primarily a soil and water borne pathogen. It is disseminated by soil, contaminated irrigation water, surface water and infected plant material (Janse, 1996; Champoiseau *et al.,* 2010) taking cuttings without disinfecting grafting knives between plants and pinching buds of plants. The pathogen early plant infection involves root entry, mostly via lateral root emergence sites or by root damage caused by handling or soil-borne organisms or stem injuries from insects, handling, or tools. Once the bacterium is inside the plant, it rapidly develops within intercellular spaces of the inner cortex; then, it crosses the natural barrier of the endodermis and penetrates into the vascular cylinder where it multiplies within vascular parenchyma to finally invade protoxylem vessels via cell wall degradation (Vasse *et al.,* 1995). Bacterial proliferation synchronizes with a massive exopolysaccharide synthesis that obstructs the vessels and blocks the circulation of raw sap (water and salts) from roots to aerial parts of the plant. This induces the typical wilting symptoms and eventually plant death. The functional analysis of pathogenicity genes indicates that several hydrolytic enzymes might be

necessary to promote the intercellular progression of the bacterium within the inner cortex and during translation towards the xylem vessels (Denny, 2006; Genin, 2010).

### 2.4.6 Symptoms on potatoes

External characteristic symptoms of bacterial wilt are mostly wilting, stunting and yellowing of the foliage; however expression of the symptoms and rate of disease development may vary according to the influence by environmental conditions, susceptibility and growth conditions of the host plants (Smith, 1914; Kelman, 1953). In potatoes the early visible symptoms at the first stages of diseases usually appear on foliage of plants. They consist of wilting of the youngest leaves at the ends of the branches during the heat of the day with recovery at night when temperatures are cooler, but soon wilting becomes irreversible and plant's death follows. The stems of young plants may collapse and/or have narrow dark streaks. Vascular discoloration of the stem appears to be grey or brown and bacterial ooze is present (Champoiseau *et al.,* 2010). A presumptive diagnostic value test can be done in the field where cut surface of a potato stem is kept in a beaker with water. A white, slime mass of bacteria exudes from vascular bundles oozes spontaneously from the broken stem. Such threads are not formed by other potato bacterial pathogens (EPPO/CABI, 1996).

On tubers, depending on the state of development of the disease the external symptoms may or may not be visible, and may be confused with those of ring rot due to other pathogens as *Clavibacter michiganensis* subsp. *Sepedonicus* (EPPO/CABI, 1996). Tubers infected with *R. solanacearum* can be distinguished by the bacterial ooze that often emerges from the eyes and stem-end attachment. When this bacterial exudate dries, a mass of soil adheres to the tubers at the eyes. Cutting the diseased tuber will reveal a browning and necrosis of the vascular ring and immediately surrounding

tissues. A creamy fluid exudate usually appears spontaneously on the vascular ring of the cut surface a few minutes after cutting. Plants with foliar symptoms of *R. solanacearum* may sometimes bear healthy and diseased tubers while others show no disease signs may produce diseased tubers (EPPO/CABI, 1996). In the case of ring rot, tuber has to be squeezed to press out mass of yellowish dissolved vascular tissue and slime. Initially the vascular ring appears yellow to light brown, but as the infection progresses the ring will become browner (Champoiseau *et al.,* 2010) (Plate 1).

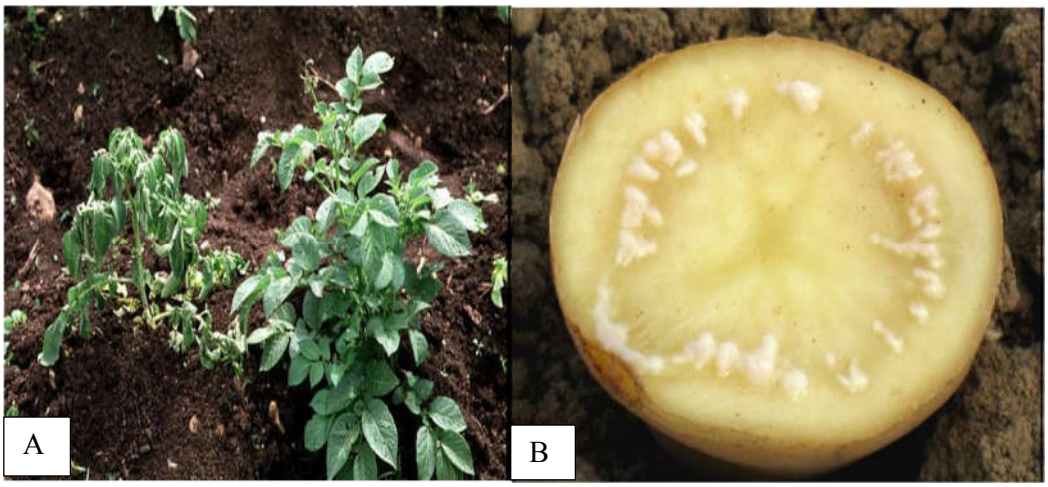


**Plate 1: Symptoms caused by R*alstonia solanacearum.* (A) Wilting and stunting of potato vegetative plant parts. (B) Bacterial ooze from vascular tissues (vascular ring) in potato tuber. Source: The International Potato Center, Lima, Peru (A), P. Champoiseau, (B) University of Florida (2010)**.

### 2.4.7 Development and field screening of bacterial wilt resistant cultivars

Resistance breeding to *R. solanacearum* in solanaceous crops appears to be regional or associated to climatic conditions (Hayward, 1991). Cultivars showing resistance to *R. solanacearum* at high altitudes often becomes susceptible when grown in the lowlands at warmer temperatures (French & De Lindo, 1982; Tung *et al.,* 1990). This suggests

the existence of latent infections which further adds complexity to breeding programs for resistance to bacterial wilt. The quantitative resistance available does not bear the high disease pressure caused by climatic conditions, especially under high soil humidity and high temperature (Nielsen & Haynes, 1960; Tung *et al.,* 1990; French, 1994). Additionally, wilting symptoms have been known not to constantly correlate with the amount of bacteria present in the plant (Angot *et al.,* 2006; Hirsch, *et al.,* 2002). To assure success of long-term breeding programs, it is extremely vital to develop screening methods that permit easy tracking and quantification of bacterial colonization and latency in plants (Lopes *et al.,* 2018).

Currently, potato breeding program worldwide are focused on the development of improved potato varieties with high levels of field resistance to bacterial wilt (Gonzalez *et al.,* 2013; Narancio *et al.,* 2013; Lopes *et al.,* 2018). The wild potato species related to *S. tuberosum* are used to generate bacterial wilt resistant cultivars (Sequeira & Rowe, 1969; Narancio *et al.,* 2013). Bacterial wilt resistance have been reported in *Solanum phureja* (Sequeira & Rowe, 1969; French & De Lindo, 1982; Fock *et al.,* 2000), *S. stenotomum* (Fock *et al.,* 2001), *S. chacoense* (Chen *et al.,* 2013) and *S. commersonii* Dun (Laferriere *et al.,* 1999; Kim-Lee *et al.,* 2005; Carputo *et al.,* 2009; Gonzalez *et al.,* 2013). In recent times, *S. commersonii* exhibits high genetic diversity (Pianzzola *et al.,* 2005; Siri *et al.,* 2009) with desirable traits like tolerance to low temperatures and resistance to various pathogens, including *R. solanacearum*. Hybridization of *S. commersonii* × *S. tuberosum* is hindered by complex interspecific crossing barriers, hybrids with partial resistance to bacterial wilt obtained using diverse strategies for ploidy manipulation (Kim-Lee *et al.,* 2005; Guidot *et al.,* 2009; Carputo *et al.,* 2009; Narancio *et al.,* 2013; Gonzalez *et al.,* 2013; Zuluaga-Cruz *et al.,* 2014). This therefore,

calls for the use of induced mutations to create new genetic variations within the potato crop varieties where natural genetic variation is limited to obtain desired mutants with important agronomic traits (Jain, 2005).

In Kenya, Ateka *et al.,* (2001) found that varieties, Kenya Baraka, Kenya Sifa, Kenya Karibu, Kenya Dhamana (CIP-800228), Mauritius (CIP- 89016), and Cruza-148 (CIP-720118) were rated as more resistant to bacterial wilt than all the other varieties grown in Kenya. They also identified that the most productive and most popular potato varieties, Tigoni (CIP-381381.13), Nyayo, and Dutch Robyjin were the most susceptible. Rotich *et al.,* (2010), screened five potato cultivars in Kenya found that none of the potato cultivars was resistant and reactions to bacterial wilt varied from cultivar to cultivar and environment to environment. Kenya karibu, Kenya Sifa and Asante (CIP-381381.20) were rated as the most tolerant cultivars while Tigoni (CIP-381381.33) and Dutch Robjin were the least tolerant to bacterial wilt. Muthoni *et al.,* (2014), screened 36 genotypes for 3 seasons in Kenya and when all the genotypes were ranked across seasons found that the most resistant genotypes were Kenya Karibu followed by Kenya Sifa, Ingabire, clone 394034.7 and Kenya Baraka was the fifth in that order. Continuous development of resistant varieties is needed to manage bacterial wilt of potatoes better (Fock *et al.,* 2001; Champoiseau *et al.,* 2010).

Induced mutations generates new genetic variations within the crop varieties that can be evaluated for useful traits. Hence, the focus of the research study is to induce mutation on three Kenyan potato varieties to create desirable allelic variants and screen for bacterial wilt resistance in the field.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Effects of induced mutation and the dose of mutagen on agronomic traits in potato mutants

### 3.1.1 Development of mutant population

**Parental lines**

Three commercial potato varieties namely; Asante, Kenya Mpya and Kenya Sherekea were obtained from Kisima farm. The three potato varieties used had various characteristics as described. Asante has intermediate dormancy, fairly tolerant to late blight, long oval white smooth skin colour, shallow eye depth, poor tuber storage, matures between 90 – 120 days and tuber yield is between 35 – 45 t/ha. Kenya Mpya has short dormancy, highly tolerant to late blight, shallow eye depth, cream white skin colour with pink eyes, early tuberization and matures early at 90 - 105 days, oval or round tubers with large tuber size yielding between 35 – 45 t/ha. Kenya Sherekea has intermediate dormancy, highly tolerant to late blight and viruses, good storability, medium eye depth, red skin colour and oblong or round high tuber number per plant that matures between 105 – 120 days yielding between 40 – 50 t/ha (NPCK, 2013).

A total of 30 tubers each of the three potatoes were sent to the Plant Genetics and Breeding Laboratories (PGBL) at IAEA/FAO Seibersdorf, Vienna, Austria, where they were grown at the greenhouse to initiate *in vitro* shoot cultures as described by Bado *et al.*, (2016).

**Irradiation methods**

A radio-active cobalt-60 ($Co^{60}$) (gamma source) with a low dose rate of 2 gray per minute (Gy/min) were used to induce mutations. The optimal dosage for mutation induction, $GR_{30}$ and $GR_{50}$ (30 % and 50 % growth reduction respectively) as well as $LD_{30}$ and $LD_{50}$ (30 % and 50 % lethality dose) were determined for each approach as described by Owoseni *et al.,* (2006); Mba *et al.,* (2010); Kodym *et al.,* (2012); Bado *et al.,* (2016) to define the susceptibility of each potato cultivar with regard to mutation induction. Two *in-vitro* radio-sensitivity tests were developed involving different tissues for irradiation of potato mutation induction as described by Bado *et al.,* (2016) namely: 1) Irradiation of *in vitro* nodal cuttings (without leaf) followed by *in vitro* shoot propagation to dissolve chimeras. A dose range of 0, 5, 10, 15, 20 and 30 Gy was used, and 2) Irradiation of *in vitro* nodal cuttings (with leaf) followed by direct *in vitro* micro-tuber production. The dose range used was 0, 3, 6, 9, 12, and 15 Gy.

The irradiation dose rates performed on the two *in-vitro* radio-sensitivity tests for the three potato varieties were 0, 3, 6, 9, 12 and 15 gray (Gy) for Asante, 0, 5, 6, 10 and 15 Gy for Kenya Mpya and 0, 3, 5, 10, 12, 15, 20, and 30 Gy for Kenya Sherekea according to Bado *et al.,* (2016). After mutation induction, a total of 570 mutant microtubers (Asante 230, Kenya Mpya 160, Kenya Sherekea 180) were developed from the three potato varieties and was transported to Kenya, University of Eldoret for establishment.

### 3.1.2 Establishment and testing of potato mutants

**Establishment of M1V1 and coding of surviving putative mutants in the greenhouse**

A total of 570 mutant microtubers (M1V1) were received from Seibersdorf laboratories, Vienna, Austria, and were established at the University of Eldoret (UoE), Biotechnology Green House Research facility on autoclaved loam sandy soil. Each mutant at M1V1 generation was planted on 10 × 9 mm polythene bag. There was no replication of the mutant minitubers because each does not maintain the same genetical constitution after irradiation. Induced potato plants derived from minitubers were therefore assumed to be different. The coding of the M1V1 plants were developed based on the number of irradiated stakes that survived and was then advanced to the subsequent generations (Table 1). This was done in sequence. The first letter in the name for each variety (A, M, and S for Asante, Kenya Mpya and Kenya Sherekea respectively) was used to separate the specific mutants.

**Table 1: Coding of the potato irradiated stakes that survived M1V1 generation**

| Mutants | Dose rates | Number of Irradiated tubers | Survived at M1V1 with tubers | Coding of tubers |
|---|---|---|---|---|
| **Asante (AP)** | **0** | 1 | 1 | AP |
| | **3** | 44 | 12 | A1 - A12 |
| | **6** | 53 | 18 | A13 - A30 |
| | **9** | 24 | 7 | A31 - A37 |
| | **12** | 18 | 2 | A38 - A39 |
| | **15** | 90 | 33 | A40 - A72 |
| **Kenya Mpya (MP)** | **0** | 1 | 1 | MP |
| | **5** | 17 | 2 | M1 - M2 |
| | **6** | 104 | 36 | M3 - M38 |
| | **10** | 18 | 2 | M39 - M40 |
| | **15** | 20 | 2 | M41 - M42 |
| **Kenya Sherekea (SP)** | **0** | 1 | 1 | SP |
| | **3** | 12 | 4 | S1 - S4 |
| | **5** | 14 | 6 | S5 - S10 |
| | **10** | 46 | 11 | S11 - S21 |
| | **12** | 15 | 2 | S22 - S23 |
| | **15** | 62 | 20 | S24 - S43 |
| | **20** | 16 | 2 | S44 - S45 |
| | **30** | 14 | 1 | S46 |

**Asante, Kenya Mpya and Kenya Sherekea were coded AP, MP and SP respectively**

**Experimental site**

The experiment was carried out at the University of Eldoret (UoE) which is at an altitude of 2153 metres above sea level (masl), latitude of 0°34'N and longitude 35°18'E. The average annual rainfall is 1295 mm with a bimodal distribution. The mean air temperature ranges from 15 to 28 °C. The soil type is rhodic ferralsol (UNESCO, 1977).

**Planting of M1V2 and M1V3 mutants in the field**

The tubers obtained at M1V1 were advanced to M1V2 and M1V3 generations of mutants by planting at the University of Eldoret research field.

**Experimental design, layout and planting in field plots**

In M1V2 and M1V3 generation five tubers per plot/mutant were planted replicated 3 times in alpha lattice design.

The linear model for alpha design was:

$y_{ijtl} = \mu + g_i + r_j + \alpha_t +, \alpha(r)_{jl} + \varepsilon_{ijtl}$

Where;

$y_{ijtl}$ represent the observations,

$\mu$ is the population mean,

$g_i$ the genotypic effects,

$r_j$ the resolvable replicate effects,

$\alpha_t$ the latinized block effects,

$\alpha(r)_{jl}$ the incomplete block effects within replicates and

$\varepsilon_{ijtl}$ the random errors.

The experimental area was divided into fifteen blocks where each block consisted of 11 plots. Each plot consisted of a mutant selection from a single variety and were planted in 1 × 0.6 metre (m) spacing. This setup was set in 3 replicates and the distance between replicates was 3 m. All agronomic practices were carried out according to recommended practices (Kabira *et al.,* 2006) . The M1V2 generation was established in April to July 2015 and the M1V3 generation was planted between January to May 2016.

### 3.1.3 Data collection

Data was collected at M1V1 mutants on:

**The number of irradiated shoots that survived and produced tubers**; this was the number of mutant plants that produced tubers in each plot divided by the control (parent) as a percentage.

**Plant height** is the average measure in centimeters from ground to tip of the main stem for 3 randomly selected plants in each plot.

**Stem number;** was done by counting the number of main stems in each mutant for 3 randomly selected plants in each plot.

**Tuber number** which is the average of total count of tubers from each plant for 3 randomly selected plants in each plot.

**Weight per mutant,** this was the average weight for 3 randomly selected plants in each plot and converted into tons per hectare.

Data were also collected on each of the plants in M1V2 and M1V3 mutants. Standard potato descriptors according to International Union for the Protection of New Varieties of plants (UPOV, 2010) were used to describe the potato mutant selections in M1V2 and confirmed in M1V3. The descriptors are presented in Table 2 below.

**Table 2: Morphological descriptors used for scoring potato accessions**

| Descriptor | Characteristic | Score |
|---|---|---|
| **Plant** | Growth habit | 1=very upright, 3=upright, 5=semi-upright, 7=spreading, 9=very speading |
| | Stem number per plant (Average) | Counting |
| | Plant height per plant (Average) | in centimetres |
| **Stem** | Anthocyanin colouration | 1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong |
| **Leaf** | Outline openness | 1=closed, 3=intermediate, 5=open |
| | presence of secondary leaflets | 1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong |
| | green colour | 1=very light, 3=light, 5=medium, 7=dark, 9=very dark |
| | anthocyanin colouration on midrib of upperside | 1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong |
| **Flower corolla** | Anthocyanin colouration | 1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong |
| **Inflorescence** | Anthocyanin colouration on penducle | 1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong |
| **Tuber** | Weight per plant (Average) | in kilograms |
| | Number per plant (Average) | Counting |

**(Source: UPOV, 2010)**

### 3.1.4 The effects of induced mutation on the genetic variance estimates

The M1V2 mutant plants were selected at harvesting of tubers based on the average tuber weight per plot. The M1V2 mutant plants that produced 25 % higher on average plant tuber weight compared with the control/parent were selected. Each of the selected individual mutant plants were labeled and advanced to M1V3 generation. The selected M1V3 generation was grown in plots of 1 × 0.6 m. The morphological data was used as described in Table 2 above to determine the diversity of the selections.

### 3.1.5 Statistical analysis

Data on the number of irradiated shoots that survived and produced tubers at M1V1 generation, plant height, stem number, tuber number and weight per mutant was calculated as the number of micro-tuber survived to produce tubers/ number of control

micro-tubers sprouted x 100 and presented in graphic form. The morphological data of M1V2 and M1V3 generations was subjected to analysis of variance (ANOVA) using R software. The means were compared using Fisher's Protected Least Significance Difference (FPLSD) whenever effects were significant at 95 % confidence level. Principal component analysis of the qualitative and quantitative traits was evaluated to examine the percentage contribution of each trait to total genetic variation.

The data on effects of the different induction levels on the potato characters of the selected M1V2 and M1V3 generation were used to generate means for the two generations. Combined ANOVA was performed for the traits by SAS software. Differences in means were compared by Duncan multiple range test (DMRT). Variance components were extracted from the expected mean squares (EMS) of main effects. Table 3 shows the format of ANOVA and of the expected mean squares.

**Table 3: Expected mean squares and extraction of variance components format of analysis of variance for obtaining estimates of variance**

| Source of variation | df | MS | Expected Mean Square |
|---|---|---|---|
| **Block** | (r-1) | | |
| **Genotype (t)** | t-1 | $M_2$ | $\sigma^2e + r\sigma^2g$ |
| **Error** | (r-1)(t-1) | $M_3$ | $\sigma^2e$ |

Where r and t, are numbers of genotypes and blocks, respectively

Genotypic variance = $\sigma^2_g = (M_2 - M_3)/r$

Phenotypic variance = $\sigma^2_p = (\sigma^2_g + \sigma^2_e)$

Error variance = $\sigma^2_e$

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Mulualem *et al.,* (2012) as:

Genotypic coefficients of variation (GCV)

$$\mathbf{GVC} = \frac{\sqrt{\boldsymbol{\delta}_{\boldsymbol{g}}^{\mathbf{2}}}}{\boldsymbol{\mu}} \times \mathbf{100}$$

Phenotypic coefficients of variation (PCV)

$$\mathbf{PVC} = \frac{\sqrt{\boldsymbol{\delta}_{\boldsymbol{p}}^{\mathbf{2}}}}{\boldsymbol{\mu}} \times \mathbf{10}$$

Where $\mu$ is the grand mean value of the trait

Broad sense heritability ($H^2$) in percentage was estimated in each character using variance components as described by DeLacy *et al.,* (1996).

$$\mathbf{H^2} = \mathbf{G_G^2/\delta_P^2}$$

The expected gain or genetic advance with one cycle of selection, assuming the selection intensity of 25 %, as described by Galwey, (2006).

$$\mathbf{GA} = (\mathbf{K})(\boldsymbol{\delta}\mathbf{p})(\mathbf{H^2})$$

Genetic advance in percentage of the mean (GAM) was calculated to compare the extent of predicted genetic advance of different traits under selection, using the following formula:

$$\mathbf{GAM} = \left(\frac{\mathbf{GA}}{\boldsymbol{\mu}}\right) \times \mathbf{100}$$

## 3.2 Assessment of the ploidy level diversity by chloroplast counts in stomatal guard cells of potato mutants

### 3.2.1 Plant Materials and Sample Preparation

Representative plants from the M1V1, M1V2 and M1V3 mutant generations for each clone as described in section 3.1 were randomly selected at 2 months after planting and subjected to ploidy level determination as described by Ordoñez, (2014). The experiment was carried out at least 5 times on each sample per treatment (clone) (section 3.1). One to five leaves of the apical part of the plant were collected from selected plants. The samples were placed in a petri-dish containing a filter paper moistened with distilled water covering the bottom of the lid. One or two drops of iodine solution (iodine-potassium iodide) were placed in the center of a slide. The surface cell layer from the under surface of the leaf was peeled off. Then, epidermal peels taken from the abaxial side near the vein structure using a pair of fine tweezers was immediately placed on the slide. After 2 min, a cover slip was mounted and gently pressed down and observations were made under a light microscope at 100X magnification.

### 3.2.2 Sample observation and data collection

Chloroplast counts in each of the two guard cells of stomata (Table 4) was scored in ten stomata per sample indicating average ploidy level using a scale as described by Ordoñez, (2014).

**Table 4: Scale to determine the ploidy level of potato genotypes**

| Chloroplast number in stomatal guard cells | Ploidy |
|---|---|
| 6 to 8 | Diploid (2n=2X=24) |
| 9 to 11 | Triploid (2n=3X=36) |
| 12 to 14 | Tetraploid (2n=4x=48) |

**Source: Ordoñez, 2014**

### 3.2.3 Data analysis

Data on the number of chloroplast counts on the stomatal guard cells per mutant was calculated as a percentage of the parents or control.

The data on chloroplast number in stomatal guard cells were analyzed separately for M1V1, M1V2 and M1V3 and subjected to ANOVA using SAS version 9.1. The means were compared based on Duncan Multiple Range Test (DMRT) whenever differences were significant at 95 % confidence level. The ploidy level distribution by chloroplast counts were also computed using descriptive analysis (Box and Whisker plots).

Dendrograms were generated on chloroplast number in stomatal guard cells data using the unweighted pair group method with arithmetic mean (UPGMA) and the hierarchical clustering method based on the estimates of genetic similarity. The genetic similarity matrix of the potato mutants and controls were calculated using the dissimilarity matrix calculated with Manhattan index, as executed in the DARwin software (version 6.0.9).

## 3.3 Screening of potato mutant populations against bacterial wilt races (pathogens) using pathogenicity test

### 3.3.1 Plant Materials

A total of 160 potato mutant tubers at M1V4 generation that were generated from M1V3 generation from the 3 parents were used. The 3 non irradiated parents acted as controls.

### 3.3.2 Experimental site

The experiment was carried out at National Research Laboratories (NARL), Kabete Station of the Kenya Agricultural and Livestock Research Organization (KALRO). The KALRO-Kabete station is at an altitude of 1795 m above sea level, latitude of 1°15' 31.64" S and longitude 36° 46' 17.96" E (Jaetzold *et al.,* 2006). The average annual rainfall is 1295 mm with a bimodal distribution. The mean air temperature ranges from 15.3 to 28.6 °C. The soil type is humic nitosol derived from quartz trachyte (UNESCO, 1977). The experiment was carried out for one season during December 2015 to 12 April, 2016.

### 3.3.3 Field Layout and Experimental design

A total of one hundred and sixty (160) M1V4 mutant potato genotypes and three controls were planted for screening for bacterial wilt resistance. The experimental design used was an alpha lattice with twenty four blocks of seven plots each and replicated three times.

The linear model for alpha design, Latinized by block was:

$$y_{ijtl} = \mu + g_i + r_j + \alpha_t +, \alpha(r)_{jl} + \varepsilon_{ijtl}$$

The $y_{ijtl}$ represent the observations, $\mu$ is the population mean, $g_i$ the genotypic effects, $r_j$ the resolvable replicate effects, $\alpha_t$ the latinized block effects, $\alpha(r)_{jl}$ the incomplete block effects within replicates and $\varepsilon_{ijtl}$ the random errors.

### 3.3.4 Inoculum collection

*Ralstonia solanacearum* inoculums were obtained from naturally infected potato plants in farmer's field in Kitale, Trans-Nzoia County, Kenya. The wilted plants were collected from the field and preliminary diagnostic test carried out in the field to rule out the presences of other bacteria. Diagnosis in the field was easily accomplished through the vascular flow test (Priou *et al.,* 1999). A piece of stem about 2-3 cm long were cut from the base of a wilting potato plant and suspending in clear water in a glass container. The cut stem is held with an opened paper clip to maintain a vertical position. After a few minutes, the smoke-like milky threads streaming downward from the cut stem confirms the presence *R. solanacearum* within the vascular system (French *et al.,* 1995; CIP, 2007; Champoiseau *et al.,* 2010). The positive plants were taken to the laboratory where resistance assay of *R. solanacearum* isolates were obtained as described by Carputo *et al.,* (2009).

### 3.3.5 Inoculum preparation

The infected potato tubers was washed with water to remove soil particles and later immersed in 70 % ethanol for 2 to 3 minutes to remove any other bacteria from the plant surface. It was cut aseptically and left for 5 minutes for the bacterial exudates to ooze. Culturing was done by streaking the oozing bacterial exudates onto a SMSA agar plates. The agar plates were then incubated at 28 – 30 °C or at room temperature for 5 days. Plates were incubated in an inverted position to avoid water condensation which

may cause colonies to flow into each other, thereby limiting separation. Populations of *R. solanacearum* were determined using a modified Semi-Selective Media South Africa (SMSA) method (Englebrecht, 1994) before inoculating the field during the time of planting of the crop.

The *Ralstonia solanacearum* colonies were then grown on Triphenyl Tetrazolium Chloride (TTC) medium to obtain pure cultures (Kelman, 1954). The stock inoculum solution was prepared in sterile conical flask containing 2100 ml of sterile distilled water and thoroughly mixed for 30 min and then allowed to stand for 5 min. From the stock solution, 0.1 ml was drawn and put in sterile Eppendorf tube which already contained 0.9 ml of sterile distilled water which formed the stock solution ($10^0$) for serial dilution. From the stock solution ($10^0$), 0.1 ml was drawn and put in sterile Eppendorf tube which already contained 0.9 ml of sterile distilled water. This formed the first dilution of the stock solution ($10^{-1}$). This serial dilution was continued up to $10^{-7}$. The 0.1 ml suspension of the serial dilution ($10^{-3}$, $10^{-5}$ and $10^{-7}$) was drawn and plated on semi-selective media for *R. solanacearum* and was replicated twice. The plates were incubated at 30 °C for 48 hour after which the bacterial colonies were counted and used for inoculation.

**3.3.6 Planting, inoculation and Crop Management**

Planting was done on ridges spaced at 75 cm inter-row and 30 cm intra row for each genotype (mutant/control). Five plants were planted per plot/clone in an alpha lattice design with seven blocks each having twenty one plots with three replications. Di-ammonium phosphate (DAP) fertilizer (18:46:0) were applied in furrows at a rate of 500 kg/ha and thoroughly mixed with soil before planting. Bacterial suspensions

concentrated at $3.0 \times 10^9$ cfu/ml were poured into the planting furrows (during planting of tubers but before covering them) at a rate of approximately 300 ml per plot to boost the innoculum concentration in the soil. Two border rows were planted around the plot. All standard agronomic practices were carried out according to recommendations for potato production in Kenya (Kabira *et al.,* 2006). To ensure disease progression, supplemental irrigation was provided during the dry spells. In addition, workers' shoes as well as working tools were disinfested by dipping in a footbath containing 1 % sodium hypochlorite when entering and leaving the field.

### 3.3.7 Data Collection

Data were collected on:

**Days to onset of wilting (DTOW);** was done by counting the number of days after planting in which the aerial part of the plant starts showing symptoms of wilting in each plot and were then done after every 7 days.

**Final bacterial wilt incidence (BWI):** This is whereby at each evaluation date (after every 7 days), all the wilting plants on each plot were counted and then expressed as a percentage of all the plants in the plot to give bacterial wilt incidence (BWI).

**Area under the disease progress curve (AUDPC),** were calculated using the BWI scores (CIP, 2007; Forbes *et al.,* 2014) using the formula below:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} \quad t_i)$$

Where $y_i$ is the BWI at $i^{th}$ days, and n is the total number of sampling times, t is the number of days after planting.

**Total tuber numbers (TTN):** Harvesting of potato tubers was done when the latest maturing genotype had reached 75 % senescence. During harvest, the plants per plot

were harvested each plant separately. The total number of tubers was counted from each of the plants.

**Proportion of symptomatic tubers based on total tuber number (PSTTN):** In addition to TTN, the number of symptomatic tubers (i.e. showing rotting or bacterial ooze in the tuber eyes or soil sticking to the eyes of the tubers) and healthy looking tubers (asymptomatic) were determined by counting from each of the plants per plot. The healthy looking tubers were then categorized based on size i.e. ware (>45 mm diameter), seed and chatts (<45 mm diameter) and the weights and number documented.

**Total tuber weight in tons ha $^{1}$ (TTW),** was done immediately after harvesting of potato tubers was done when the latest maturing genotype had reached 75 % senescence. During harvest, the plants per plot were harvested each plant separately. The total number of tubers was weight from each of the plants per plot.

**Proportion of symptomatic tubers based on total tuber weight (PSTTW),** this was done by taking the weights of symptomatic and ware tubers and were then expressed as percentage of the total yields.

**Proportion of ware sized tubers based on total tuber weight (PWTTW),**

The percent of symptomatic tubers were expressed as a weight, a value which is helpful to establish yield loses (tons ha $^{1}$) and as a number of infected tubers, a value used to calculate infection tuber rates.

### 3.3.8 Data analysis

Data on days to onset of wilting (DTOW), final bacterial wilt incidence (BWI), total tuber numbers (TTN), total tuber weight in tons ha $^{1}$ (TTW), proportion of symptomatic tubers based on total tuber numbers (PSTTN), proportion of symptomatic tubers based on total tuber weight (PSTTW), and proportion of ware sized tubers based on total tuber

weight (PWTTW) values were subjected to analysis of variance using SAS statistical package, version 9.1. Data on TTN, TTW, PWTTW, PSTTN and PSTTW were first averaged on plot basis; the average values were then used to extrapolate values per hectare. Where analysis of variance showing significant differences mean separation was done using Duncan Multiple Range Test.

Potato mutants were also ranked based on AUDPC, DTOW, TTN, TTW, PWTTW, PSTTW and PSTTN. Genotypes with low AUDPC, low PSTTW low PSTTN, more DTOW, high TTN, high TTW and high PWTTW were considered better and hence ranked high. Resistance of mutants to bacterial wilt was determined using ranking based AUDPC, DTOW, PSTTW and PSTTN. Genotypes with low AUDPC, low PSTTW, low PSTTN and more DTOW were more resistant to bacterial wilt and hence ranked high.

**Molecular phylogenetic analysis**

Phylogenetic trees were produced using phenotypic data of selected agronomic and bacterial wilt traits. The unweighted UPGMA (Sokal and Michener, 1958) and the hierarchical clustering method was used based on the dissimilarity matrix calculated with Manhattan index in the DARwin software version 6.0.9.

## 3.4 Determination of genetic variability of potato mutants using simple sequence repeat (SSR) markers

### 3.4.1 Plant Materials

A total of 160 potato mutants at M1V4 generation tubers that were generated from M1V3 generation and the three parents were used. The tubers were planted at the University of Eldoret, Biotechnology Green House Research facility to raise plants to be used for DNA extraction. Young fresh tender potato leaves were picked from one month old plants for DNA extraction using modified Dellaporta *et al.,* (1983).

### 3.4.2 DNA Extraction and Polymerase Chain Reaction (PCR)

Approximately 0.2 - 0.25 g per genotype/clone tissues were ground in a mortar and pestle and placed in a 1.5 millimeter (ml) Eppendorf® tube containing 600 µl of extraction buffer [0.1 M of Tris-Hydrochloric acid (Tris-HCl) pH 8.0, 0.05 M of (w/v) Ethylene diaminetetraacetate (EDTA), 0.5 M Sodium chloride (NaCl), 1 % of Polyvinylpirrodine (PVP), 0.07 % β mercaptoethanol and 20% (0.7 µl) of (w/v) sodium dodecyl sulphate (SDS) added separately]. The mixture was incubated at 65 °C for 15 minutes with agitation every 5 minutes. Then the samples were placed at room temperature for 5 minutes followed by addition of 350 µl of ice-cold 5M potassium acetate then incubated at -20 °C for 20 minutes. After incubation the samples were centrifuged for 15 minutes at 13,000 revolutions per minute (rpm) at room temperature. The supernatant was transferred to another tube and then 480 µl of ice-cold isopropanol was added and mixed gently. The mixture was kept at -20 °C for one hour or overnight and then centrifuged for 15 minutes at 13,000 rpm. The pellet was left to dry at room temperature by inverting the tubes on paper towels until all isopropanol droplets

disappeared from the walls of the tubes. The supernatant was removed and the pellet was washed with 700 µl of 70 % ethanol and the pellet dried at room temperature followed by a brief centrifugation of 5 minutes at 13,000 rpm. The above process from the addition of ice-cold isopropanol was done twice and the incubation was done for 20 minutes at -20 °C. The pellet was then air dried and later resuspended in 50 µl of Tri-EDTA (TE) 10:1 mM buffer and then incubated at -4 °C.

### 3.4.3 DNA Quantification

DNA quantity and quality of each accession was determined by running samples on 1 % (w/v) agarose gels for 1 hour at 80 volts diluted in 100 ml 1X TAE buffer (0.89 M Tris base, 0.89 M boric acid, 20 mM EDTA pH 8.0) and 900 ml of distilled water. A standard undigested lambda DNA with a range variation of 10, 20, 50, 80 and 100 ng was used as a comparison to determine the DNA concentration of the potato accessions by comparing band sizes and intensities. The gel was stained in ethidium bromide (10mg/ml) for 30 minutes and later de-stained in distilled water for 20 minutes before viewing under ultraviolet transilluminator. Between 0.5 µg and 1µg of high quality DNA was obtained and was diluted to 0.01 µg/µl with deionized distilled water for PCR amplification.

### 3.4.4 PCR Amplification

A subset of 20 SSR markers (Table 5) were selected from a data base of SSRs applied in previous studies based on their high polymorphic content and broad coverage of the potato genome (Ghislain *et al.,* 2004; Feingold *et al.,* 2005; Reid & Kerr, 2007; Ghislain *et al.,* 2009; Rocha *et al.,* 2010; Muthoni *et al.,* 2014; Biniam *et al.,* 2016).

**Table 5: Description of the 20 SSR loci used to characterize 163 potato mutants**

| Locus | Forward/ Reverse | Ta (°C) | Range Size (bp) | Chr Pos |
|---|---|---|---|---|
| STG0016 | AGCTGCTCAGCATCAAGAGA | 56 | 135-175 | I |
| | ACCACCTCAGGCACTTCATC | | | |
| STM5114 | AATGGCTCTCTCTGTATGCT | 55 | 295-325 | II |
| | GCTGTCCCAACTATCTTTGA | | | |
| STM1053 | TCTCCCCATCTTAATGTTTC | 55 | 170-195 | III |
| | CAACACAGCATACAGATCATC | | | |
| STI0012 | GAAGCGACTTCCAAAATCAGA | 56 | 180-235 | IV |
| | AAAGGGAGGAATAGAAACCAAAA | | | |
| STI0032 | TGGGAAGAATCCTGAAATGG | 60 | 125-150 | V |
| | TGCTCTACCAATTAACGGCA | | | |
| STI0004 | GCTGCTAAACACTCAAGCAGAA | 56 | 80-130 | VI |
| | CAACTACAAGATTCCATCCACAG | | | |
| STM0031 | CATACGCACGCACGTACAC | 60 | 110-210 | VII |
| | TTCAACCTATCATTTTGTGAGTCG | | | |
| STM1104 | TGATTCTCTTGCCTACTGTAATCG | 60 | 180-200 | VIII |
| | CAAAGTGGTGTGAAGCTGTGA | | | |
| STM1052 | CAATTTCGTTTTTTCATGTGACAC | 55 | 220-255 | IX |
| | ATGGCGTAATTTGATTTAATACGTAA | | | |
| STM1106 | TCCAGCTGATTGGTTAGGTTG | 60 | 165-200 | X |
| | ATGCGAATCTACTCGTCATGG | | | |
| STM0037 | AATTTAACTTAGAAGATTAGTCTC | 55 | 85-108 | XI |
| | ATTTGGTTGGGTATGATA | | | |
| STI0030 | TTGACCCTCCAACTATAGATTCTTC | 56 | 73-122 | XII |
| | TGACAACTTTAAAGCATATGTCAGC | | | |
| STM2013 | TTCGGAATTACCCTCTGCC | 55 | 160-185 | VII |
| | AAAAAAAGAACGCGCACG | | | |
| STM1049 | CTACCAGTTTGTTGATTGTGGTG | 57 | 136-212 | I |
| | AGGGACTTTAATTTGTTGGACG | | | |
| STM5127 | TTCAAGAATAGGCAAAACCA | 55 | 255-305 | I |
| | CTTTTTCTGACTGAGTTGCCTC | | | |
| STI0046 | CAGAGGATGCTGATGGACCT | 55 | 196-230 | XI |
| | GGAGCAGTTGAGGGCTTCTT | | | |
| STI0036 | GGACTGGCTGACCATGAACT | 55 | 130-160 | II |
| | TTACAGGAAATGCAAACTTCG | | | |
| STI0023 | GCGAATGACAGGACAAGAGG | 55 | 80-220 | X |
| | TGCCACTGCTACCATAACCA | | | |
| STWAX-2 | CCCATAATACTGTCGATGAGCA | 53 | 230-260 | VIII |
| | GAATGTAGGGAAACATGCATGA | | | |
| STPoAc58 | CAGAGGATGCTGATGGACCT | 57 | 240-255 | V |
| | GGAGCAGTTGAGGGCTTCTT | | | |

**Ta (°C) = Annealing temperature in degree centigrade, Chr Pos= Chromosome position (Source: Ghislain *et al.,* 2004; Feingold *et al.,* 2005; Reid and Kerr, 2007; Biniam *et al.,* 2016)**

The PCR reactions were performed in a Mastercycler (Eppendorf®) using in a final volume of 20 µl Bioneer AccuPower® containing 4 µl pre-mix (1U Top DNA, 250 µM each dNTP, 10 mM Tris-HCl pH 9.0, 30 mM KCl, 1.5 mM $MgCl_2$, stabilizer and tracking dye), 0.5 ng/µl of each f orward and reverse primer, 0.5 ng of DNA template, and 6 µl of double distilled water (ddH2O). The PCR cycles consisted of initial denaturation at 95 °C for 5 minutes, followed by 35 cycles at 95 °C for 30 seconds, annealing at 45 or 60 °C (depending on primer) for 1 minute, extension at 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. The DNA fragments were separated on 2 % agarose gel run at 100 volts (V) for 3 hours (h) using 1 M TAE buffer. The DNA fragments in gel was visualized by staining at 0.5 ug/mg ethidium bromide for 30 minutes and rinsed with distilled water for 20 minutes, visualized and photographed using ultraviolet (UV) transilluminator at 312 nm. Allele sizes were scored using a 100 base pair (bp) molecular size ladder.

**Genetic diversity data score**

The bands generated from SSR markers were scored for presence or absence of bands for all the potato mutants and controls.

### 3.4.5 Statistical analysis

The PowerMarker software package (Liu & Muse, 2005) was used to calculate the following summary statistics; percentage of polymorphic loci, mean number of alleles per polymorphic locus, observed heterozygosity (HO), expected heterozygosity (HE) and polymorphic information content (PIC). The genetic variance within and among populations was analyzed using the ARLEQUIN 3.01 software by analysis of molecular variance (AMOVA) (Excoffier *et al.,* 1992).

**Population structure analysis**

The population structure analysis of the entire germplasm was carried out based on Bayesian model (Hubisz *et al.,* 2009) as implemented in the STRUCTURE program version 2.3.4 (Pritchard *et al.,* 2010). The number runs for K values ranging from 1 to 10 were performed with a burn-in length of 100,000 followed by 1,000,000 Monte Carlo Markov Chain (MCMC) interactions using admixture model. The number of subpopulations was determined using the Delta *K* (Δ*K*) *ad hoc* method proposed by Evanno *et al.,* (2005) and implemented in the online tool Structure Harvester (Earl & VonHoldt, 2012) to estimate the most likely *K* in each set of potato mutants. The number of sub-populations (ΔK) was determined using the ad-hoc statistical method, based on the rate of change in the log probability of data between successive K values (Evanno *et al.,* 2005). Mutants were assigned to a subpopulation if the probability of membership was greater than 70 % (Liu *et al.,* 2003). If membership was ≤70 %, the mutants were allocated to the mixed subpopulation.

**Molecular phylogenetic and principal coordinate analyses**

Phylogenetic trees were produced using genotyping data with 20 SSR markers using the hierarchical clustering method based on the dissimilarity matrix calculated with Manhattan index, as implemented in the DARwin software (version 6.0.9) (Perrier & Jacquemoud-Collet, 2006). The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA) based on the estimates of genetic similarity of the potato mutants and controls. Principal coordinate analyses were also performed with DARwin 6.0.9 (Perrier & Jacquemoud-Collet, 2006).

### 3.4.6 Consensus tree analysis for comparing the relationship of the agronomic and bacterial wilt resistant traits versus SSR markers

Data from phylogenetic trees from section 3.3 (agronomic and bacterial wilt traits) and 3.4 (SSR data) was used to calculate the genetic distance of potato mutants using calculated with Manhattan index, as implemented in the DARwin software (version 6.0.9) (Perrier & Jacquemoud-Collet, 2006). Similarity matrices data were subjected to Unweighted Pair-Group method (UPGMA) (Sokal & Michener, 1958) clustering and dendrograms were constructed.

The cophenetic correlation coefficient (r) for each dendrogram was computed between the genetic similarity matrix (original distances) and the cophenetic values using Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1 (Rohlf, 2000). The relatedness between two matrices is measured by '*r'*, a product moment correlation coefficient. A higher *'r*' - value indicates a higher degree of similarity and vice versa. The significance of association between distance matrices was tested using Mantel matrix test as described by (Mantel, 1967) to test the goodness of fit between the similarity and the cophenetic matrices (Sneath & Sokal, 1973).

Consensus tree analysis was used to compare the different dendrogram types using MAJRUL method by estimation of consensus fork index (*CIc*) using the DARwin software (version 6.0.9) (Perrier & Jacquemoud-Collet, 2006). which provides an indication of similarity of the dendrograms (Duarte *et al.,* 1999).

# CHAPTER FOUR

# RESULTS

## 4.1 Effects of the dose rates on agronomic traits of potato mutants at different generations

### 4.1.1 Effects of dosage on percentage survival and tuberization of irradiated potato mutants

The total number of irradiated potato mutants that survived during planting after irradiation treatment were far much less than half of each respective potato mutants that were initially irradiated and varied with dosage rates across the three genotypes used (Figure 1). The Asante mutants at dosage 15 Gy had higher percentage survival and tuber number compared the Kenya Mpya and Kenya Sherekea mutants. The total number of irradiated Asante, Kenya Mpya and Kenya Sherekea genotypes were 230, 160 and 180 mutants respectively but the number of survived to produce M1V2 plants were 73 (Asante), 44 (Kenya Mpya) and 48 (Kenya Sherekea) mutants (Figure 1).

Figure 1, shows that the percentage of stem and tuber number of Asante mutants increased from 3 Gy to 9 Gy and reduced from 12 Gy to 15 Gy. The tuber number increased again at 15 Gy. The percent tuber weight was high at 6 Gy dose rate in Asante mutants. The percentage stem and tuber number, tuber weight and plant height was decreasing as the dosage rates increased in both Kenya Mpya and Kenya Sherekea mutants.

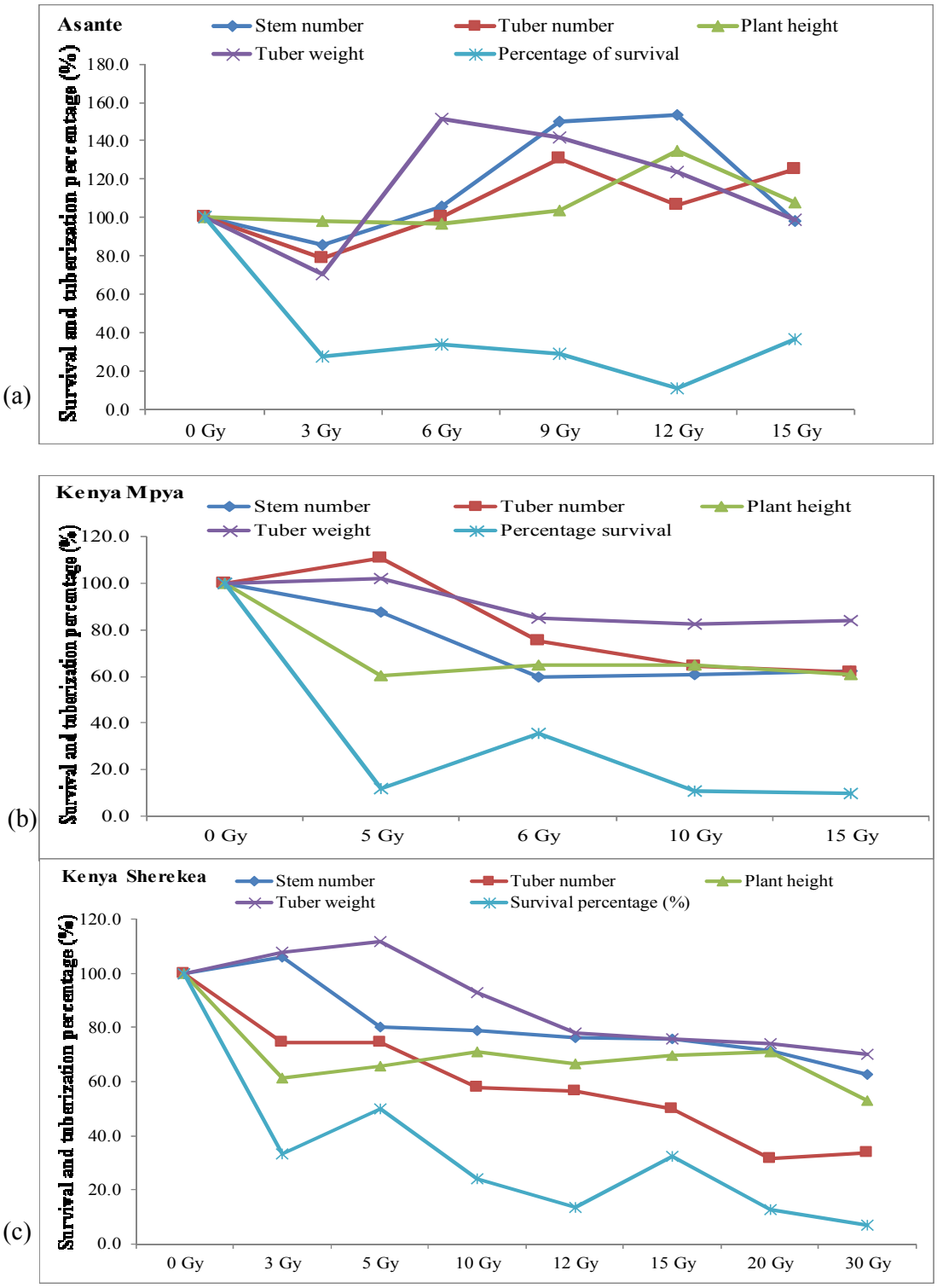


**Figure 1: Effects of dosage rates on survival rate of irradiated (a) Asante, (b) Kenya Mpya and (c) Kenya Sherekea potato mutants at the first generation**

### 4.1.2 Effects of dosage rate on various agronomic traits

The Asante mutants at M1V2 generation and M1V3 generation in Table 6 showed no significant difference between the different mean dose rates in the following traits; stem anthocyanin colouration (SAC), leaf outline openness (LOO), leaf presence of secondary leaflets (LPSL), leaf green colour (LGC) and flower corolla anthocyanin colouration (FCAC). Plant height (PH) and mean per plant tuber number (PTN) in M1V2 generation and mean plant tuber weight (PTW) in M1V3 generation were also not significantly different. There was significant difference at $p \leq 0.05$ in mean plant stem number (PSN) and mean plant tuber weight (PTW) in M1V2 generation and plant height (PH), mean plant tuber number (PTN) in M1V3 generation for the different mean dose rates (Table 6).

**Table 6: Effects of dosage rates on various morphological traits of Asante potato mutants at M1V2 and M1V3 generation**

| Generation | Dosage (Gray) | GH | SAC | LOO | LPSL | LGC | FCAC | PH | PSN | PTN | PTW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M1V2 | 0 | 3.7a | 1.7a | 3a | 4.3a | 3.7a | 1a | 72.3a | 6.7b | 17a | 15.7a |
| | 3 | 3.1a | 2.1a | 3.1a | 4.6a | 3.6a | 1.1a | 79.4a | 4.6ab | 22.5a | 19.5ab |
| | 6 | 3a | 1.7a | 3.1a | 5a | 3.1a | 1.1a | 78.7a | 2.9a | 25.3a | 19.8ab |
| | 9 | 3a | 1.1a | 3.1a | 5a | 3a | 1a | 84.8a | 3.8ab | 20.2a | 22.0b |
| | 12 | 3a | 1.7a | 4a | 5a | 3a | 1a | 83a | 6.3ab | 13.3a | 17.3a |
| | 15 | 3a | 1.2a | 3.6a | 5a | 3a | 1a | 86.7a | 5.1ab | 14.8a | 20.3ab |
| | Grand mean | 3.1 | 1.6 | 3.2 | 4.8 | 3.2 | 1.03 | 80.8 | 4.9 | 18.9 | 19.1 |
| | CV % | 15.3 | 39.3 | 30.3 | 10.1 | 15 | 9.2 | 30.4 | 38.4 | 37.9 | 16.3 |
| | EMS | ns | ns | ns | ns | ns | ns | ns | 3.2* | ns | 84* |

| Generation | Dosage (Gray) | SAC | LOO | LPSL | LGC | FCAC | IAC | PH | PSN | PTN | PTW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M1V3 | 0 | 1.7a | 3a | 4.3a | 3.7a | 1.7a | 1a | 113b | 6.7a | 46.3a | 67.5a |
| | 3 | 2.1a | 2.5a | 4.6a | 3.6a | 1.4a | 1.3a | 95.5a | 5.8a | 55.1b | 53.5a |
| | 6 | 1.7a | 3.1a | 5a | 3a | 1a | 1.1a | 87.8a | 5.8a | 51a | 53.5a |
| | 9 | 1.1a | 3.1a | 5a | 3a | 1a | 1a | 94.6a | 5.7a | 55.8b | 59.3a |
| | 12 | 1.7a | 4a | 5a | 3a | 1a | 1a | 88.3a | 6.5a | 53a | 56.9a |
| | 15 | 1.3a | 3.6a | 5a | 3a | 1a | 1a | 99.5ab | 7b | 52a | 57.0a |
| | Grand mean | 1.6 | 3.2 | 4.8 | 3.3 | 1.2 | 1.1 | 96.5 | 6.2 | 52.2 | 58.3 |
| | CV % | 9.2 | 15.2 | 10.1 | 14.7 | 18.3 | 13.4 | 9.2 | 13.2 | 24.1 | 14.6 |
| | EMS | ns | ns | ns | ns | ns | ns | 3.4* | 5.4* | 14.3* | ns |

**ns= not significant, *=significant at $p \leq 0.05$, Growth habit (GH), Stem anthocyanin colouration (SAC), Leaf Outline openness (LOO), Leaf presence of secondary leaflets (LPSL), Leaf green colour (LGC), Flower corolla anthocyanin colouration (FCAC), Plant height in cm (PH), Average Plant stem number (PSN), Average Plant Tuber number (PTN), Average Plant Tuber weight in t/ha (PTW). Error mean squares (EMS), Coefficient of variation (CV), within each column, means having the same letters are not significantly different at $p \leq 0.05$**

In Table 7 Kenya Mpya mutants did not show significant difference between the different dose rates for leaf outline openness, leaf green colour, flower corolla anthocyanin colouration, plant height and plant tuber weight in t/ha. While growth habit, leaf anthocyanin colour midrib of upperside, in M1V2 generation and potato tuber number in M1V3 generation also did not differ significantly between the different mean dose rates. In M1V2 generation stem anthocyanin colouration and potato tuber number differed significantly at $p \leq 0.05$ while plant height, growth habit, stem anthocyanin colouration, leaf presence of secondary leaflets, leaf anthocyanin colour midrib of upperside and potato tuber number differed significantly in M1V3 generation.

**Table 7: Effects of dose rates on various morphological traits of Kenya Mpya potato mutants at M1V2 and M1V3 generation**

| Generation | Dosage (Gy) | GH | SAC | LOO | LPSL | LGC | LACM | FCAC | PH | PSN | PTN | PTW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M1V2 | 0 | 2.3a | 3b | 4.7a | 4.3a | 3.7a | 3b | 4.7a | 79.3a | 3.7a | 27.7a | 27.7a |
| | 5 | 3a | 1a | 4.8a | 5a | 3.3a | 1.3a | 5a | 73.8a | 3.8a | 34b | 30.8a |
| | 6 | 3a | 1a | 5a | 5a | 3a | 1a | 5a | 81.1a | 3.3a | 30.3a | 30.0a |
| | 10 | 3a | 1a | 5a | 5a | 3a | 1a | 5a | 73.5a | 3a | 31.7a | 31.0a |
| | 15 | 3a | 1a | 5a | 5a | 3a | 1a | 5a | 84.7a | 3.2a | 21.7a | 31.0a |
| | Grand mean | 2.9 | 1.4 | 4.9 | 4.9 | 3.2 | 1.5 | 4.9 | 78.5 | 3.4 | 29.1 | 30 |
| | CV % | 18 | 11.5 | 6.2 | 10.6 | 18.9 | 9.7 | 5.2 | 9.1 | 13.7 | 16.5 | 12.9 |
| | EMS | ns | 0.7* | ns | ns | ns | ns | ns | ns | ns | 7.3* | ns |

| Generation | Dosage (Gy) | GH | SAC | LOO | LPSL | LGC | LACM | FCAC | PH | PSN | PTN | PTW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M1V3 | 0 | 1.7a | 5b | 4.7a | 3.7a | 3.7a | 3b | 4.6a | 82b | 5a | 38b | 43.1a |
| | 5 | 3b | 1a | 4.7a | 5a | 3.3a | 1.3a | 5a | 68.7a | 4a | 33.7a | 36.9a |
| | 6 | 3b | 1a | 5a | 5a | 3a | 1a | 5a | 70.3a | 5.7a | 34a | 37.2a |
| | 10 | 3b | 1a | 5a | 5a | 3a | 1a | 5a | 88.3b | 3a | 21.3a | 31.6a |
| | 15 | 3b | 1a | 5a | 5a | 3a | 1a | 5a | 88b | 5.3a | 33.7a | 46.8a |
| | Grand mean | 2.7 | 1.8 | 4.9 | 4.6 | 3.2 | 1.5 | 4.9 | 79.5 | 4.6 | 31.9 | 39.1 |
| | CV % | 18.9 | 6.1 | 6.9 | 10.9 | 18.9 | 9.7 | 5.2 | 17.2 | 17.8 | 16.7 | 24.3 |
| | EMS | 0.3* | 2.4* | ns | 0.3* | ns | 0.8* | ns | 1.2* | ns | 0.2* | ns |

**ns= not significant, *=significant at $p \leq 0.05$, Gray (Gy), Growth habit (GH), Stem anthocyanin colouration (SAC), Leaf Outline openness (LOO), Leaf presence of secondary leaflets (LPSL), Leaf green colour (LGC), Leaf anthocyanin colour midrib of upperside (LACM), Flower corolla anthocyanin colouration (FCAC), Plant height in cm (PH), Plant stem number (PSN), Plant Tuber number (PTN), Plant Tuber weight (PTW). Error mean squares (EMS), Coefficient of variation (CV), within each column, means having the same letters are not significantly different at $p \leq 0.05$**

The Kenya Sherekea mutants exposed to different dose rates at M1V2 generation and M1V3 generation (Table 8) showed significant difference at $p \leq 0.05$ for leaf outline openness and plant tuber number and plant tuber weight (M1V3 generation) and at $p \leq 0.01$ for plant height and plant tuber weight (M1V2 generation). Most of the qualitative traits did not differ significantly among the different mean dose rates of this mutant.

**Table 8: Effects of dose rates on various morphological traits of Kenya Sherekea potato mutants at M1V2 and M1V3 generation**

| Generation | M1V2 | | | | M1V3 | | | |
|---|---|---|---|---|---|---|---|---|
| Dosage (Gy) | LOO | PH | PTN | PTW | LOO | PH | PTN | PTW |
| 0 | 3.4 ab | 97.7 cd | 55.3 b | 40.9 bc | 3.6ab | 94.7bcd | 51.3bc | 38.4abc |
| 3 | 3.8 b | 89.8 bc | 58.6 b | 41.1 bc | 3.8b | 89.8bc | 58.6c | 37.6abc |
| 5 | 3.6 ab | 85.4 ab | 59 b | 43.1 bc | 3.5ab | 82.1ab | 52.1bc | 46.3bc |
| 10 | 3.4 ab | 95.3 bcd | 54 b | 48.4 c | 3.4ab | 92.3bcd | 54c | 49.0c |
| 12 | 3.6 ab | 86 abc | 39.3 a | 38.7 b | 3.6ab | 86abc | 39.3ab | 33.4ab |
| 15 | 3.4 ab | 95.7 bcd | 49.6 ab | 39.3 b | 3.8b | 95.7cd | 49.6abc | 41.8abc |
| 20 | 3.7 ab | 102.5 d | 48.3 ab | 42.2 bc | 3.8b | 103.8d | 48.3abc | 44.9abc |
| 30 | 3a | 74.67 a | 38.9 a | 22.6 a | 3a | 74.7a | 36.4a | 30.0a |
| Grand mean | 3.5 | 90.9 | 50.4 | 39.5 | 3.5 | 89.9 | 48.7 | 40.1 |
| CV % | 11.5 | 7.7 | 13.2 | 12.9 | 10.6 | 8.6 | 15.7 | 22.7 |
| EMS | 1.5* | 40** | 6.7* | 234** | 0.14* | 59.6** | 58.4* | 718* |

***=significant at $p \leq 0.05$, **=significant at $p \leq 0.01$, Gray (Gy), Leaf Outline openness (LOO), Plant height (PH), Average Plant Tuber number (PTN), Average Plant Tuber weight (PTW). Error mean squares (EMS), Coefficient of variation (CV), within each column, means having the same letters are not significantly different at $p \leq 0.05$**

### 4.1.3 Principal component analysis

The principal component analysis (PCA) in table 9 showed that the quantitative traits had higher percentage of total variation compared to qualitative traits in all the three potato mutants in the two generations. The total percentage variation for the first three PC at M1V2 generation for both qualitative and quantitative traits accounted for 92.14 and 97.92 %, 90.76 and 99.96 %, 88.06 and 95.98 % in Asante, Kenya Mpya and Kenya Sherekea respectively. The traits that contributed to the first PCA for the 3 mutants were tuber weight (Appendix I and II).

**Table 9: Principal component analysis of Asante, Kenya Mpya and Kenya Sherekea potato mutants for M1V2 and M1V3 generations showing the qualitative and quantitative traits contribution to the total percentage (%) variation**

| Generations | | M1V2 | | M1V3 | |
|---|---|---|---|---|---|
| Mutants | PCA | Qualitative (%) | Quantitative (%) | Qualitative (%) | Quantitative (%) |
| Asante | 1 | 74.23 | 82.01 | 67.12 | 84.67 |
| | 2 | 12.54 | 10.54 | 18.41 | 11.21 |
| | 3 | 5.37 | 5.37 | 3.41 | 0.11 |
| | **Total** | **92.14** | **97.92** | **88.94** | **95.99** |
| Kenya Mpya | 1 | 72.64 | 81.44 | 72.41 | 76.41 |
| | 2 | 14.2 | 17.23 | 12.2 | 18.2 |
| | 3 | 3.92 | 1.29 | 4.36 | 1.36 |
| | **Total** | **90.76** | **99.96** | **88.97** | **95.97** |
| Kenya Sherekea | 1 | 70.56 | 80.33 | 72.54 | 78.68 |
| | 2 | 15.12 | 12.32 | 5.15 | 9.87 |
| | 3 | 2.38 | 3.33 | 0.28 | 2.11 |
| | **Total** | **88.06** | **95.98** | **77.97** | **90.66** |
| All mutants | 1 | 71.54 | 78.56 | 70.3 | 74.56 |
| | 2 | 13.41 | 11.15 | 11.41 | 9.15 |
| | 3 | 2.24 | 4.28 | 1.66 | 4.28 |
| | **Total** | **87.19** | **93.99** | **83.37** | **87.99** |

The total percentage variation at M1V3 generation for both qualitative and quantitative traits accounted for 88.94 and 95.99 %, 88.97 and 95.97 %, 77.97 and 90.66 % in Asante, Kenya Mpya and Kenya Sherekea respectively (Table 9). The traits that contributed in the first principal component for the three mutants were tuber weight. The traits that did not contribute to the variation are inflorescence anthocyanin colouration in Kenya Mpya, flower corolla anthocyanin colouration and leaf anthocyanin colour midrib of upperside in Asante and Kenya Sherekea and stem anthocyanin colouration in Kenya Sherekea mutants. When all the mutants were combined (of Asante, Kenya Mpya and Kenya Sherekea) across M1V2 and M1V3 generations for both qualitative and quantitative traits it accounted for 87.19 and 93.99 %, 83.37 and 87.99 %, respectively (Table 9, Appendix I and II).

### 4.1.4 Estimating the effect of induced mutations in improvement of potato

The genetic estimate effects in Table 10 showed that there were significant difference in plant height, stem number, tuber number and tuber weight in Asante, Kenya Mpya and Kenya Sherekea potato mutants. The genetic estimates of Kenya Sherekea in growth habit, stem anthocyanin colouration, leaf outline openness, leaf anthocyanin colour midrib of upperside, leaf green colour and flower corolla anthocyanin colouration did not differ significantly and therefore not included in the analysis. The potato mutants were selected based on average potato tuber weight in ton/ha. The selected number of mutants was 20, 12 and 16 for Asante, Kenya Mpya and Kenya Sherekea mutants respectively (Table 10).

**Table 10: Genetic estimate effects for various agronomic traits in selected population in Asante, Kenya Mpya and Kenya Sherekea potato mutants**

| Source of Variation | DF | Asante Mean squares | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GH | SAC | LOO | LPSL | LGC | FCAC | PH | PSN | PTN | PTW |
| Blocks (Generation) | 1 | 0.01 | 0.02 | 0.02 | 0.2 | 0.03 | 0.01 | 1491** | 54.7*** | 362.2*** | 183.8*** |
| Genotypes | 19 | 0.13* | 1.26* | 1.6*** | 0.36 | 0.53 | 0.36* | 1164*** | 11.4*** | 76.7* | 11.8* |
| Error | 20 | 0.06 | 0.6 | 0.5 | 0.19 | 0.34 | 0.19 | 199 | 3.9 | 126.4 | 55.5 |

| Source of Variation | DF | Mpya Mean squares | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GH | SAC | LOO | LPSL | LGC | LACM | FCAC | PH | PSN | PTN | PTW |
| Blocks | 1 | 0.009 | 0.02 | 0.02 | 0.2 | 0.22 | 0.06* | 0.01 | 0.01** | 10.89* | 9430*** | 60.5*** |
| Genotypes | 11 | 0.4* | 3.63* | 0.16 | 0.4* | 0.65 | 2.72** | 0.1* | 526* | 6.18* | 204.3* | 2.1* |
| Error | 12 | 0.19 | 1.74 | 0.1 | 0.04 | 0.44 | 0.72 | 0.01 | 125.2 | 0.33 | 95.1 | 0.38 |

| Source of Variation | DF | Sherekea Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | LPSL | IAC | PH | PSN | PTN | PTW |
| Blocks | 1 | 5.02 | 0.04 | 52.5 | 0.01 | 153 | 183.8*** |
| Genotypes | 15 | 0.04 | 0.04 | 963.1*** | 9*** | 84.6* | 24* |
| Error | 16 | 0.63 | 0.04 | 322.4 | 1.9 | 55.7 | 7 |

***= significant at $p \leq 0.05$, **=significant at $p \leq 0.01$, ***=significant at $p \leq 0.001$. Degree of freedom (DF), Growth habit (GH), Stem anthocyanin colouration (SAC), Leaf Outline openness (LOO), Leaf presence of secondary leaflets (LPSL), Leaf green colour (LGC), Flower corolla anthocyanin colouration (FCAC), Plant height (PH), Plant stem number (PSN), Plant Tuber number (PTN), Plant Tuber weight (PTW).**

The highest positive heritability percentage ($H^2$) estimates in Kenya Mpya and Kenya Sherekea mutants were in plant height with 81.51 % and 87.7 % respectively, while Asante had 86.96 % in stem anthocyanin colouration (Table 11). Negative percentage heritability estimates was shown in leaf presence of secondary leaflets trait (-1.63 %) in Asante mutants and stem number (-23.49 %) trait in Kenya Sherekea mutants. The highest genetic advance as a percentage of the mean (GAM %) in Asante potato mutants was 27.87 % (stem number), Kenya Mpya mutants was 63.67 % (stem anthocyanin colouration) while Kenya Sherekea mutants was 43.94 % (tuber weight). Kenya Mpya mutants recorded high GAM % in the quantitative traits (stem anthocyanin colouration and leaf anthocyanin colour midrib of upperside). The quantitative traits recorded the lowest GAM % in all the 3 potato mutants (Table 11). The Kenya Sherekea genetic estimates in growth habit, stem anthocyanin colouration, leaf outline openness, leaf green colour and flower corolla anthocyanin colouration did not differ significantly and therefore not included in the analysis.

**Table 11: Estimates of Genetic parameters for various agronomic traits in Asante, Kenya Mpya and Kenya Sherekea potato mutants**

| Asante | | | | | | |
|---|---|---|---|---|---|---|
| **Traits** | **Mean±SE** | **PCV** | **GCV** | **$H^2$ (%)** | **GA** | **GAM (%)** |
| **GH** | 3.03±0.15 | 4.04 | 3.30 | 33.33 | 10.78 | 4.04 |
| **SAC** | 1.37±0.45 | 24.75 | 27.31 | 86.96 | 77.85 | 24.75 |
| **LOO** | 3.18 ±0.41 | 15.72 | 11.03 | 20.00 | 26.40 | 15.72 |
| **LPSL** | 4.9±0.25 | 8.00 | 4.42 | -1.63 | -1.68 | 8.00 |
| **LGC** | 3.17±0.46 | 6.31 | 7.73 | 37.50 | 19.80 | 6.31 |
| **FCAC** | 1.1±0.25 | 17.31 | 19.28 | 34.48 | 17.33 | 17.31 |
| **PH** | 88.3±8.14 | 20.51 | 6.53 | 2.51 | 120.24 | 20.51 |
| **PSN** | 4.88±1.14 | 27.87 | 9.16 | 2.70 | 9.70 | 27.87 |
| **PTN** | 40.5±6.49 | 9.31 | 5.52 | 82.30 | 819.15 | 6.09 |
| **PTW** | 21.76±4.30 | 13.01 | 11.09 | 53.33 | 398.55 | 13.01 |

| Kenya Mpya | | | | | | |
|---|---|---|---|---|---|---|
| **Traits** | **Mean±SE** | **PCV** | **GCV** | **$H^2$ (%)** | **GA** | **GAM (%)** |
| **GH** | 2.89±0.25 | 8.48 | 3.46 | 25.00 | 16.17 | 8.48 |
| **SAC** | 1.3±0.76 | 63.67 | 7.69 | 62.04 | 135.56 | 63.67 |
| **LOO** | 4.89 ±0.19 | 2.40 | 2.04 | 18.18 | 5.63 | 2.40 |
| **LPSL** | 4.89±0.25 | 5.46 | 2.04 | 52.63 | 37.09 | 5.46 |
| **LGC** | 3.22±0.39 | 7.03 | 3.11 | 4.88 | 2.92 | 7.03 |
| **LACM** | 1.47±0.56 | 46.76 | 6.80 | 11.64 | 21.12 | 46.76 |
| **FCAC** | 4.94±0.13 | 2.68 | 2.02 | 57.14 | 19.96 | 2.68 |
| **PH** | 82.1±10.91 | 10.28 | 0.12 | 81.51 | 1815.59 | 10.28 |
| **PSN** | 3.5±1.04 | 29.98 | 2.86 | 65.61 | 181.76 | 29.98 |
| **PTN** | 26.4±8.25 | 17.32 | 0.38 | 61.24 | 739.16 | 17.32 |
| **PTW** | 3.48±0.84 | 18.34 | 2.87 | 11.04 | 18.61 | 18.34 |

| Kenya Sherekea | | | | | | |
|---|---|---|---|---|---|---|
| **Traits** | **Mean±SE** | **PCV** | **GCV** | **$H^2$ (%)** | **GA** | **GAM (%)** |
| **LPSL** | 5.02±0.12 | 18.18 | 1.99 | 0.00 | 0.00 | 18.18 |
| **IAC** | 1.02±0.12 | 8.90 | 4.80 | 0.00 | 0.00 | 4.90 |
| **PH** | 93.5±6.39 | 11.29 | 0.11 | 87.70 | 2443.04 | 11.29 |
| **PSN** | 6.3±0.79 | 22.51 | 1.59 | -23.49 | -87.96 | 22.51 |
| **PTN** | 48.6±16.79 | 5.22 | 0.21 | 24.47 | 163.88 | 5.22 |
| **PTW** | 34.48±0.86 | 43.94 | 2.23 | 19.35 | 100.58 | 43.94 |

**Standard error (SE), phenotypic and genotypic coefficient of variation (PVC and GVC), Heritability ($H^2$), Genetic advance (GA), Genetic advance as a percentage of the mean (GAM %) Growth habit (GH), Stem anthocyanin colouration (SAC), Leaf Outline openness (LOO), Leaf presence of secondary leaflets (LPSL), Leaf green colour (LGC), Flower corolla anthocyanin colouration (FCAC), Plant height (PH), Plant stem number (PSN), Tuber number (PTN), Tuber weight (PTW).**

## 4.2 Assessment ploidy level diversity of potato mutants by chloroplast counts in stomatal guard cells

### 4.2.1 Ploidy level percentage (%) distribution

**Chloroplast counts percentage distribution in the three generations of mutants**

In figure 2, all the three potato mutant populations; Asante, Kenya Mpya and Kenya Sherekea, showed that the distribution percentage of chloroplast counts in stomatal guard cells decreased significantly from M1V1 through M1V2 to M1V3 generation. The M1V1 generation distribution percentage of chloroplast counts in stomatal guard cells of Asante mutants ranged from 42 to 90 % and was the most widely distributed compared to Kenya Mpya (range from 60 to 96 %) and Kenya Sherekea (range from 60 to 98 %) mutants. The Asante mutants at M1V3 generation was the least distributed with only (range from 82 % to 96 %) tetraploids (2n=4x=48) as shown by the box and whisker plot compared to Kenya Mpya and Kenya Sherekea mutants at the same generation which had triploids (2n=3x=36) and tetraploids (2n=2x=24).

The Asante mutants, Figure 2 showed that the percent of chloroplast counts in stomatal guard cells from M1V1, M1V2 and to M1V3 generation was decreasing and reducing ploidy distribution. The Kenya Sherekea potato mutants showed that the percent of chloroplast counts in stomatal guard cells was also decreasing from M1V1, M1V2 and to M1V3 generation but the ploidy distribution was constant. The Kenya Mpya potato mutants at M1V2 and M1V3 generation showed that the percent of chloroplast counts distribution was constant. The mutant's generations showing longer box and whiskers had wider range of chloroplast counts percent distribution compared to the ones with shorter box and whiskers depicting the degree of dispersion in the population.

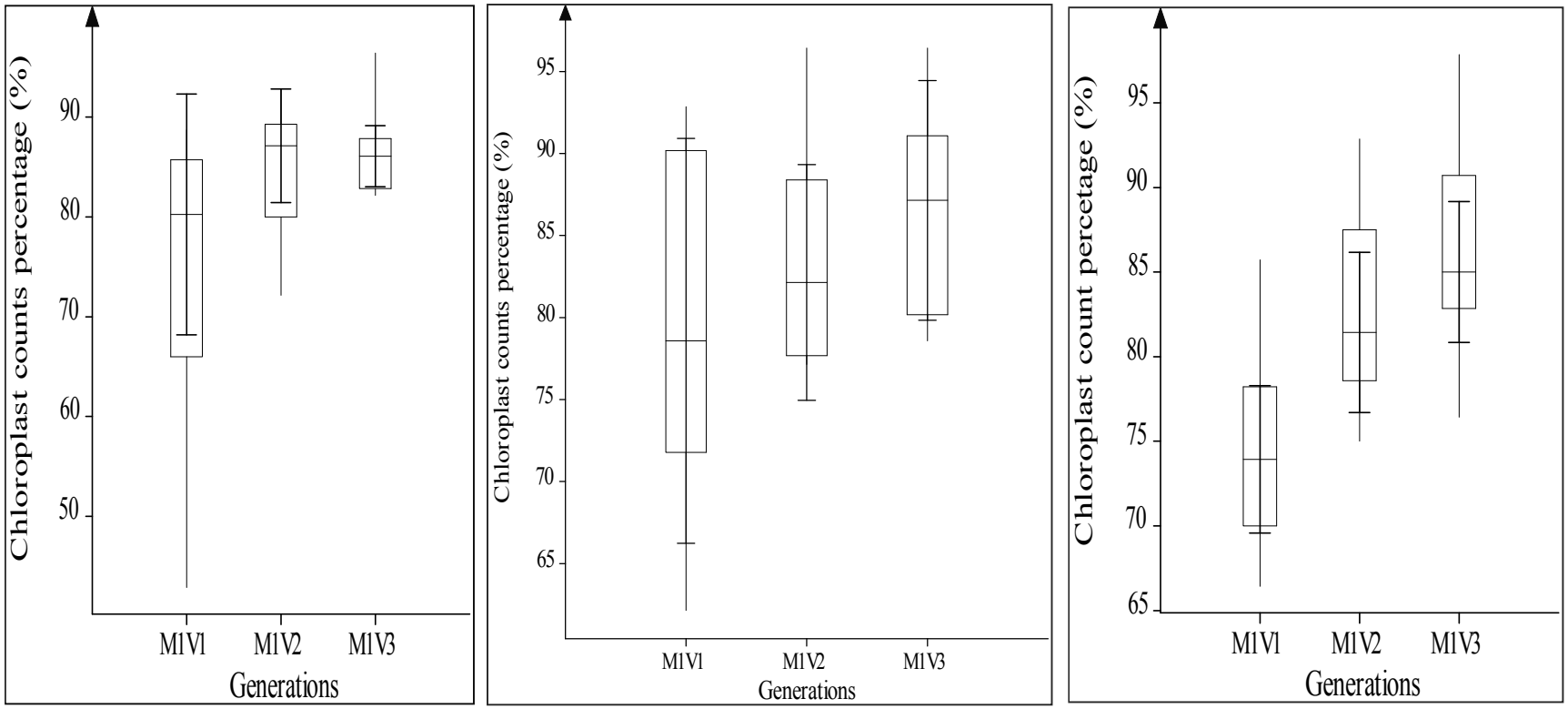


**Figure 2: The ploidy level distribution by chloroplast counts in stomatal guard cells of Asante, Kenya Mpya and Kenya Sherekea potato mutant populations across all the generations (M1V1, M1V2 and M1V3) using Box and Whisker plot.**

**Chloroplast counts percentage distribution in dosage rates applied**

Figure 3 shows the percentage distribution of chloroplast counts based on the dosage rates showed that 12 Gy in Asante, 5 Gy in Kenya Mpya and 3 Gy in Kenya Sherekea potato mutants displayed the largest percent chloroplast counts distribution. In Asante dosage rates of 0, 3, 6 and 9 Gy had high percent of chloroplast counts of 76 % to 96 % with low distribution while 15 Gy had moderate distribution.

The Kenya Mpya potato mutants at 6 Gy (75 % to 87 %) and 15 Gy (78 % to 89 %) dosage rates had almost similar percentage distribution of chloroplast counts. The 10 Gy dosage rate (Kenya Mpya) was the least percent distributed in chloroplast counts. The Kenya Sherekea potato mutants was decreasing percent in chloroplast counts as the dosage rates increases from 0 to 30 Gy (Figure 3). In Kenya Sherekea mutants the dosage rates; 0, 3 and 5 Gy showed wide distribution of chloroplast counts while 10, 12, 15, 20 and 30 Gy display narrow percent distribution of chloroplast counts in stomatal guard cells (Figure 3).

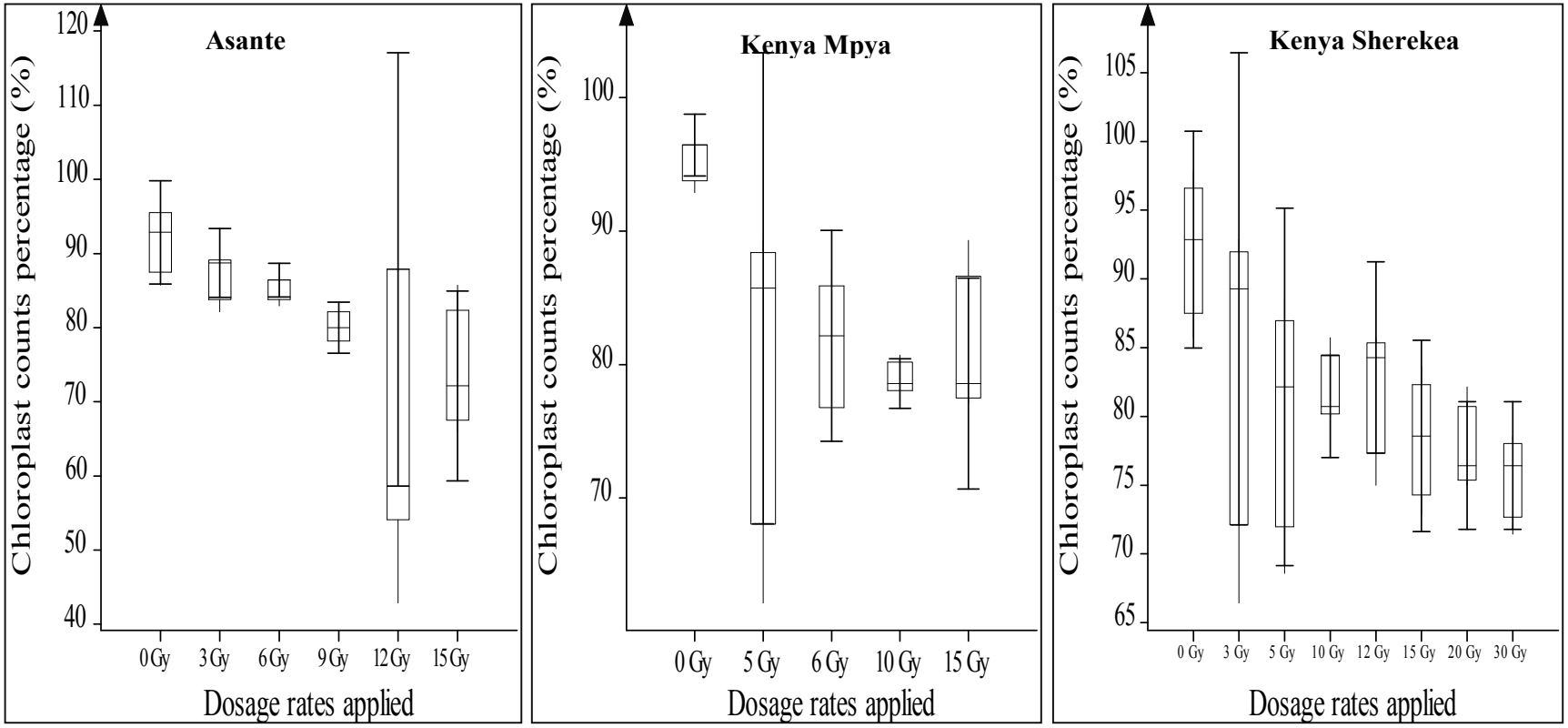


**Figure 3: The ploidy level distribution by chloroplast counts in stomatal guard cells of Asante, Kenya Mpya and Kenya Sherekea potato mutant populations across the various dosage rates applied using Box and Whisker plots**

The percentage number of mutant's distribution based on chloroplast counts in stomatal guard cells from M1V1, M1V2 to M1V3 generation was decreasing in diploids and triploids while increasing in tetraploids across all the three potato mutants (Figure 4, Plate 2). In the three potato mutant populations the percentage number of mutants was generally increasing from diploids to tetraploids. Plate 2 shows the Chloroplast counts in stomatal guard cells of Asante potato mutants (a) mutant A56 at M1V1 generation showing 8 and 6 chloroplast counts in stomatal guard cells with a mean of nine chloroplasts counts (diploid, 2n=2x=24) (b) A24 at M1V2 generation showing 10 and 8 chloroplast counts in stomatal guard cells with a mean of nine chloroplasts counts (tetraploid, 2n=3x=36).

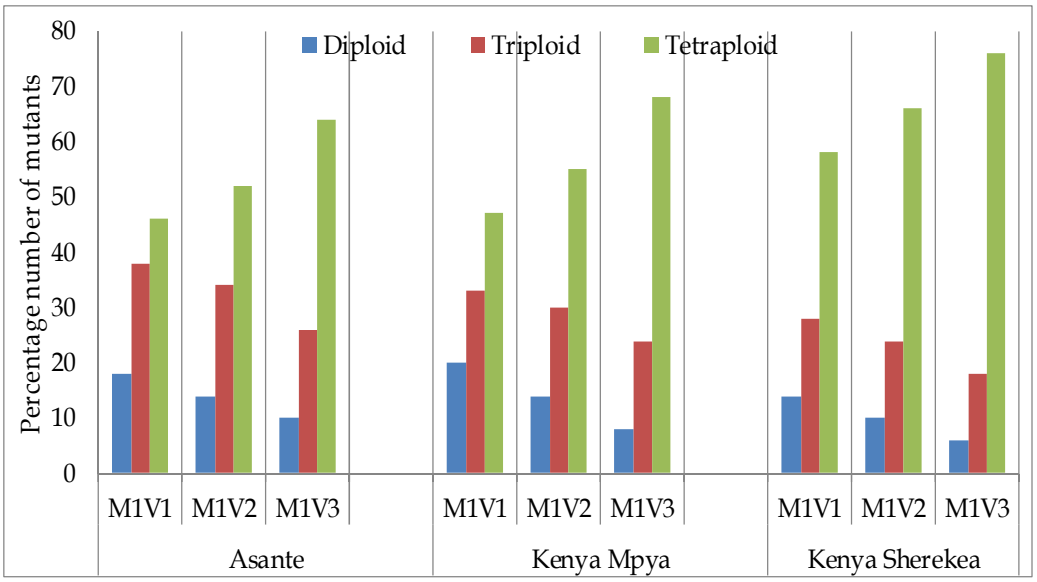


**Figure 4: Percentage number of Asante, Kenya Mpya and Kenya Sherekea potato mutants at various generations (M1V1, M1V2 and M1V3) based on chloroplast counts in stomatal guard cells.**

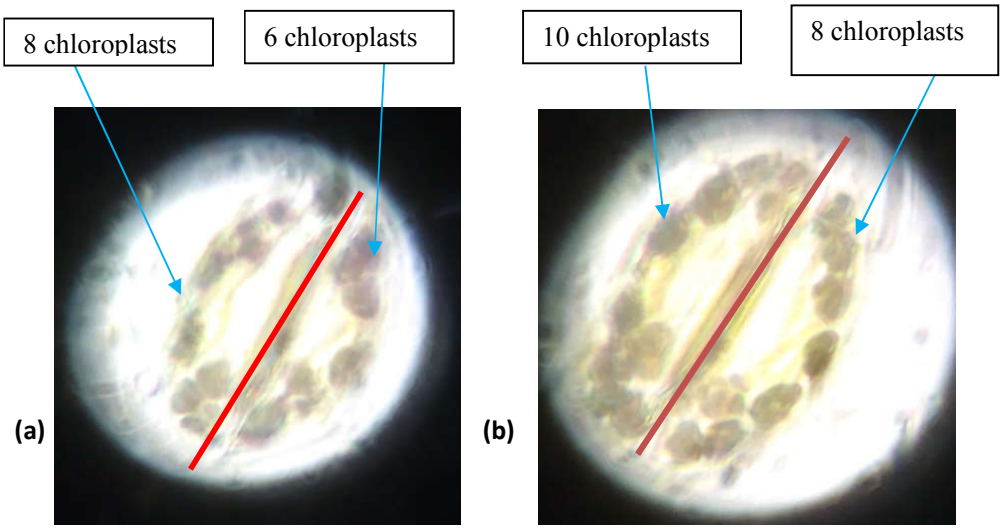


**Plate 2: Chloroplast counts in stomatal guard cells of Asante potato mutants (a) mutant A56 at M1V1 generation showing 8 and 6 chloroplast counts in stomatal guard cells with a mean of seven chloroplasts counts (diploid, 2n=2x=24) (b) A24 at M1V2 generation showing 10 and 8 chloroplast counts in stomatal guard cells with a mean of nine chloroplasts counts (triploid, 2n=3x=36) Source: Author, 2017.**

### 4.2.2 Cluster analysis of 160 potato mutants and 3 parents based on chloroplast counts at M1V3 generation

In Figure 5, the unrooted dendrogram showed that the potato mutants at M1V3 generation had a common origin and clustered randomly. The clustering of the potato mutants generated 2 groupings. Group I consisted of an average of 10 chloroplast counts while Group II consisted of the parents (red, Figure 5) with an average of 12 chloroplast counts in the stomatal guard cells. The clustering was random though majorly associated with the dosage rate applied in all the mutants. Most mutants in cluster I was generated from higher dosage rates which gave low number of chloroplast counts in the stomatal guard cells (Figure 5).

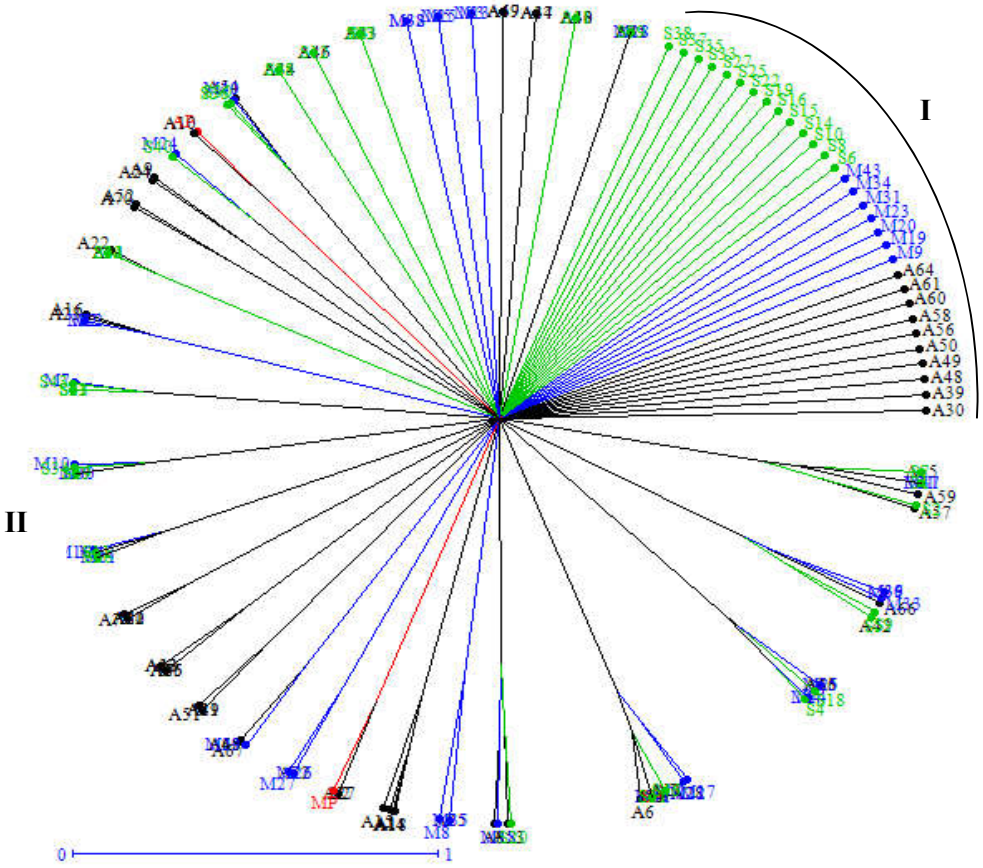


**Figure 5: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the ploidy level relationship among 160 potato mutants (Asante – black, Kenya Mpya – blue and Kenya Sherekea – green); and 3 (parents - red) based on chloroplast counts in the stomatal guard cells**

## 4.3 Screening of the potato mutants populations against bacterial wilt using pathogenicity test

### 4.3.1 The response of potato mutant dosage rates to Bacterial wilt disease

Table 12 showed that the days to onset of wilting (DTOW) was significantly different at $p \leq 0.05$ (Asante), $p \leq 0.01$ (Kenya Mpya) and $p \leq 0.001$ (Kenya Sherekea) potato mutant dosage rates. The area under the disease progress curve (AUDPC) was significantly different in Asante ($p \leq 0.05$), Kenya Mpya ($p \leq 0.01$) and Kenya Sherekea ($p \leq 0.01$). Total tuber number (TTN) also exhibited significant difference in Asante ($p \leq 0.01$), Kenya Mpya ($p \leq 0.05$) and non-significant in Kenya Sherekea mutants. Total tuber weight (TTW) was significantly different ($p \leq 0.05$) in Kenya Sherekea and non-significant for Asante and Kenya Mpya mutants. Kenya Mpya and Asante mutants dose rates were significantly different ($p \leq 0.05$) in percentage of symptomatic ware sized tubers of total tuber weight in t/ha (PWTTW) and percentage of symptomatic tubers of total tuber number (PSTTN) per ha while percentage of symptomatic tubers of total tuber weight per ha (PSTTW) were non-significant in Asante but significant for Kenya Mpya mutant dosage rates. Kenya Sherekea mutant dose rates were significantly different in percentage of symptomatic tubers of total tuber number (PSTTN) ($p \leq 0.01$), percentage of symptomatic tubers of total tuber weight per ha (PSTTW) ($p \leq 0.001$) and non-significant for percentage of symptomatic ware sized tubers of total tuber weight in t/ha (PWTTW) (Table 12).

**Table 12: Effect of different dose rates on Asante, Kenya Mpya and Kenya Sherekea potato mutants for selected agronomic and bacterial wilt resistance parameters at KALRO NARL**

| Mutants | Dosage (Gy) | DTOW | AUDPC | TTN | TTW | PSTTN | PSTTW | PWTTW |
|---|---|---|---|---|---|---|---|---|
| **Asante** | **0** | 49a | 560ab | 24.7bc | 39.3a | 10.3a | 6.7a | 4.76 |
| | **3** | 50.7a | 573.6ab | 15.1a | 37.9a | 11.5ab | 7.1a | 17.5c |
| | **6** | 51.5a | 536.7a | 25.9bc | 40.6a | 22.3c | 8.6a | 12.7bc |
| | **9** | 55.3ab | 530a | 35.2d | 44.3a | 19.5bc | 10.5a | 11.4abc |
| | **12** | 53.6a | 546.7a | 30.7cd | 33.5a | 9.3a | 5.9a | 8.3ab |
| | **15** | 63.2b | 662.4b | 22.3ab | 44.4a | 19.9abc | 7.6a | 17.3c |
| | **Grand mean** | 59.3 | 568 | 25.6 | 40 | 14.47 | 7.7 | 12 |
| | **CV %** | 8.4 | 10.2 | 17.1 | 22.9 | 22.2 | 23.9 | 20.5 |
| | **EMS** | 20.7* | 3354* | 19** | ns | 21.6* | ns | 13.4* |
| **Kenya Mpya** | **0** | 74.7b | 410a | 19.7ab | 61.2a | 19.5ab | 7.2ab | 4a |
| | **5** | 51.3a | 561.7bc | 30.5b | 47a | 9.1a | 5.9ab | 10.04b |
| | **6** | 54.4a | 538.6bc | 27.7ab | 42a | 18.4ab | 9.2ab | 9.07b |
| | **10** | 51.3a | 595c | 30.7b | 59a | 10.3a | 5.1a | 10.09b |
| | **15** | 44.7a | 501.7b | 14.2a | 40.6a | 29.9b | 10.6b | 13.23b |
| | **Grand mean** | 55.3 | 521 | 24.5 | 50 | 17.4 | 7.6 | 9.3 |
| | **CV %** | 12.7 | 8.7 | 25 | 13.3 | 14.3 | 27.4 | 16.1 |
| | **EMS** | 49.7** | 2035** | 73* | ns | 59.6* | 8* | 11* |
| **Kenya Sherekea** | **0** | 49a | 436.7a | 25a | 35.5ab | 9.4a | 5.2a | 8.2a |
| | **3** | 52.5ab | 560bc | 25.2a | 36.9ab | 22.5b | 5.4ab | 9.3a |
| | **5** | 62.3c | 526.7b | 28a | 40.5ab | 15.1ab | 8.5b | 9.5a |
| | **10** | 56.9bc | 517.6b | 33.6a | 44.2b | 9.5ab | 7.6ab | 4.8a |
| | **12** | 50.2ab | 560bc | 31.8a | 42.3b | 10.5ab | 7.3ab | 6.6a |
| | **15** | 56.4abc | 535.4b | 26.8a | 40.4ab | 11.7ab | 6.8ab | 9.6a |
| | **20** | 61.8c | 525b | 37.7a | 32.3ab | 18.6ab | 6.3ab | 7.8a |
| | **30** | 77d | 613.3c | 34.7a | 27.9a | 37.3c | 15c | 10.5a |
| | **Grand mean** | 58.3 | 534.3 | 30.3 | 37.5 | 16.8 | 7.8 | 8.3 |
| | **CV %** | 7.4 | 6.3 | 28.9 | 19.2 | 44.7 | 23.7 | 22.6 |
| | **EMS** | 18.7*** | 1121** | ns | 51.8* | 56.4** | 3.4*** | ns |

**ns=not significant, *=significant at $p \leq 0.05$, **=significant at $p \leq 0.01$, ***=significant at $p \leq 0.001$; DTOW= Days to onset of wilting; PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha$^{-1}$); TTW= Total tuber weight (t ha$^{-1}$); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha$^{-1}$); TTN= Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve; Error mean square (EMS), Percentage Coefficient of variation (CV %), Within each column, means having the same letter are not significantly different at $p \leq 0.05$.**

### 4.3.2 Bacterial wilt incidence

Bacterial wilt incidences (BWI) exhibited significant difference ($p \leq 0.05$) among Asante and Kenya Mpya and non-significant in Kenya Sherekea potato mutants at 42 days after planting (Table 13). The bacterial wilt incidences at 49 and 84 days after planting were highly significant ($p \leq 0.001$) in Asante and non-significant in Kenya Mpya and Kenya Sherekea potato mutants. Table 13 showed that the potato mutants of Asante, Kenya Mpya and Kenya Sherekea potato mutants showed non-significant bacterial wilt incidence at 56, 63, 70 and 77 days after planting.

**Table 13: Effects of Bacterial wilt incidences (BWI) among Asante, Kenya Mpya and Kenya Sherekea potato mutants at 42 to 84 days after plating at KALRO NARL at M1V4 generation**

| | Days after planting | | | | | | |
|---|---|---|---|---|---|---|---|
| Clones | 42 | 49 | 56 | 63 | 70 | 77 | 84 |
| Asante | 9.3* | 13.3*** | 11.9 ns | 11.9 ns | 10.6 ns | 7.7 ns | 32.5*** |
| Kenya Mpya | 9.6* | 15 ns | 10.7 ns | 11.5 ns | 10.7 ns | 6.7 ns | 35.5 ns |
| Kenya Sherekea | 8.2 ns | 13.6 ns | 11.2 ns | 10.6 ns | 11.5 ns | 6.7 ns | 36.9* |

**Ns=non-significant, *=significant at $p \leq 0.05$, **=significant at $p \leq 0.01$, ***=significant at $p \leq 0.001$**

Figure 8 illustrates the bacterial wilt incidence at 65 days after planting of the vegetative plant parts of the various potato mutant symptoms on a score of 1 to 5 disease index.

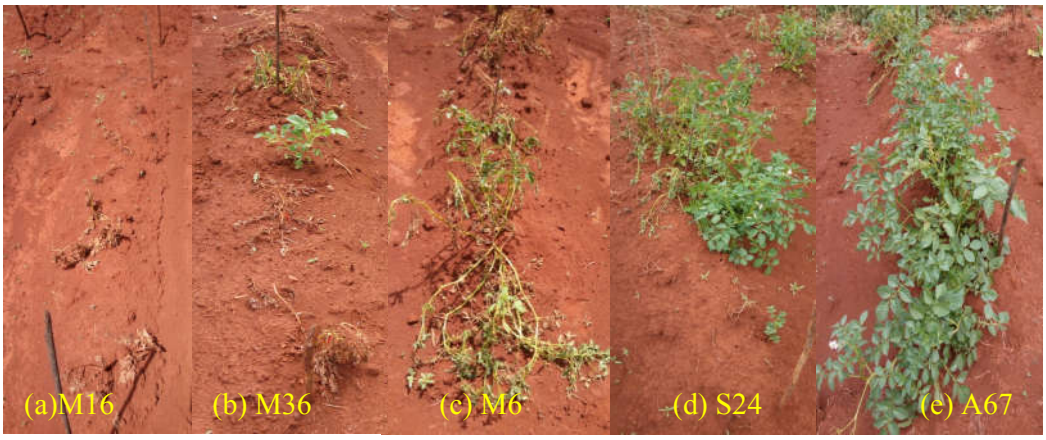


**Plate 3: Bacterial wilt incidence of the vegetative parts of potato mutants at 65 days after planting of various plots symptoms score of 1 to 5 disease index: (a) 5 indicates 75 to 100 % of leaves or plants wilted (mutant M16) (b) 4 indicates 50 to 75 % of leaves or plants wilted (mutant M36 (c) 3 indicates 25 to 50 % of leaves or plants wilted (mutant M6) (d) 2 indicates 10 to 25 % of leaves or plants wilted (mutant S24) and (e) 1 indicates 1 to 10 % of leaves or plants wilted (mutant A67). Source: Author, 2017**

### 4.3.3 Correlation analysis

In Table 14 the correlations between days to onset of wilting (DTOW) and area under disease pressure curve (AUDPC) were positive and significant in Asante ($p \leq 0.01$) and Kenya Sherekea ($p \leq 0.05$) and negative but non-significant in Kenya Mpya mutants. Correlations between days to onset of wilting and percentage of symptomatic of total tuber weight in t/ha (PSTTW), percentage of symptomatic tubers of total tuber number per ha (PSTTN) and total tuber number (TTN) were positive and significant in Kenya Sherekea mutants, positive and non-significant in Asante's mutants and negative and non-significant in Kenya Mpya mutants. On the other hand, correlations between PSTTW and PSTTN; TTN and total tuber weight (TTW) were positive and significantly different in all the mutant populations. Correlations between PSTTW and

with all the other traits were positive and non-significant across the mutant populations. Kenya Sherekea showed more positive significant correlation among traits versus Asante and Kenya Mpya mutants.

**Table 14: Pearson correlation coefficients for various agronomic traits in Asante, Kenya Mpya and Kenya Sherekea potato mutants**

| | | AUDPC | DTOW | PSTTN | PSTTW | PWTTW | TTN | TTW |
|---|---|---|---|---|---|---|---|---|
| **Asante** | **AUDPC** | 1 | | | | | | |
| | **DTOW** | 0.64** | 1 | | | | | |
| | **PSTTN** | 0.02 ns | 0.15 ns | 1 | | | | |
| | **PSTTW** | -0.01ns | 0.14 ns | 0.71** | 1 | | | |
| | **PWTTW** | 0.31 ns | 0.3 ns | 0.23 ns | 0.24ns | 1 | | |
| | **TTN** | -0.23ns | 0.03 ns | -0.15ns | -0.23ns | -0.37ns | 1 | |
| | **TTW** | 0.03 ns | 0.16 ns | -0.19ns | -0.37ns | 0.14 ns | 0.5* | 1 |
| **Kenya Mpya** | **AUDPC** | 1 | | | | | | |
| | **DTOW** | -0.77ns | 1 | | | | | |
| | **PSTTN** | -0.27ns | -0.27ns | 1 | | | | |
| | **PSTTW** | -0.09ns | -0.21ns | 0.43* | 1 | | | |
| | **PWTTW** | 0.37ns | 0.67 ns | 0.28ns | 0.02ns | 1 | | |
| | **TTN** | 0.35ns | -0.07ns | -0.56ns | -0.34ns | -0.07ns | 1 | |
| | **TTW** | 0.11ns | 0.15 ns | -0.4ns | 0.23ns | -0.45ns | 0.17* | 1 |
| **Kenya Sherekea** | **AUDPC** | 1 | | | | | | |
| | **DTOW** | 0.46* | 1 | | | | | |
| | **PSTTN** | 0.50* | 0.64*** | 1 | | | | |
| | **PSTTW** | 0.62** | 0.71*** | 0.59** | 1 | | | |
| | **PWTTW** | 0.12ns | 0.17 ns | 0.35ns | 0.12ns | 1 | | |
| | **TTN** | 0.14ns | 0.4* | -0.04ns | -0.03ns | -0.33ns | 1 | |
| | **TTW** | -0.06ns | -0.16ns | -0.5* | -0.28ns | -0.25ns | 0.52** | 1 |

***=Significant at $p \leq 0.05$; **=Significant at $p \leq 0.01$; ns=Non-significant; DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha-1); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t $ha^{-1}$); TTN=Total tuber number per ha; TTW= Total tuber weight (t/ha).**

### 4.3.4 Ranking of potato mutants for tolerance to bacterial wilt based on selected agronomic and bacterial wilt traits

Table 15 shows the ranking of the top five (Asante, Kenya Mpya and Kenya Sherekea) potato mutants for tolerance to bacterial wilt based on selected agronomic and bacterial wilt traits. The ranking of the mutants were based on the mean of each of the selected agronomic and bacterial wilt traits. The mutant A67 of Asante genotype was ranked first overall and in total tuber weight. Mutant A57 was ranked second overall and first in days to onset of wilting. In Kenya Mpya mutants, M6 was ranked first overall and in percentage of symptomatic tubers of total tuber number per ha. The M4 mutant was ranked fifth overall but ranked first in days to onset of wilting and area under disease progress curve. In Kenya Sherekea mutants, mutant S20 was ranked first overall and in area under disease progress curve and total tuber weight (Appendix III, IV and V).

**Table 15: Asante, Kenya Mpya and Kenya Sherekea potato mutants ranked based on some agronomic and bacterial wilt resistance parameters**

| Genotypes | Clones | DTOW | AUDPC | TTN | TTW | PSTTN | PSTTW | PWTTW | Overal Rank |
|---|---|---|---|---|---|---|---|---|---|
| Asante | A67 | 18.0 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 40.0 | 1 |
| | A57 | 1.0 | 2.0 | 5.0 | 9.0 | 11.0 | 28.0 | 16.0 | 2 |
| | A40 | 7.0 | 27.0 | 10.0 | 11.0 | 2.0 | 1.0 | 52.0 | 3 |
| | A59 | 3.0 | 7.0 | 14.0 | 47.0 | 9.0 | 32.0 | 7.0 | 4 |
| | A58 | 18.0 | 40.0 | 3.0 | 8.0 | 9.0 | 25.0 | 50.0 | 5 |
| Kenya Mpya | M6 | 5.0 | 12.0 | 14.0 | 17.0 | 5.0 | 1.0 | 3.0 | 1 |
| | M39 | 17.0 | 25.0 | 2.0 | 1.0 | 3.0 | 2.0 | 22.0 | 2 |
| | M30 | 4.0 | 8.0 | 5.0 | 3.0 | 12.0 | 19.0 | 27.0 | 3 |
| | M25 | 23.0 | 18.0 | 4.0 | 8.0 | 2.0 | 9.0 | 19.0 | 4 |
| | M4 | 1.0 | 1.0 | 9.0 | 10.0 | 18.0 | 25.0 | 22.0 | 5 |
| Kenya Sherekea | S20 | 11.0 | 12.0 | 1.0 | 1.0 | 8.0 | 9.0 | 2.0 | 1 |
| | S14 | 29.0 | 4.0 | 4.0 | 12.0 | 5.0 | 19.0 | 3.0 | 2 |
| | S21 | 11.0 | 12.0 | 2.0 | 3.0 | 21.0 | 28.0 | 9.0 | 3 |
| | S29 | 36.0 | 12.0 | 2.0 | 5.0 | 3.0 | 2.0 | 27.0 | 4 |
| | S34 | 1.0 | 2.0 | 29.0 | 15.0 | 6.0 | 20.0 | 24.0 | 5 |

**DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha-1); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t $ha^{-1}$); TTN=Total tuber number per ha; TTW= Total tuber weight (t/ha).**

### 4.3.5 Genetic diversity of mutant clones based on selected traits

Four groups were formed in the dendrogram based on UPGMA cluster analysis of potato mutants constructed from selected agronomic and bacterial wilt traits using DARwin software package. Group I, II and IV had equal proportionate number of Asante, Kenya Mpya and Kenya Sherekea mutant populations. Group III contain large population of Asante's mutants (Figure 6). Group 1 was the most diverse containing sub-clusters with mutant A45 being clustered alone.

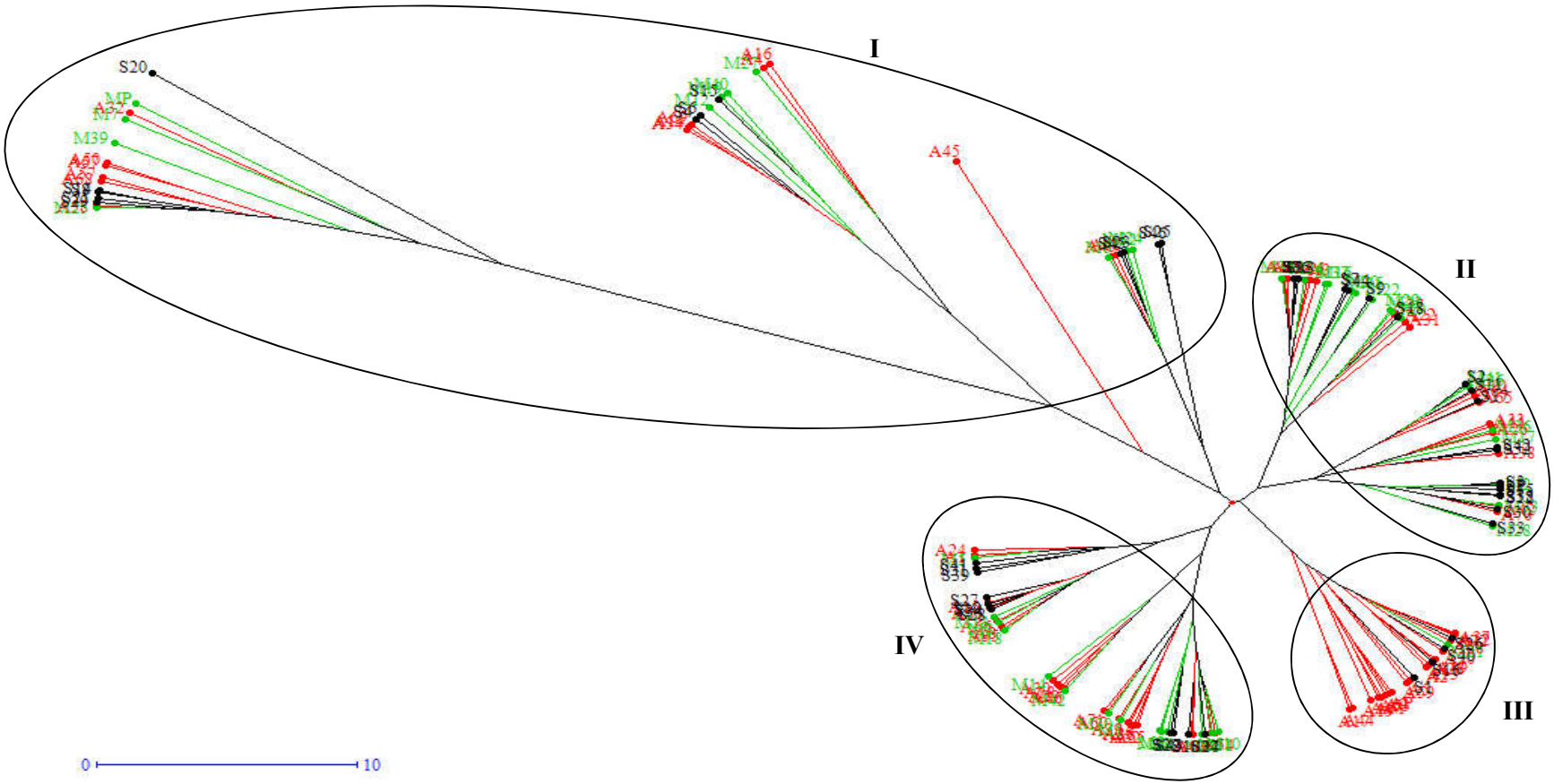


**Figure 6: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among 163 potato mutants (Asante – red, Kenya Mpya – green and Kenya Sherekea – black) based on selected agronomic and bacterial wilt resistance parameters**

## 4.4 Determining the genetic variability of potato mutants using SSR markers

### 4.4.1 Genetic Diversity

In Table 16, all the 20 SSR primers gave polymorphic bands, Marker STM5127 (Figure 7) gave the highest allele number and gene diversity of 19 and 0.81 respectively. Marker STWAX-2 gave the highest allele frequency of 0.95 and the lowest allele number of 6. The STI0032 marker had the highest heterozygosity of 0.82 while STPoAc58 marker had the highest polymorphic information content (PIC) of 0.89. A total number of 211 alleles (average of eleven alleles) were generated with Asante generating 69 alleles (average of three alleles), Kenya Mpya was 75 alleles (average of four alleles) and Kenya Sherekea with 67 alleles (average of three alleles) (Table 16, Appendix VI, VII and VIII). The average values of allele frequency and genetic diversity were 0.68 (ranging from 0.32 to 0.99) and 0.20 (ranging from 0.00 to 0.74) respectively for Asante; 0.78 (ranging from 0.21 to 0.98) and 0.22 (ranging from 0.00 to 0.84), respectively, for Kenya Mpya; and 0.80 (ranging from 0.23 to 0.98) and 0.22 (ranging from 0.00 to 0.78), respectively, for Kenya Sherekea. The heterozygosity and polymorphic information content (PIC) average values were 0.42 and 0.52 respectively for Asante; 0.43 and 0.50, respectively, for Kenya Mpya; and 0.26 and 0.36 respectively, for Kenya Sherekea.

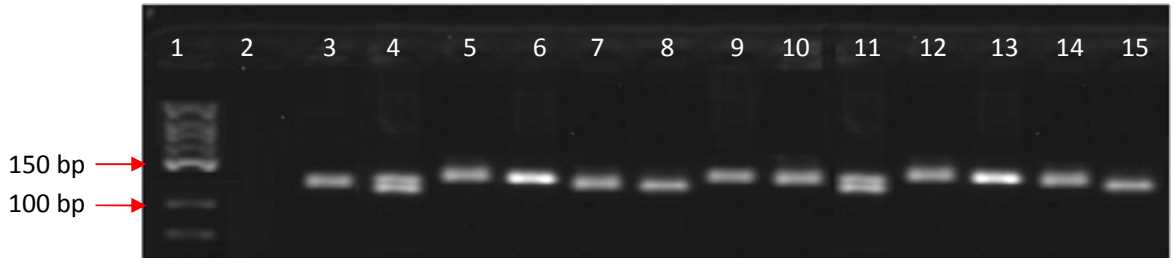


**Figure 7: SSR markers profile of Kenya Mpya potato mutants generated by primer STM5127; (1 = ladder, 2 = water, 3 to 15 are mutants from M1 to M15); bp – base pair**

**Table 16: Summary of statistical analysis of genetic diversity across all the 160 potato mutants and 3 parents' genotypes based on 20 microsatellite loci**

| Marker | Allele Frequency | Allele Number | GeneDiversity | Heterozygosity | PIC |
|---|---|---|---|---|---|
| **STG0016** | 0.86 | 12 | 0.26 | 0.81 | 0.79 |
| **STM5114** | 0.49 | 9 | 0.63 | 0.61 | 0.56 |
| **STM1053** | 0.48 | 7 | 0.67 | 0.57 | 0.61 |
| **STI0012** | 0.42 | 9 | 0.69 | 0.76 | 0.64 |
| **STI0032** | 0.45 | 10 | 0.66 | 0.82 | 0.59 |
| **STI0004** | 0.69 | 12 | 0.06 | 0.55 | 0.59 |
| **STM0031** | 0.79 | 8 | 0.06 | 0.45 | 0.59 |
| **STM1104** | 0.39 | 13 | 0.01 | 0.60 | 0.81 |
| **STM1052** | 0.45 | 11 | 0.65 | 0.34 | 0.57 |
| **STM1106** | 0.58 | 6 | 0.50 | 0.25 | 0.85 |
| **STM0037** | 0.42 | 9 | 0.68 | 0.56 | 0.61 |
| **STI0030** | 0.73 | 10 | 0.42 | 0.61 | 0.70 |
| **STM2013** | 0.96 | 8 | 0.08 | 0.64 | 0.79 |
| **STM1049** | 0.29 | 17 | 0.76 | 0.59 | 0.73 |
| **STM5127** | 0.34 | 19 | 0.81 | 0.68 | 0.79 |
| **STI046** | 0.63 | 11 | 0.48 | 0.67 | 0.74 |
| **STI0036** | 0.53 | 9 | 0.52 | 0.32 | 0.77 |
| **STI0023** | 0.50 | 16 | 0.68 | 0.58 | 0.65 |
| **STWAX-2** | 0.97 | 6 | 0.06 | 0.24 | 0.58 |
| **STPoAc58** | 0.95 | 9 | 0.09 | 0.65 | 0.89 |
| **Combined Mean** | 0.60 | 11 | 0.44 | 0.57 | 0.69 |
| **Asante Mean** | 0.68 | 3 | 0.22 | 0.06 | 0.24 |
| **Mpya Mean** | 0.78 | 4 | 0.45 | 0.11 | 0.19 |
| **Sherekea Mean** | 0.80 | 3 | 0.23 | 0.16 | 0.18 |

### 4.4.2 Analysis of Molecular Variance (AMOVA)

A population diversity analysis was performed to explore the genetic variations among and within groups of the potato mutants (Table 17). AMOVA revealed that the diversity within populations of Asante, Kenya Mpya, Kenya Sherekea and all the three combined (160) potato mutant clones and the 3 parents were 83 %, 79 %, 87.4 % and 91.4 % respectively. The Kenya Sherekea mutants were the most diverse within populations with 87.4 %. The minimum diversity attributed to genetic differentiation among groups of Asante, Kenya Mpya, Kenya Sherekea and all the three combined (160) potato mutant clones and parents were 17 %, 21 %, 12.6 % and 6.2 % respectively (Table 17).

**Table 17: Analysis of Molecular Variance (AMOVA) based on SSR markers for each population of potato mutants**

| Population | Sources of variations | Degrees of freedom | Sum of Squares | Variance components | Percentage (%) of variation |
|---|---|---|---|---|---|
| Asante | Among Populations | 5 | 99887.4 | 96 | 17 |
| | Within Individuals | 72 | 156945.4 | 1160 | 83 |
| | **Total** | **78** | **256832.8** | **1184** | **100** |
| Kenya Mpya | Among Populations | 4 | 18356.4 | 106 | 21 |
| | Within Individuals | 42 | 124432.6 | 964 | 79 |
| | **Total** | **46** | **142789** | **1140** | **100** |
| Kenya Sherekea | Among Populations | 7 | 76243.2 | 68 | 12.6 |
| | Within Individuals | 46 | 142654.3 | 679 | 87.4 |
| | **Total** | **53** | **218897.5** | **986** | **100** |
| Combined | Among Populations | 2 | 42589.7 | 24 | 6.2 |
| | Among sub-populations | 18 | 86426 | 66 | 2.4 |
| | Within Individuals | 162 | 114986.3 | 128 | 91.4 |
| | **Total** | **180** | **244002** | **245** | **100** |

### 4.4.3 Population structure analysis

The Bayesian clustering method with admixed model indicated that the 73 mutant accessions (including 1 parent) of Asante were clustered into five genetic groups ($K$ = 5) (Figure 8 and 9). Subpopulation (SP) SP5 was the largest group with 41 % of all the Asante potato mutants while SP1, SP2, SP3 and SP4 consisted of 15, 11, 26 and 8 % respectively. The figure 8 and 9 shows the estimated population structure based on Delta $K$ ($\Delta K$) when it reaches its maximum value following the *ad-hoc* method and subpopulation clusters (K) that are represented by different colors, respectively.

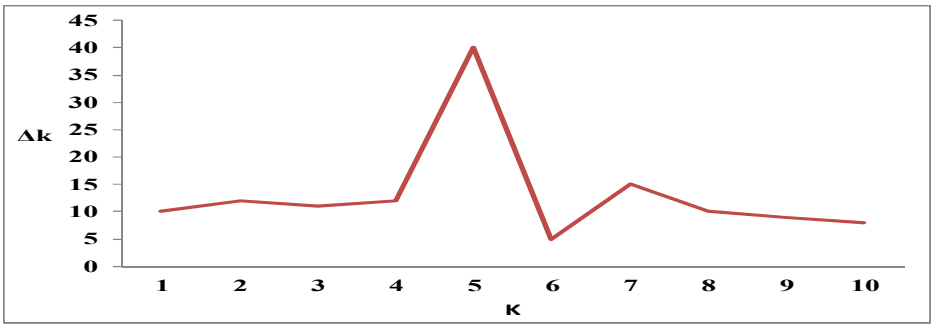


**Figure 8: STRUCTURE estimation of the number of subgroups for the K values ranging from 1 to 10, by delta K ($\Delta$K) = 5 values of the 73 Asante potato mutants**

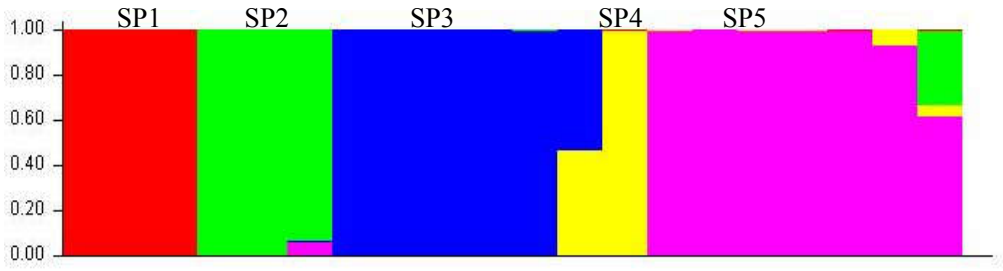


**Figure 9: The estimated Population structure of the 73 Asante Potato mutant's obtained with the STRUCTURE program based on based on SSR markers for K = 5. Five subpopulation clusters (K) are represented by different colors (Subpopulation 1 (SP1) = Red, SP2 = Green, SP3 = Blue, SP4 = Yellow and SP5 = Purple) and the length of the colored segment shows the estimated membership proportion of each sample to designed group.**

The Bayesian clustering method with admixed model indicated that the 43 mutant accessions (including 1 parent) of Kenya Mpya (Figure 10 and 11) clustered into four genetic groups ($K$ = 4) with estimated membership probability threshold ($q$) values of between 0.25 and 0.53. The subpopulation (SP) 1 was largest group with 86 % of the total Kenya Mpya potato mutants, SP2 and SP3 had 4 % each while SP4 had 6 % mutants. SP1 and SP4 contain an admix populations.

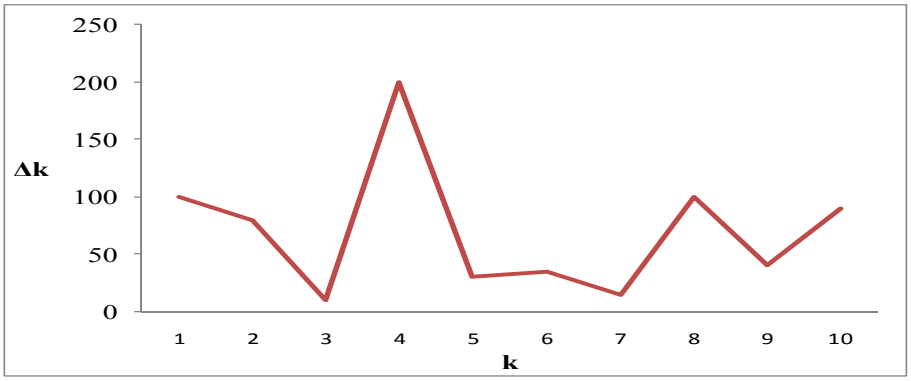


**Figure 10: STRUCTURE estimation of the number of subgroups for the K values ranging from 1 to 10, by delta K (*Δ*K) = 4 values of the 43 Kenya Mpya potato mutants**

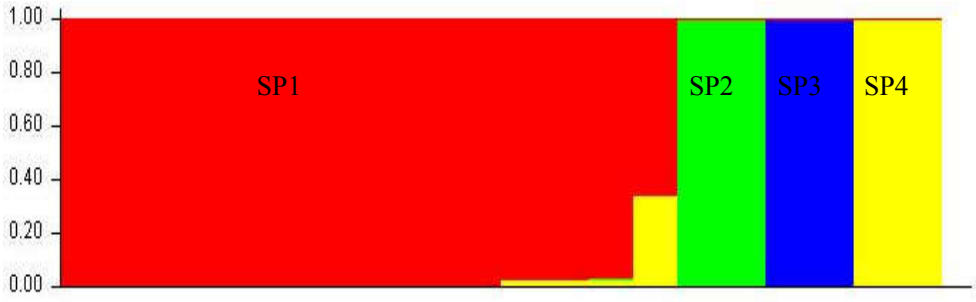


**Figure 11: The estimated Population structure of the 43 Kenya Mpya Potato mutants obtained with the STRUCTURE program based on based on SSR markers for K = 4. Four subpopulation clusters (K) are represented by different colors (Subpopulation 1 (SP1) = Red, SP2 = Green, SP3 = Blue and SP4 = Yellow) and the length of the colored segment shows the estimated membership proportion of each sample to designed group.**

The Bayesian clustering method with admixed model indicated that the 47 mutant accessions (including 1 parent) of Kenya Sherekea (Figure 12, 13) clustered into four

genetic groups ($K$ = 4) with estimated membership probability threshold ($q$) values of between 0.24 and 0.63. The SP4 was largest group with 64 % of the total Kenya Sherekea potato mutants; SP3 had 15 % mutants while SP2 and SP1 had 6 % mutants each and 9 % mutants respectively. The SP4 contained the largest admix groups.

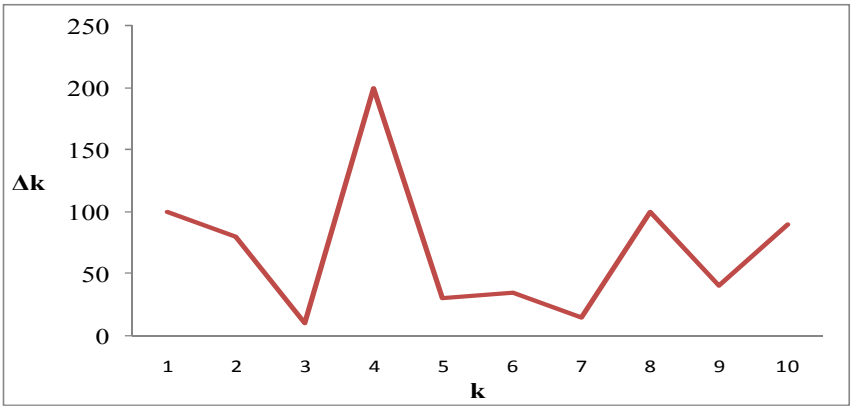


**Figure 12: STRUCTURE estimation of the number of subgroups for the K values ranging from 1 to 10, by delta K ($\Delta$K) = 4 values of the 47 Kenya Sherekea potato mutants**

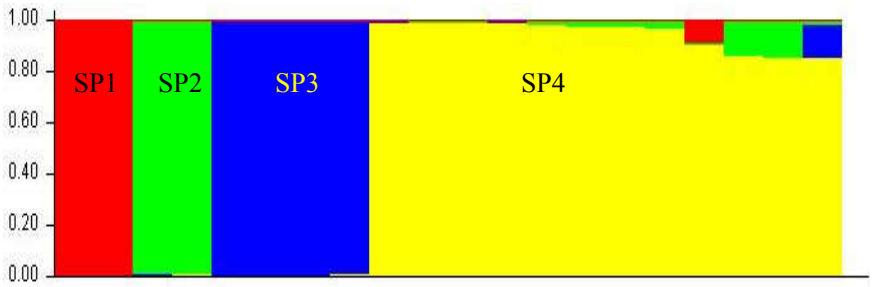


**Figure 13: The estimated Population structure of the 47 Kenya Sherekea Potato mutants obtained with the STRUCTURE program based on based on SSR markers for K = 4. Four subpopulation clusters (K) are represented by different colors (Subpopulation 1 (SP1) = Red, SP2 = Green, SP3 = Blue and SP4 = Yellow) and the length of the colored segment shows the estimated membership proportion of each sample to designed group.**

The Bayesian clustering method with admixed model indicated that 160 mutant accessions and 3 parents of Asante (72), Kenya Mpya (46) and Kenya Sherekea (42) were clustered into six genetic groups ($K$ = 6) with estimated membership probability threshold ($q$) values of between 0.34 and 0.60 (Figure 14 and 15). The estimated membership probability threshold (q) indicated that 146 (89.6 %) of the 163 individuals belonged to groups SG1 to SG5, while the remaining 17 (10.4 %) potato mutants were assigned into an admix group. The accessions in admix group presented the probability of belonging to more than one subgroup, demonstrating that these accessions shared some degree of genetic information with some other groups. The SP2 was the largest group and consisted mainly of mutants from Asante (38 mutants) and Kenya Sherekea (22 mutants), which accounted for 52 % and 46% of the mutants, respectively. The figures 14 and 15 shows the estimated population structure based on Delta $K$ ($\Delta K$) reaches its maximum value following the *ad-hoc* method and subpopulation clusters (K) that are represented by different colors, respectively.

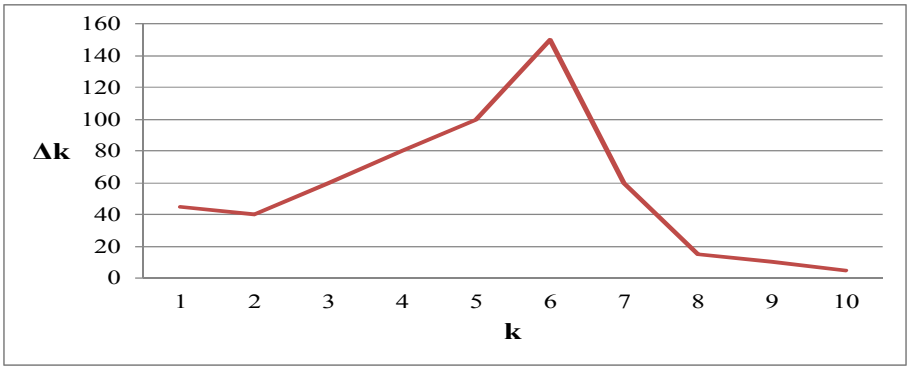


**Figure 14: STRUCTURE estimation of the number of subgroups for the K values ranging from 1 to 10, by delta K ($\Delta$K) values of the 160 potato mutants and 3 parents**

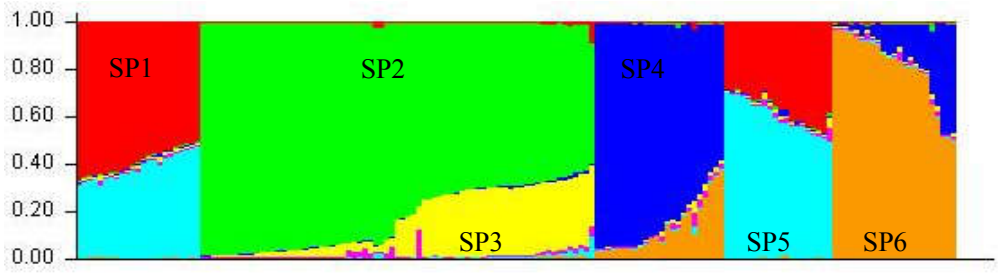


**Figure 15: Population structure of 160 potato (Asante, Kenya Mpya and Kenya Sherekea) mutants and 3 parents obtained with the STRUCTURE program based on SSR markers for K = 6. Each colour represents one subpopulation (Subpopulation 1 (SP1) = Red, SP2 = Green, SP3 = Yellow, SP4 = Blue, SP5 = Light blue and SP6 = Orange) and the length of the coloured segment shows the estimated membership proportion of each sample to designed group.**

### 4.4.4 Molecular phylogenetic analysis

The dendrogram based on UPGMA cluster analysis constructed from SSR markers in DARwin software package among the Asante potato mutants were grouped into five sub-groups (Figure 16) as revealed by population structure in Figure 8 and 9. Group I and II gave the largest cluster with equal number of potato mutants. Group III and V gave the smallest cluster populations of 6 % and 4 % respectively. The dendrogram showed that group I consisted of 38 % of the total Asante mutant population while group II and IV consisted of 35 % and 17 % respectively.

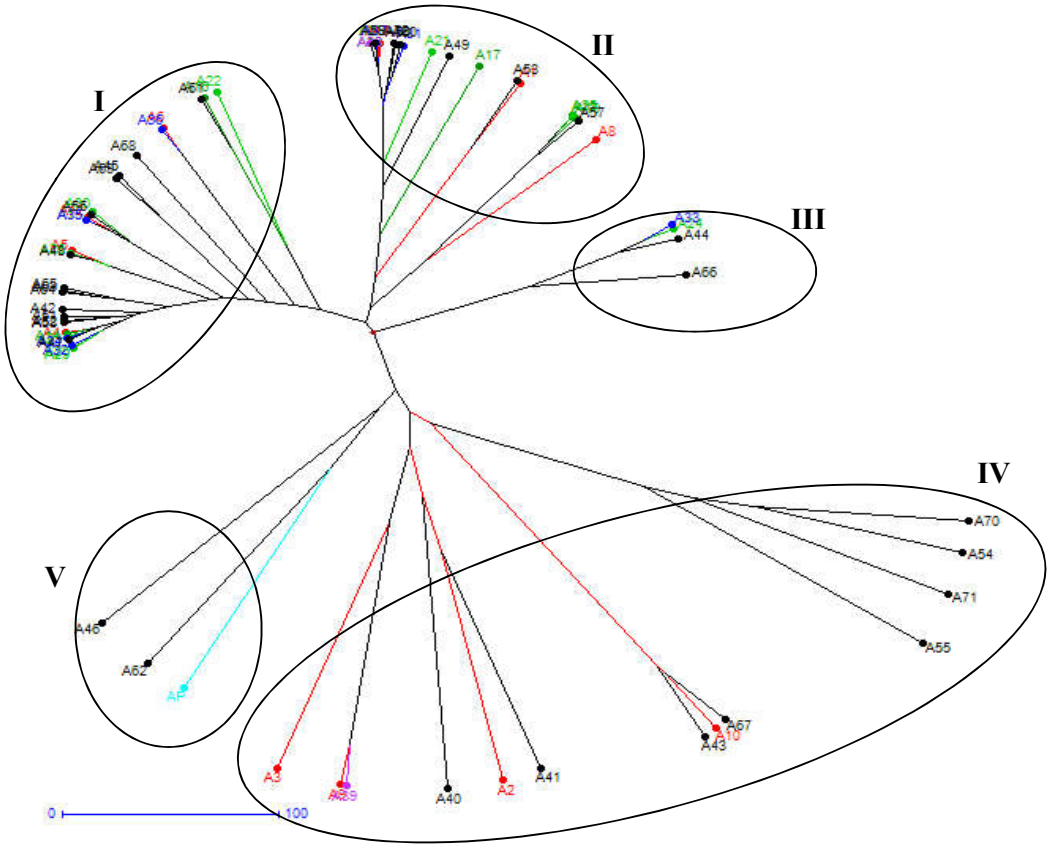


**Figure 16: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among Asante potato mutants based on 20 SSR markers with different dosage rates represented by different colours**

The dendrogram based on UPGMA cluster analysis constructed from SSR markers in DARwin software package among the Kenya Mpya potato mutants were grouped into four major groups (Figure 17) and supported by population structure in Figure 10 and 11. Group III was the most diverse consisting of the parent and all the potato mutants from all the dose rates used. Group II gave the smallest cluster populations of 9 % of total mutants. The dendrogram showed that group I consisted of 21 % of the total Kenya Mpya mutant population while group III and IV consisted of 37 % and 33 % respectively.

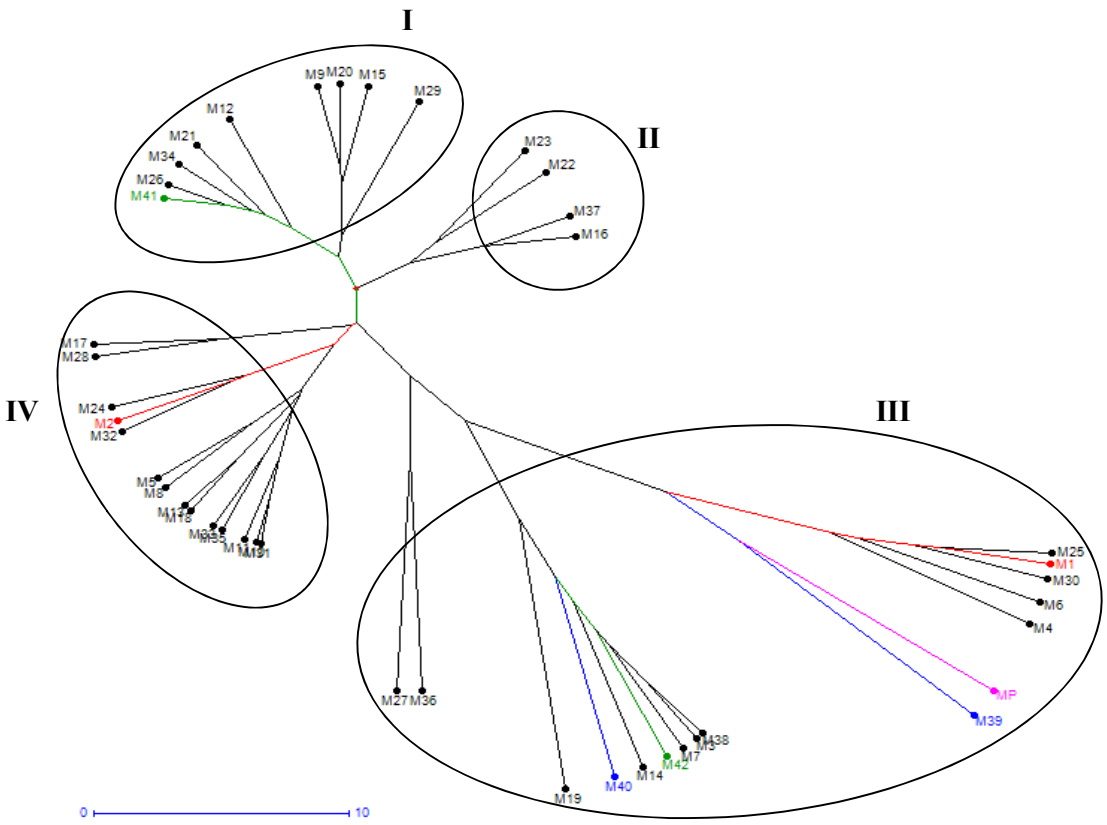


**Figure 17: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among Kenya Mpya potato mutants based on 20 SSR markers with different dosage rates represented by different colours**

The dendrogram based on UPGMA cluster analysis constructed from SSR markers in DARwin software package among the Kenya Sherekea potato mutants were grouped into four major groups (Figure 18) and corroborated by population structure in Figure 12 and 13. Group IV was the most diverse consisting of the parent and all the potato mutants from all the dose rates used. Group II and III gave the smallest cluster populations of 6 and 11 % of the total mutants. While group I and IV consisted of 30 and 53 % respectively, of the total Kenya Sherekea mutant populations.

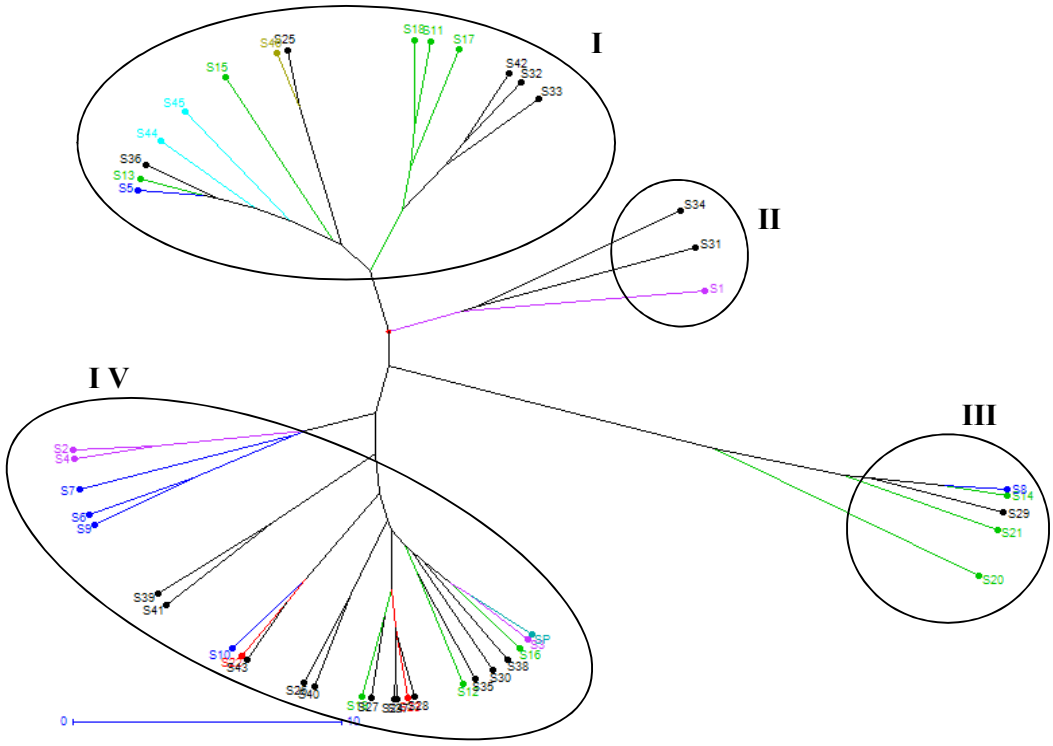


**Figure 18: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among Kenya Sherekea potato mutants based on 20 SSR markers with different dosage rates represented by different colours**

The dendrogram based on UPGMA cluster analysis constructed from SSR markers in DARwin software package among potato mutants were grouped into three groups (Figure 19). Group I was the most diverse group consisting of four subgroups (SG) A, B, C and D while group II group III consisted of similarly equal number of potato mutants (Figure 19). These makes a total of six subgroups as revealed by population structure (Figure 14 and 15). The dendrogram showed that group I consisted of 74 % of the total mutant population while group II and III consisted of 15 % and 11 % respectively.

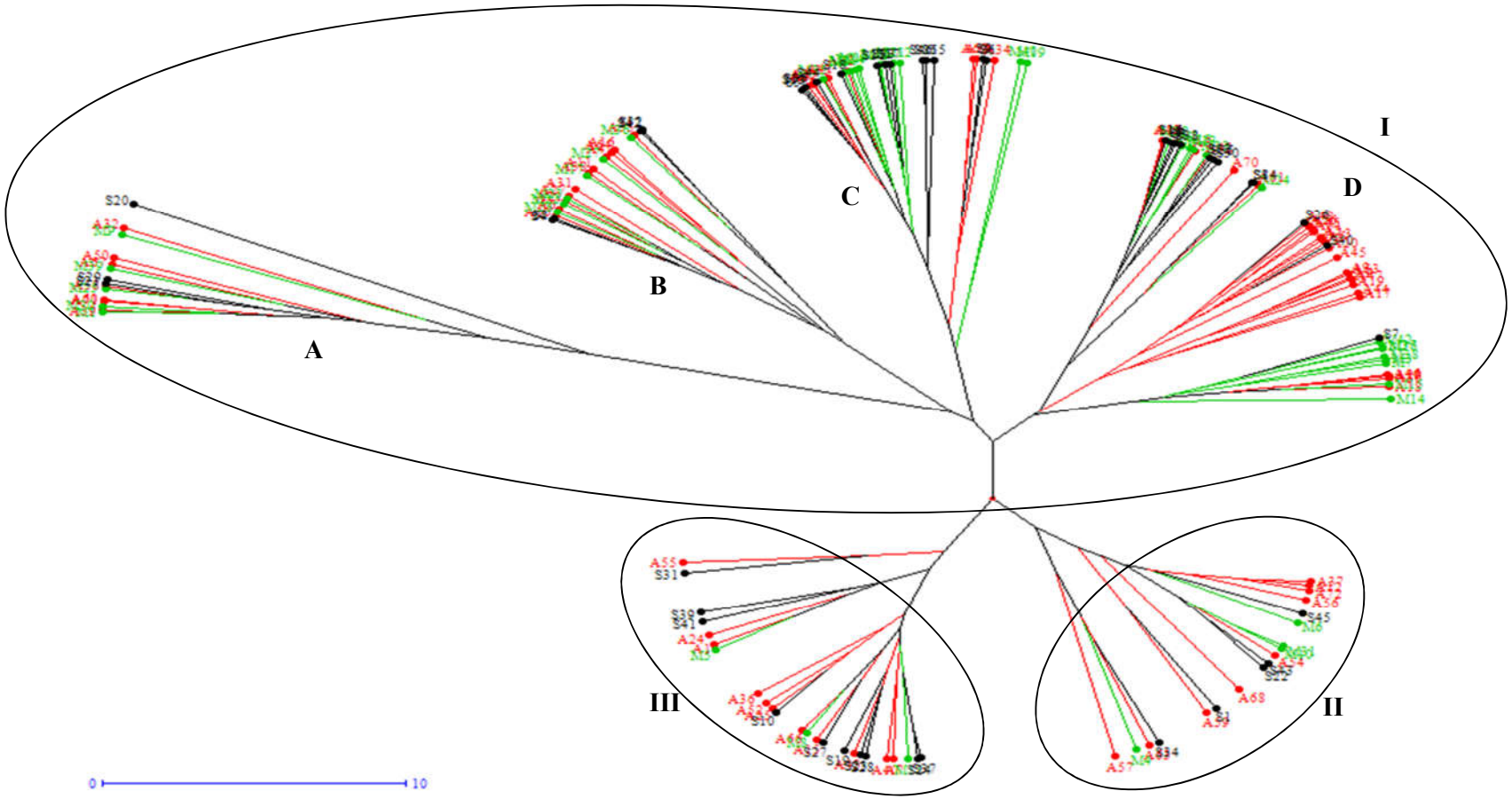


**Figure 19: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among 160 potato mutants (Asante – red, Kenya Mpya – green and Kenya Sherekea – black) based on 20 SSR markers**

### 4.4.5 Principal co-ordinate analysis

A further molecular analysis using SSR data in Figure 20 shows the scatter plot that splits the potato mutants in three main clusters defined by the first two coordinates. The principal co-ordinate analysis resembled more strongly the dendrogram obtained with SSR data analysis (Figure 19) with mutants contributing more to the first principal component analysis. The first and second principal components of the potato mutants comprised 54.2 % and 18.6 % respectively, accounting to 72.8 % of the total variation (Figure 20).

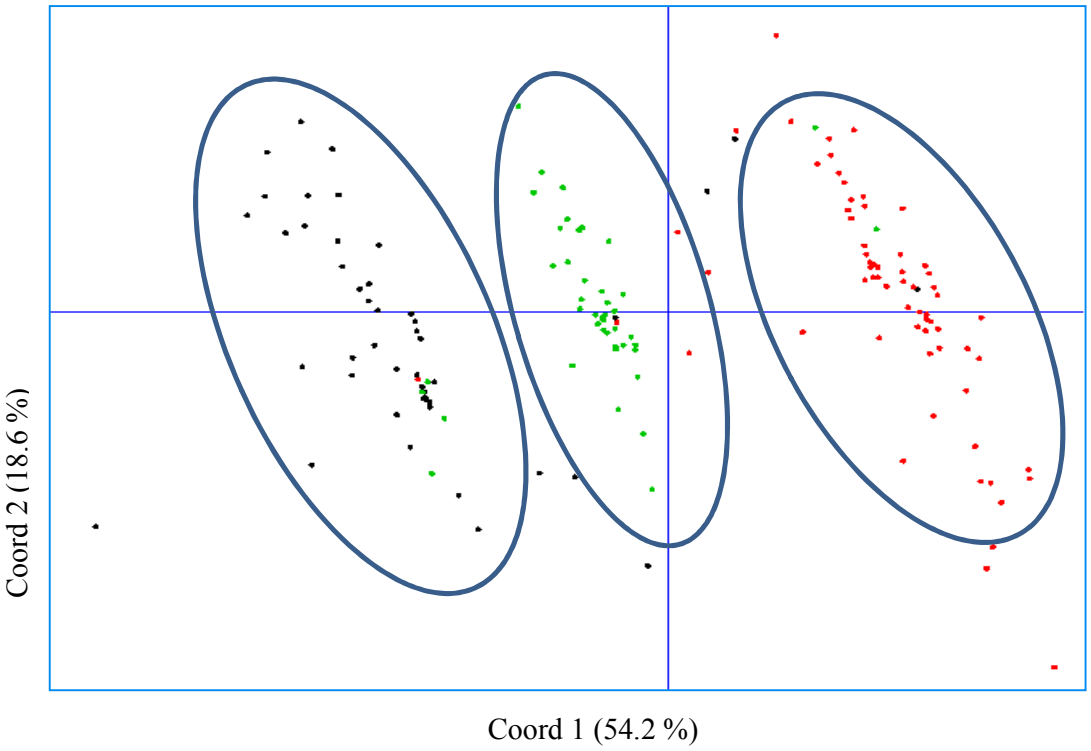


**Figure 20: A scatter plot of 160 potato mutants and 3 parents based on first and second components of principal coordinate analysis using 20 SSR data (Red represent Asante mutants, Green represent Kenya Mpya mutants and Black represent Kenya Sherekea mutants.**

**Consensus tree analyses from agronomic and bacterial wilt resistance traits and SSR markers**

The dendrogram was generated from agronomic and bacterial wilt resistance traits with SSR data using consensus clustering method. The comparison of the two data sets revealed three main clusters Figure 21 demonstrating that the combined SSR and agronomic and bacterial wilt resistance traits dendrogram resembled the SSR dendrogram (Figure 19) more than the dendrogram generated from agronomic and bacterial wilt resistance traits (Figure 6) generating three clustering patterns of the mutants. However, Group I in all the three dendrograms; agronomic and bacterial wilt resistance traits (Figure 6), SSR (Figure 19) and consensus tree from combined SSR and agronomic and bacterial wilt resistance traits (Figure 21) showed that most of the mutants maintained their positioning.

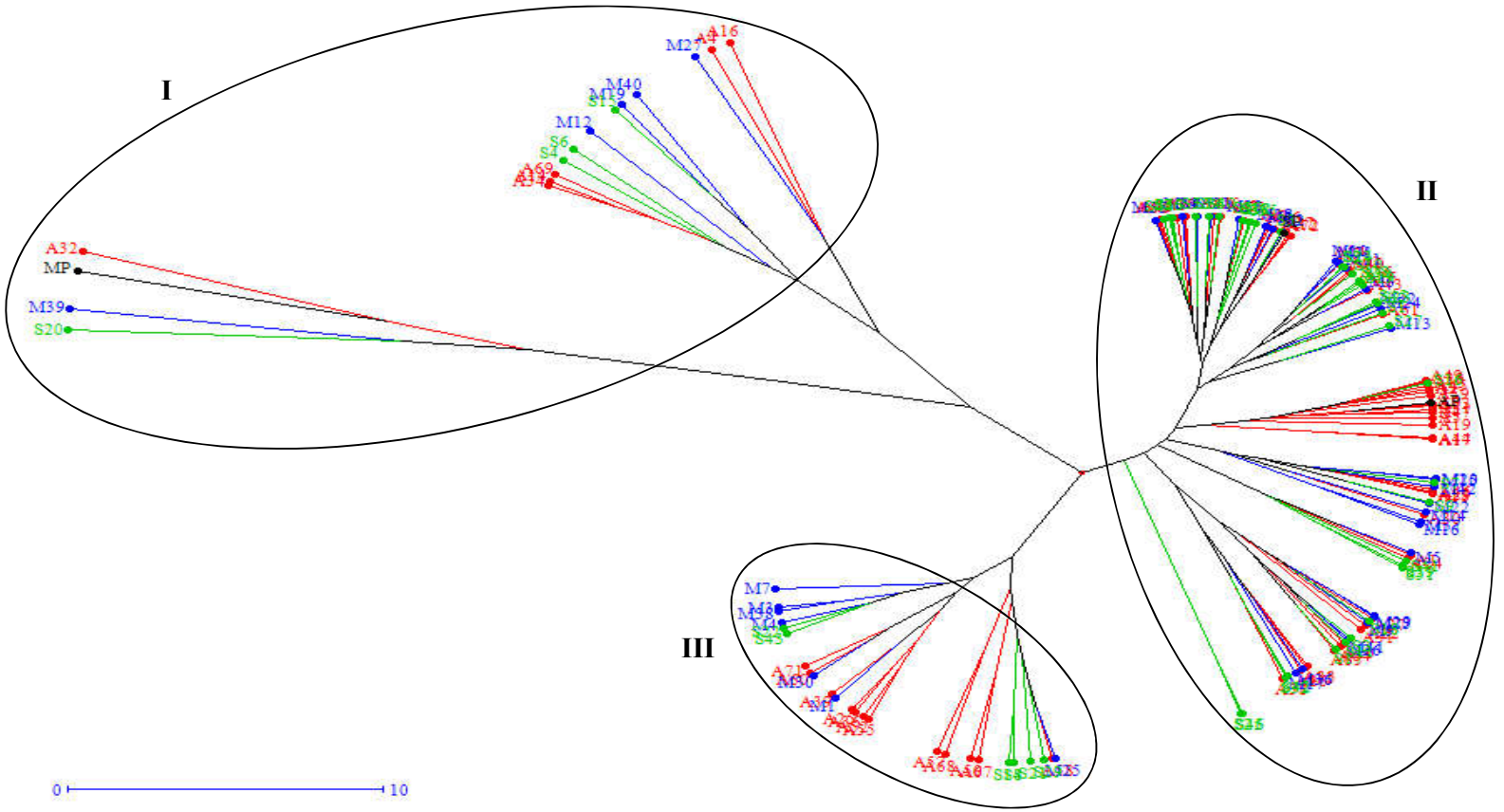


**Figure 21: Unrooted consensus tree analysis using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among the potato mutants (Asante – red, Kenya Mpya – blue and Kenya Sherekea – green) based on agronomic and bacterial wilt traits and 20 SSR markers**

The cophenetic correlation coefficients (r) were used to objectively compare matrices generated using the Mantel test. The cophenetic correlation coefficients (r) between the similarity matrices and cophenetic matrices obtained from the combined SSR with agronomic and bacterial wilt resistance traits versus SSR data and combined SSR with agronomic and bacterial wilt resistance traits versus agronomic and bacterial wilt resistance traits was 0.67 and 0.48 respectively. These dendrograms were compared using consensus tree analysis. When the consensus fork index *CIc* equals one the dendrograms is considered identical. Dendrograms obtained from SSR and agronomic and bacterial wilt resistance traits data was 20 % identical (*CIc*= 0.20).

# CHAPTER FIVE

# DISCUSSION

## 5.1 Effects of mutagen dose on selected agronomic traits in of potato mutants

The results showed that the parents were significantly different from the mutants in terms of stem number (M1V1) and plant height (M1V2) in Asante mutants and stem anthocyanin colouration, leaf anthocyanin colour midrib of upperside, growth habit, plant height and tuber number in Kenya Mpya mutants. This demonstrates that induced mutations are highly effective in enhancing natural genetic resources and efficient tool to rectify or amend certain characters without altering other traits of the crop plants (Mba, 2013). Induced mutation has been used successfully to develop improved cultivars in cereals, fruits, Dolichos and other crops (Kinyua *et al.,* 2000; Kamau *et al.,* 2011; Kinyua, 2014; Ulukapi & Nasircilar, 2015; Kumar *et al.,* 2018).

The study showed that the total number of potato tubers that survived to produce tubers at the first generation was lower (approximately 50 % of irradiated) compared to the total (original) number of microtubers that were initially irradiated. The high mortality rates observed on potato mutant plants after transplanting in the glasshouse could be as a result of poor adaptability, effects of irradiation or environmental conditions (temperature). This results agrees with those of Malebana (2014) who observed uniform death on Monate mutant sweetpotato plants after transplanting the plantlets in the glasshouse from both single and re-irradiated treatments. Similarly, Kumar *et al.,* (2018) in dolichos reported increased mortality at M1 generation with increasing doses of gamma radiations.

The findings also showed that the survival and tuberization rate of M1V1 was much lower than that of M1V2 plants. The low survival rate and decreased vigour of M1V1 plants could be because they were directly acquired from minitubers exposed to acute irradiation and the lingering effects of the genotypes. This might have suppressed the enzymatic activity and membrane integrity minitubers leading to reduced metabolic activity and failure of germination and tuberization. The induced M1V1 plants that survived the irradiation effects gave rise to induced M1V2 plants and were advanced to subsequent generations. The higher survival rate of M1V2 plants is because they had already stabilized and selection had favoured them. This finding agrees with the observations made by Bado *et al.,* (2016) and Ahloowalia, (1994) in their research on the effects of irradiation on tuberization capacity.

The Asante mutants showed increased tuberization parameters (plant height, tuber weight, stem and tuber number) as the dosage rates increased from 3 Gy to 12 Gy and decreased at 15 Gy except tuber number at 12 and 15 Gy compared to Kenya Mpya and Kenya Sherekea mutants that were at a decreasing rate. The variation in response of potato genotypes to increasing gamma dose rates could be due to the sensitivity (Kenya Mpya and Kenya Sherekea) or persistence of the genotypes to irradiation methods used which affects plant growth and development. Higher doses of radiation can cause chromosomal damage in plant meristematic cells, cell cycle deceleration and mitotic delay, which might have brought down the rate of cell division, hence considerably influence general plant regeneration and development. Similar trends were observed in *Crossandra infundibuliformis* var. Danica (Hewawasam *et al.,* 2004), potato (Yaycili & Alikamanoğlu, 2012; Bado *et al.,* 2016) and dolichos (Kumar *et al.,* 2018).

Regarding the relationship between the gamma rays dosage and morphological traits, it was found that relatively low doses of gamma irradiation (3 to 9 Gy) had a positive effect in plant height, plant tuber number and plant tuber weight in all the three mutant populations at M1V2 and M1V3 generations. This is consistent with the observations made by Li *et al.,* (2005) and Roy *et al.,* (2009) whose reports indicated that low doses of gamma irradiation stimulate plant growth through enhanced physiological activity. Bado *et al.,* (2016) reported that low dose gamma irradiation (3 and 6 Gy) increased tuberization rate of potato cultivars; BP1, Mpya, Mondial, and Basotho Pink *in vitro*. Other studies on different potato genotypes have also shown that gamma irradiation stimulated micro-tuber induction (Al-Safadi *et al.,* 2000; Al-Safadi & Arabi, 2003; Mahfouze *et al.,* 2012).

At relatively higher doses of gamma irradiation, (6 to 15 Gy for Asante and 10 to 30 Gy Kenya Sherekea mutants), there was an observed decline in the trends of stem number, tuber weight and tuber number with increased dosage. This can be attributed to the inhibitory effect of gamma radiation on physiological and physical properties that lead to reduced cell division and elongation of the plant. Reports by other researchers indicate the higher gamma irradiation can cause chromosomal damage in plants and therefore have significant effects on plant development (Yaycili & Alikamanoğlu, 2012; Bado *et al.,* 2016). Abdul *et al.,* (2010) working with yams reported a decline in stem number with increasing doses of gamma irradiation. Similar trends were observed by Ellafa *et al.,* (2007) in snap bean, (Khan & Goyal, 2009) green beans, (Gnanamurthy & Dhanavel, 2014) cowpea and (Anchalee, 2011) Wishbone Flower (*Torenia fournieri*).

The potato mutants showed significant differences in stem number (Asante), stem anthocyanin colouration and tuber number (Kenya Mpya) and leaf outline openness, plant height, tuber number and tuber weight (Kenya Sherekea) mutants at M1V2 and M1V3 generation. The genetic variation observed within genotypes and generations may be due the effects of mutation induction on the physical and biochemical tissue contents such as DNA size, water content, DNA content, nuclear volume. Various reports showed that different genotypes of the same crop respond differently to different irradiation dosages due to genotypic effects (Owoseni *et al.,* 2006). Several authors have reported variation in yield contributing traits due to different doses of mutagens used in different crops such as green gram (Khan & Wani, 2006), sweet potato (Shin *et al.,* 2011) and African wrinkled pepper (*Capsicum annuum* var *abbreviatum* Fingerh) (Falusi *et al.,* 2013).

The first principal component analysis was effective in discrimination of potato mutants as it showed positive correlation in most of the traits studied. The highest correlation value of plant tuber weight (0.9) across the three mutants and generations indicated that the trait was the most reliable for phenotypic discrimination of the accessions under study. The first PCA across all the mutants contributed more than 60 % variation and can be interpreted to mean the reliability in discrimination based on the trait that contributed more to the variation.

The association between phenotypic and genotypic values is important in predicting the outcome of selection in a collection of genotypes. For all the traits studied in all the 3 potato mutants, the estimates of phenotypic coefficient of variation (PCV) were slightly higher than the corresponding genotypic coefficient of variation (GCV). This indicates

that these characters were less influenced by the environment or generation and phenotype selection alone on the basis of these traits can be effective for the improvement. The results on high PCV values compared GCV values agreed with Wera *et al.* (2014) in sweet potatoes and those of Reddy *et al.,* (2013) in linseed (*Linum usitatissimum* L.) genotypes.

Heritability estimates has been classified as low (below 30 %), medium (30-60 %) and high (above 60%) (Allard, 1999). In the present study, the characters studied expressed all the heritability estimates from low to high ranging from -23.49 to 87.7 percent. The disparity in heritability is not only a property of a trait but also of the mutant population, generation and the genotypes circumstances which indicates that the induced variability in mutant population can be fixed by selection. High heritability estimates of more than 60 % was reported in stem anthocyanin colouration (Asante and Kenya Mpya) and tuber number (Asante), plant height, stem and tuber number in Kenya Mpya mutants and only plant height in Kenya Sherekea mutants. Similar findings on high heritability have been reported in different crops in various traits studied by different authors (Sureja & Sharma, 2000; Panwar & Singh, 2000; Asif *et al.,* 2004; Rasal *et al.,* 2008; Wera *et al.,* 2014; Nang *et al.,* 2015).

High heritability could be due to high contribution of genotypic component hence heritability alone is not a reliable parameter to predict the effective selection. High heritability for plant height have been reported in various crops such as wheat (Panwar & Singh, 2000), spring wheat (Asif *et al.,* 2004) and sorghum (Nang *et al.,* 2015). High heritability accompanied by high genetic advance signifies the predominance of additive gene.

All the three mutant populations showed significant genotypic variation on the tuber yield components such plant height, tuber number, stem number and tuber weight. The existence of variability in the mutant populations can be attributed to the effect of induced mutation. Similar findings have been reported in mung bean for quantitative traits which showed that mutagenic treatments could alter mean values and create additional genetic variability (Khan & Goyal, 2009). The varietal differences have also been reported with respect to mutagen sensitivity in various crops such as *Lens culinaris* (Sharma & Sharma, 1981), *Arachi hypogyea* (Mensah & Obadoni, 2007) and sweetpotato (Shin *et al.,* 2011). The sensitivity of an organism (plant) depends upon the type of mutagen employed, plant genetic makeup, DNA amount and replication time in the initial stages beside physical factors such as moisture, oxygen pH, and temperature (Konzak *et al.,* 1965; Lagoda, 2007; Kodym *et al.,* 2012; Shu *et al.,* 2012; Bado *et al.,* 2016).

With regard to genetic advance, yield related traits (plant height, stem number, tuber number and tuber weight) were observed to be relatively higher compared to other traits. High genetic advance of the mean, high genotypic coefficient of variation along with high heritability gives important information in terms of selection advance of each parameter and therefore helps the breeder to predict the rate of improvement that can be achieved in different characters (Lynch & Walsh, 1998).

For successful genetic improvement of a crop, high heritability together with high genetic advance is helpful in assessing the nature of gene action and positive effects of selection. Gaul and Hesemann, (1966) reported that high genetic gain estimates can be obtained by comparing each generation of the mutant population or line with the best

lines selected from control population. High heritability and high genetic advance in genotype affects have also been reported by in sorghum by different authors (Veerabadhiran & Kennedy, 2001; Unche *et al.,* 2008; Nang *et al.,* 2015). Low genetic advance with moderate heritability observed tuber weight shows that its most probably governed by non-additive gene action and the presence of intra and inter allelic interactions in the appearance of such character (Falconer & Mackay, 1996).

**5.2 Assessment of potato mutants diversity based on chloroplast counts in stomatal guard cells**

The chloroplast counts distribution in M1V1 generation in the three mutant populations was found to differ for diploids, triploids and tetraploids compared with M1V2 and M1V3 generations. This can be attributed to damage or shock absorbed by the cells after irradiation or confounding effects which include reliance on dominant or hemizygous alleles in the first generation. Phenotypic observations at first generation can be influenced by environmental conditions, epigenetic variation and the presence of genotypic heterogeneity or chimerism after mutagenesis (Owoseni *et al.,* 2006; Wang *et al.,* 2007).

The results from the box and whisker plots showed that the chloroplast number in the stomatal guard cells ranged from diploids ($2n = 2x = 24$) through triploids ($2n = 3x = 36$) to tetraploids ($2n = 4x = 48$) in the different potato mutants in all the generations and could be attributed to mutation effects. At M1V3 generation, the average chloroplast number counts showed that the mutant populations were tetraploids ($2n = 4x = 48$). These observations were in agreement with Ugent, (1970) who reported that cultivated potatoes have frequent genetic exchange among ploidy levels, a high

incidence of interspecific hybridization and a high level of diversity among cultivars. Other studies have shown consistency with the present findings that cultivated potato is an autotetraploid ($2n = 4x = 48$) species (Bradshaw & Mackay, 1994; Sleper & Poehlman, 2006). Jaskani and Khan, (2000) in potato and Usman *et al.,* (2012) in grapefruit reported that the number of stomatal guard cells was directly proportional to ploidy level of the plants.

The three potato mutant populations showed increasing trends in terms of ploidy level from diploids ($2n = 2x = 24$) to tetraploids ($2n = 4x = 48$) level from M1V1 to M1V3 generations. This is due to the fact that the M1V1generation were directly obtained from irradiation which shocked the cells affecting cell size as well as regeneration. At M1V3 generation, the cells had regenerated and regained their size to maintain the tetraploid nature of potatoes. Parry *et al.,* (2009) reported that high polyploidy species have a high tolerance to mutations and are less likely to show a phenotypic change due to complementation of important genes by homeologus copies. Similar studies have also confirmed that polyploids show relative increased resistance to irradiation by creating a genetic buffering effect (Kodym *et al.,* 2012; Bado *et al.,* 2016).

The cluster analysis showed that mutants were distributed randomly in different clusters or sub-clusters not based on the proximity to their dose rates. This suggests that induced mutation which is a random phenomenon might have occurred resulting in new genetic variations within crop varieties. A similar explanation has been given by Ahloowalia *et al.,* (2004) and Amenorpe, (2010) in cassava.

**5.3 Screening of the potato mutants populations against bacterial wilt disease**

This study revealed significant differences in days to onset of wilting (DTOW), area under disease progress curve (AUDPC) and percentage of symptomatic tubers of total tuber number per ha (PSTTN) in all the three potato mutant populations used. This is could be because these traits are scored directly from aerial parts and infected tubers infected with bacterial wilt disease. Similar trends have been reported by French, (1994); Rotich *et al.,* (2010) and Muthoni *et al.,* (2014) for potato cultivars.

The Asante, Kenya Mpya and Kenya Sherekea potato mutant populations displayed diverse resistance to bacterial wilt. The variation within each set of the potato mutants could be attributed the use of different dose rates and the reaction of each variety to the mutagen used. Since mutation is a random process, its effects are gigantic (Mba, 2013). Potatoes with a broad genetic background for both bacterial wilt resistance and adaptation have a tendency to exhibit a higher level of resistance and can be extra stable over environments (Tung *et al.,* 1993).

Bacterial wilt incidence responded variably within the potato mutant populations in number of days after planting. The potato mutant populations with bacterial wilt incidence were observed to be significantly different at fourty days (Asante and Kenya Mpya mutants) and eighty four days (Asante and Kenya Sherekea mutants) after planting. This could be owed to the fact that resistance to bacterial wilt is dependent on the genotypes effects and the disease progress and development. Previous studies suggest that the expression resistance to bacterial wilt in potatoes is very complex and unstable in nature being attributed to high genetic variability of *R. solanacearum* strains and possibly to greater extent interaction between genes for resistance and those for

adaptation (Schmiediche, 1985; Kloos & Fernandez, 1986; Tung *et al.,* 1990; 1992b; 1993). In addition to the genotype effects, the observed differences could also be attributed to the changes in environmental conditions which can variably affect the entry, survival and development of the pathogen in the plant (van Elsas *et al.,* 2000).

Correlation analysis among most agronomic and bacterial wilt resistant traits was not consistent among the different potato mutant populations. This could be because the potato mutants were generated from different parental lines which might also have been influenced by the environmental conditions and induced mutation effects. Similar findings have been reported in the correlation between latent infection and all the other traits were not consistent (Muthoni *et al.,* 2014). Jill *et al.,* (2004) reported that *R. solanacearum* expresses different sets of genes during latent infection and during symptomatic disease development. Other studies have shown that plant susceptibility to bacterial wilt tuber latent infection and above ground are not correlated because the potential of clone‘s latent infection does not depend only on bacterial wilt incidence but also on other factors such as environmental conditions (soil texture, humidity and temperatures) (Priou *et al.,* 2001; CIP, 2007).

The overall ranking of the three potato mutant populations with respect to selected agronomic and bacterial wilt traits showed that the best five mutants in Asante were from 15 Gy, Kenya Sherekea between 10 to 15 Gy while Kenya Mpya varied between 5 to 10 Gy. This suggests that potato mutants developed at dosages between 5 to 15 Gy could possibly result in giving better chances of obtaining potato with bacterial wilt resistance. Low dosage treatments (1 to 15 Gy) of gamma rays have been observed to stimulate growth attributed by increased cell division, and are genotype dependant (Al-

Safadi and Arabi, 2003; Bado *et al.,* 2016) which could have an effect on any plant traits. Previous ranking of potato genotypes screened against bacterial wilt disease have been reported by Harahagazwe and Nzoyihera, (2000); Ateka *et al.,* (2001); Rotich *et al.,* (2010) and Muthoni *et al.,* (2014).

The dendrogram generated based on the selected parameters (agronomic and bacterial wilt) did not group the potato mutants into different bacterial wilt resistant groups. The clustering pattern of the mutants revealed that mutants/lines originating from the same parents did not form a single cluster because of direct selection pressure and the random occurrence of the mutation induction. This is probably because bacterial wilt resistance is very unstable and complex due to strong host-pathogen-environment interactions being involved (Tung *et al.*, 1992; 1993; Muthoni *et al.,* 2014).

**5.4 Determining the genetic variability of potato mutants using SSR markers**

The SSRs studies showed there was significant genetic variability among the 160 potato mutants and 3 parents studied generating a total number of 211 alleles with a range of 6 to 19 alleles per marker. This could be due to the fact that microsatellites are often valuable for only directly related germplasm sources and some significant distortion in genetic similarity estimates can arise due to amplification of moderately divergent cross species (Peakall *et al.,* 1998). In addition, differences in laboratory procedures may have also led to the reason for the diverse results reported by these studies is mainly associated with the origin and sources of potato collections or genotypes studied (Di *et al.,* 2006), as well as the marker type applied and the appropriate platform used for resolution of amplified products (Tyagi *et al.,* 2014). These results are within the range of several studies in potato previously reported by Kassa, (2017); Biniam *et al.,* (2016);

Muhinyuza *et al.,* (2014); Liao and Guo, (2014); Muthoni *et al.,* (2014); Tiwari *et al.* (2013); Favoretto *et al.,* (2011) and Rocha *et al.,* (2010). The markers used in the present study had diverse allele numbers compared to previous studies (Muthoni *et al.,* 2014; Muhinyuza *et al.,* 2015; Biniam *et al.,* 2016).

The polymorphic information content (PIC) values were moderately high with an average 0.69 in most of the SSR markers used in this study. The individual potato mutant populations (Asante (0.24), Kenya Mpya (0.19) and Kenya Sherekea (0.18) generated low average PIC values. The results observed in the present study could be due to the fact that the potato mutant populations were generated from gamma (induced) irradiation from three parental genotypes and each mutant population are directly related to their parents. Furthermore, it could be due to the fact that SSRs are generally useful only for closely related germplasm sources and the effects of mutation as well as the amplification of moderately divergent cross species can bring about significant distortion in genetic similarity estimates (Peakall *et al.,* 1998). Similarly, high PIC values have been reported by several authors in potatoes; (Muthoni *et al.,* (2014) ranged from 0.839 to 0.208 with an average of 0.649; Biniam *et al.,* (2016) reported a mean of 0.87 with a range of 0.51 to 0.98; Wang *et al.* (2017) also reported a range from 0.9857 to 0.9897 with the average value as 0.9871; Kassa, (2017) reported a range of 0.57 to 0.93 with a mean value of 0.85 among others (Ghislain *et al.,* 2004; 2009; Feingold *et al.,* 2005; Rocha *et al.,* 2010).

In the present study, the average allele frequency (0.6), gene diversity (0.44) and heterozygosity (0.69) values were found to be moderate to high. The observation in this present study could be due to a result of narrow genetic base of potato mutants created

as a result of induced mutation. Liao and Guo (2014) reported low genetic diversity as explained by the genetic similarity matrix among 85 potato cultivars from Yunnan, China, studied using 24 SSR markers. Similarly, Barandalla *et al.,* (2006) also detected lower genetic diversity using 19 SSR markers to fingerprint 41 local potato cultivars from 10 locations of Tenerife Island. Kassa, (2017) reported low allele frequency in 53 conserved potato genotypes in Ethiopia using 12 SSRs markers. Other previous studies in potato populations have reported moderate to high genetic diversity using SSR markers in their individual countries; Europe (Gebhardt *et al.,* 2004), Argentina (Ispizúa *et al.,* 2007), Canada (Fu *et al.,* 2009), Turkey (Kandemir *et al.,* 2010), Kenya (Muthoni *et al.,* 2014), China (Liao & Guo, 2014), Rwanda (Muhinyuza, 2014), Eritrea (Biniam *et al.,* 2016) and Ethiopia (Kassa, 2017).

The Bayesian clustering method generated different clusters in the potato mutants with Asante $\Delta k = 5$, Kenya Mpya $\Delta k = 4$, Kenya Sherekea $\Delta k = 4$ and combined for all potato mutants $\Delta k = 6$. The results from the three individual potato mutants (Asante, Kenya Mpya and Kenya Sherekea) showed that the clustering were relatively based on the dosage rates applied that grouped the potato mutants into different $\Delta k$ populations. According to (Kodym *et al.* (2012), the susceptibility of seed and vegetatively propagated crops to physical and chemical mutagens varies between and within species. Similar studies reported that the potato genotype showed different responses with increasing doses of gamma rays progressively inhibited the growth of stem cuttings (Bado *et al.,* 2016).

The dendrogram and the principal co-ordinate analysis (PCoA) revealed three cluster groups of potato mutants with group (I) generating the largest and diverse mutants from

the three mutant populations compared to group II and III. The variation suggests that mutation which is a random phenomenon might have occurred resulting in variations within the mutants (Amenorpe, 2010). The genetic similarity and dissimilarity observed between and within the different clusters of mutant populations could possibly due to resemblance and differences in ancestry. The selection of primers is critical to the accuracy of potato cultivar identification since it affects the results depending on different primer(s) combinations employed. Liao and Guo, (2014) reported that a cultivar could be in a cluster with a few cultivars in a dendrogram, and possibly cluster with other cultivars in another dendrogram based on different primer combinations.

Analysis of Molecular Variance (AMOVA) revealed higher percent (91.4 %) genetic diversity within populations. The increased genetic variability effects within individual genotype could be as a result of induced mutation which could also reduce the diversity among populations. The high genetic diversity within individuals in the potato populations in the study could imply that the crop is primarily propagated vegetatively by tubers and mini-tubers have common alleles due to directional selection done on agronomic plant traits with true seed propagation being mainly for breeding purposes as suggested by Al-Safadi *et al.,* (2000). Other researchers have reported analogous findings; Gwandu *et al.,* (2012) (97 %) on sweet potato virus disease resistance variation, Tiwari *et al.,* (2013) (93 %) within the Indian Andigena potato core collection, Biniam *et al.,* (2016) (92 %) in 93 potato genotypes, Kassa, (2017) (96 %) in 53 conserved potato genotypes. Outcrossing crops like maize, as well as potato have reported most of the variation observed within rather than between populations (Hamrick & Godt, 1997).

The analysis of Molecular Variance (AMOVA) revealed high genetic diversity within the potato mutant populations (91.4 %) as a result of induced mutation. These results were also corroborated by principal co-ordinate analysis (PCoA) and the dendrogram which gave three clusters, however, the dendrogram analyses consisted of sub-clusters in group I which supported the STRUCTURE analysis giving six affiliate clusters. Zheng *et al.,* (2017) also reported comparable results on genetic diversity and population structure of Chinese natural bermudagrass (*Cynodon dactylon* (L.) Pers.) germplasm based on SRAP markers. Berdugo-Cely *et al.,* (2017) as well reported similar findings on genetic diversity and association mapping in the Colombian Central Collection of *Solanum tuberosum* L. Andigenum group using Single Nucleotide Polymorphisms (SNPs) markers. Similarly, Ahmadizadeh and Felenji, (2011) reported that principal co-ordinate analysis (PCoA) and cluster analysis yielded the same grouping of similar accessions.

With regard to the pattern of bacterial wilt resistance with the consensus tree analysis between the SSR marker clusters and agronomic and bacterial wilt resistant traits, it was observed that the phylogenetic tree generated using consensus tree analysis indicated that the consensus fork index *CIc* was 20 % identical (*CIc*= 0.20). This indicates that the dendrogram was far-off consistent in clustering potato mutants into bacterial wilt resistance groups. The dendrogram generated were more related to the SSR dendrogram than the agronomic and bacterial wilt traits dendrogram. The reason for this possibly could be that SSR primers used were not specifically for the selection of bacterial wilt resistance but probably measured different characteristics of genetic diversity. In addition, perhaps resistance to bacterial wilt in potatoes is very unstable and complex owing to strong host-pathogen-environment interaction (Tung *et al.,* 1990;

Tung *et al.,* 1992; Tung *et al.,* 1992; Muthoni *et al.,* 2014). Similarly, Carputo *et al.,* (2013) using UPGMA and maximum parsimony-based analyses provided evidences that potato genotypes resistant to a particular disease did not group together in the same cluster. Regardless of the discrepancies, the SSR markers generated valuable information that will assist in ascertaining parents to incorporate in the future potato breeding programme.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATION

## 6.1 CONCLUSION

This study aimed at investigating the relationship between the dosage rates and the agronomic traits of the potato mutants; It can be concluded that the optimum dosage rates are 9 Gy in Asante mutants, 15 Gy in Kenya Mpya mutants and 10 Gy in Kenya Sherekea mutants at M1V2 and M1V3 generation with respect to tuber weight.

With regard to the relationship between dosage rates and the percentage distribution of chloroplast counts in stomatal guard cells, it can be concluded that the number of chloroplast counts in stomatal guard cells increase with increasing dosage rates as the three generation are advanced.

This study also sought to investigate the resistance of potato mutants as a function of induced mutation; it can be concluded that resistance of potato to bacterial wilt can be achieved through application of mutation technique. Asante mutants irradiated at dosage rates of 15 Gy gave a better response than Mpya and Sherekea mutants to bacterial wilt disease resistance.

The SSR data was successfully used to group potato mutants into six distinct groups. The SSR marker STM5127 gave the highest number of alleles (19) and gene diversity (0.81). The UPGMA dendrogram revealed that mutants from the same parent were generally, but not entirely, clustered into the same cluster. Comparison of the UPGMA dendrogram and the Bayesian STRUCTURE analysis confirmed general agreement with six population subdivisions and the genetic relationships among accessions.

## 6.2 RECOMMENDATION

Based on the research findings of the current study, it is recommended that:

i. Selection and further evaluation of potato mutant families can be done to determine their yield potential and stability for release in various environments.

ii. Future studies to focus on determining the potato mutant's ploidy level diversity at molecular level to identify the presence of ploidy allelic variants.

iii. Further perform the bacterial wilt screening using pathogenicity test and also perform Quantitative Trait Loci (QTL) or Association mapping analyses to identify the presence or absence of gene(s) for bacterial wilt resistance for effective disease mapping.

iv. Further studies to focus on the use of reverse genetic technique by working backwards, from the genotype to the phenotype to improve application of induced mutagenesis to produce, handle and query large putative mutant populations with low frequency recessive events. Through Targeting Induced Local Lesions IN Genomes (TILLING) suitably characterized "Traditional Mutagenesis Meets Functional Genomics", permits the high throughput querying of putative mutants for point mutation events in specific genomic regions.

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# APPENDICES

**Appendix I: Principal component analysis of Asante, Kenya Mpya and Kenya Sherekea potato mutants across the 3 generations showing the contribution of qualitative traits to total variation**

| Generation/ | | | | | M | 1 | V | 1 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mutants | PCA | % Var | FCAC | GH | IAC | LOO | LACM | LGC | LPSL | SAC |
| Asante | 1 | 62.50 | 0.00 | 0.000 | -0.001 | 0.003 | 0.000 | -0.001 | 0.000 | 0.000 |
| | 2 | 13.40 | 0.00 | 0.000 | 0.000 | -0.010 | 0.000 | 0.002 | 0.000 | 0.016 |
| | 3 | 5.40 | 0.00 | 0.003 | -0.004 | 0.002 | 0.000 | 0.008 | 0.000 | 0.004 |
| Kenya Mpya | 1 | 72.00 | 0.00 | 0.003 | 0.000 | 0.000 | -0.006 | 0.000 | 0.003 | -0.009 |
| | 2 | 16.40 | 0.00 | -0.003 | 0.000 | -0.003 | 0.011 | 0.005 | -0.003 | 0.010 |
| | 3 | 4.70 | 0.00 | 0.001 | 0.000 | -0.022 | 0.043 | 0.044 | 0.001 | -0.003 |
| Kenya Sherekea | 1 | 73.24 | 0.00 | 0.003 | 0.000 | -0.001 | 0.000 | 0.000 | 0.000 | 0.017 |
| | 2 | 12.73 | 0.00 | -0.003 | 0.000 | 0.003 | 0.000 | -0.003 | -0.003 | 0.024 |
| | 3 | 5.30 | 0.00 | 0.001 | 0.000 | -0.020 | 0.000 | 0.006 | 0.006 | -0.040 |
| All mutants | 1 | 67.12 | 0.00 | 0.002 | 0.001 | -0.001 | -0.006 | 0.000 | 0.000 | 0.017 |
| | 2 | 18.41 | 0.00 | -0.002 | 0.000 | 0.003 | 0.011 | 0.001 | -0.003 | 0.024 |
| | 3 | 5.1 | 0.00 | 0.001 | -0.004 | 0.001 | 0.043 | 0.006 | 0.006 | -0.040 |
| Generation/ | | | | M | 1 | V | 2 | | | |
| Mutants | PCA | % Var | FCAC | GH | IAC | LOO | LACM | LGC | LPSL | SAC |
| Asante | 1 | 74.23 | 0.009 | 0.009 | 0.000 | 0.004 | 0.000 | 0.008 | -0.009 | 0.008 |
| | 2 | 12.54 | 0.020 | 0.009 | 0.008 | -0.079 | 0.000 | 0.032 | -0.020 | 0.044 |
| | 3 | 5.37 | -0.088 | -0.119 | 0.015 | 0.106 | 0.000 | -0.064 | 0.088 | 0.121 |
| Kenya Mpya | 1 | 72.64 | 0.009 | 0.022 | 0.000 | 0.007 | -0.059 | -0.018 | 0.022 | -0.063 |
| | 2 | 14.2 | 0.006 | 0.013 | 0.000 | 0.012 | -0.048 | -0.023 | 0.013 | -0.037 |
| | 3 | 3.92 | 0.017 | 0.039 | 0.000 | 0.012 | -0.104 | -0.031 | 0.039 | -0.111 |
| Kenya Sherekea | 1 | 70.56 | 0.000 | 0.000 | -0.005 | 0.001 | 0.000 | 0.005 | -0.005 | 0.000 |
| | 2 | 15.12 | 0.000 | 0.000 | 0.005 | -0.007 | 0.000 | -0.005 | 0.005 | 0.000 |
| | 3 | 2.38 | 0.000 | 0.000 | 0.039 | 0.007 | 0.000 | -0.039 | 0.039 | 0.000 |
| All mutants | 1 | 71.54 | 0.009 | 0.022 | 0.000 | 0.007 | -0.059 | -0.018 | 0.022 | -0.063 |
| | 2 | 13.41 | 0.006 | 0.013 | 0.000 | 0.044 | -0.048 | -0.023 | 0.013 | -0.037 |
| | 3 | 3.24 | 0.017 | 0.039 | 0.000 | 0.012 | -0.104 | -0.031 | 0.039 | -0.011 |
| Generation/ | | | | M | 1 | V | 3 | | | |
| Mutants | PCA | % Var | FCAC | GH | IAC | LOO | LACM | LGC | LPSL | SAC |
| Asante | 1 | 67.12 | 0.000 | 0.005 | -0.001 | 0.001 | 0.000 | 0.003 | -0.003 | -0.001 |
| | 2 | 18.41 | 0.000 | 0.015 | 0.010 | -0.059 | 0.000 | 0.037 | -0.029 | 0.015 |
| | 3 | 3.41 | 0.000 | 0.011 | -0.029 | 0.161 | 0.000 | -0.066 | 0.040 | -0.022 |
| Kenya Mpya | 1 | 72.41 | 0.000 | 0.000 | 0.000 | 0.000 | -0.001 | 0.000 | 0.000 | 0.000 |
| | 2 | 12.2 | -0.006 | -0.009 | 0.000 | 0.004 | 0.016 | -0.007 | -0.009 | 0.027 |
| | 3 | 4.36 | 0.003 | 0.009 | 0.000 | -0.005 | -0.006 | 0.006 | 0.009 | -0.027 |
| Kenya Sherekea | 1 | 72.54 | 0.000 | 0.000 | -0.005 | 0.001 | 0.000 | 0.005 | -0.005 | 0.000 |
| | 2 | 5.15 | 0.000 | 0.000 | 0.005 | -0.007 | 0.000 | -0.005 | 0.005 | 0.000 |
| | 3 | 0.28 | 0.000 | 0.000 | 0.039 | 0.007 | 0.000 | -0.039 | 0.039 | 0.000 |
| All mutants | 1 | 70.3 | 0.000 | 0.005 | -0.001 | 0.001 | 0.000 | 0.003 | -0.003 | -0.001 |
| | 2 | 18.41 | 0.000 | 0.015 | 0.010 | -0.059 | 0.000 | 0.037 | -0.029 | 0.015 |
| | 3 | 11.66 | 0.000 | 0.011 | -0.029 | 0.161 | 0.000 | -0.066 | 0.040 | -0.022 |

**Principal component analysis (PCA), Percentage (%), Growth habit (GH), Inflorescence anthocyanin colouration (IAC), Stem anthocyanin colouration (SAC), Leaf Outline openness (LOO), Leaf presence of secondary leaflets (LPSL), Leaf green colour (LGC), Flower corolla anthocyanin colouration (FCAC)**

**Appendix II: Principal component analysis of Asante, Kenya Mpya and Kenya Sherekea potato mutants across the 3 generations showing the contribution of quantitative traits to total variation**

| Generation/ | | | M | 1 | V | 1 | |
|---|---|---|---|---|---|---|---|
| Mutants | PCA | % Var | PH | PSN | SAC | PTN | PTW |
| Asante | 1 | 73.50 | 0.019 | 0.014 | 0.000 | 0.055 | 0.618 |
| | 2 | 16.04 | -0.598 | -0.004 | 0.016 | 0.055 | 0.016 |
| | 3 | 9.46 | -0.054 | 0.053 | 0.004 | -0.695 | 0.056 |
| Kenya Mpya | 1 | 86.00 | -0.130 | 0.007 | -0.009 | 0.045 | 0.611 |
| | 2 | 9.05 | 0.685 | 0.021 | 0.010 | 0.130 | 0.118 |
| | 3 | 4.77 | -0.120 | 0.081 | -0.003 | 0.485 | -0.061 |
| Kenya Sherekea | 1 | 74.73 | 0.000 | -0.064 | 0.017 | 0.118 | 0.611 |
| | 2 | 18.73 | 0.003 | 0.990 | 0.024 | 0.130 | 0.049 |
| | 3 | 6.51 | -0.006 | 0.124 | -0.040 | -0.980 | 0.140 |
| All mutants | 1 | 77.12 | 0.019 | 0.014 | 0.000 | 0.055 | 0.618 |
| | 2 | 14.33 | -0.598 | -0.004 | 0.016 | 0.055 | 0.016 |
| | 3 | 7.11 | -0.054 | 0.053 | 0.004 | -0.695 | 0.056 |
| Generation/ | | | M | 1 | V | 2 | |
| Mutants | PCA | % Var | PH | PSN | SAC | PTN | PTW |
| Asante | 1 | 82.01 | -0.131 | 0.055 | 0.008 | -0.112 | 0.483 |
| | 2 | 10.54 | -0.563 | -0.124 | 0.044 | 0.510 | -0.023 |
| | 3 | 5.37 | 0.783 | -0.112 | 0.121 | 0.534 | -0.173 |
| Kenya Mpya | 1 | 81.44 | -0.143 | -0.011 | -0.063 | 0.128 | 0.768 |
| | 2 | 17.23 | 0.696 | -0.025 | -0.037 | -0.689 | 0.185 |
| | 3 | 1.29 | 0.687 | 0.039 | -0.111 | 0.706 | -0.008 |
| Kenya Sherekea | 1 | 80.33 | 0.018 | 0.006 | 0.000 | 0.039 | 0.499 |
| | 2 | 12.32 | 0.431 | -0.089 | 0.000 | -0.598 | 0.028 |
| | 3 | 3.33 | -0.900 | -0.026 | 0.000 | -0.428 | 0.034 |
| All mutants | 1 | 78.56 | -0.143 | -0.011 | -0.063 | 0.128 | 0.768 |
| | 2 | 11.15 | 0.696 | -0.025 | -0.037 | -0.689 | 0.185 |
| | 3 | 4.28 | 0.687 | 0.039 | -0.111 | 0.706 | -0.008 |
| Generation/ | | | M | 1 | V | 3 | |
| Mutants | PCA | % Var | PH | PSN | SAC | PTN | PTW |
| Asante | 1 | 84.67 | 0.141 | 0.014 | -0.001 | 0.023 | 0.696 |
| | 2 | 11.21 | 0.982 | 0.043 | 0.015 | 0.083 | -0.143 |
| | 3 | 0.11 | 0.086 | 0.230 | -0.022 | -0.521 | 0.007 |
| Kenya Mpya | 1 | 76.41 | 0.004 | -0.002 | 0.000 | 0.030 | 0.991 |
| | 2 | 18.2 | 0.594 | 0.012 | 0.027 | -0.516 | 0.012 |
| | 3 | 1.36 | -0.515 | 0.007 | -0.027 | -0.562 | 0.028 |
| Kenya Sherekea | 1 | 78.68 | 0.018 | 0.006 | 0.000 | 0.039 | 0.699 |
| | 2 | 9.87 | 0.431 | -0.089 | 0.000 | -0.898 | 0.028 |
| | 3 | 2.11 | -0.900 | -0.026 | 0.000 | -0.428 | 0.034 |
| All mutants | 1 | 74.56 | 0.004 | -0.002 | 0.000 | 0.030 | 0.991 |
| | 2 | 9.15 | 0.464 | 0.012 | 0.029 | -0.516 | 0.012 |
| | 3 | 4.28 | -0.066 | 0.007 | -0.027 | -0.562 | 0.028 |

**Principal component analysis (PCA), Percentage (%), Plant height (PH), Plant stem number (PSN), Plant Tuber number (PTN), Plant Tuber weight (PTW).**

**Appendix III: Asante potato mutants ranked based on some agronomic and bacterial wilt resistance parameters**

| Clones | DTOW | AUDPC | TTN | TTW | PSTTN | PSTTW | PWTTW |
|---|---|---|---|---|---|---|---|
| **A1** | 11.0 | 32.0 | 51.0 | 13.0 | 21.0 | 4.0 | 70.0 |
| **A10** | 49.0 | 71.0 | 65.0 | 65.0 | 56.0 | 20.0 | 28.0 |
| **A11** | 68.0 | 65.0 | 72.0 | 68.0 | 41.0 | 6.0 | 21.0 |
| **A12** | 7.0 | 12.0 | 47.0 | 41.0 | 14.0 | 17.0 | 29.0 |
| **A13** | 33.0 | 32.0 | 39.0 | 56.0 | 27.0 | 54.0 | 13.0 |
| **A14** | 49.0 | 40.0 | 63.0 | 37.0 | 69.0 | 44.0 | 54.0 |
| **A15** | 33.0 | 53.0 | 28.0 | 20.0 | 5.0 | 2.0 | 51.0 |
| **A16** | 49.0 | 4.0 | 70.0 | 73.0 | 72.0 | 65.0 | 21.0 |
| **A17** | 64.0 | 70.0 | 58.0 | 22.0 | 55.0 | 35.0 | 8.0 |
| **A18** | 43.0 | 8.0 | 26.0 | 15.0 | 66.0 | 52.0 | 57.0 |
| **A19** | 68.0 | 60.0 | 57.0 | 69.0 | 38.0 | 48.0 | 2.0 |
| **A2** | 43.0 | 65.0 | 28.0 | 61.0 | 49.0 | 65.0 | 17.0 |
| **A20** | 43.0 | 8.0 | 34.0 | 30.0 | 53.0 | 62.0 | 23.0 |
| **A21** | 68.0 | 40.0 | 46.0 | 39.0 | 63.0 | 59.0 | 72.0 |
| **A22** | 25.0 | 40.0 | 68.0 | 42.0 | 65.0 | 52.0 | 65.0 |
| **A23** | 7.0 | 16.0 | 44.0 | 42.0 | 43.0 | 44.0 | 59.0 |
| **A24** | 24.0 | 60.0 | 61.0 | 26.0 | 38.0 | 10.0 | 67.0 |
| **A25** | 33.0 | 12.0 | 24.0 | 12.0 | 25.0 | 23.0 | 37.0 |
| **A26** | 33.0 | 53.0 | 66.0 | 62.0 | 17.0 | 10.0 | 42.0 |
| **A27** | 64.0 | 12.0 | 55.0 | 60.0 | 67.0 | 69.0 | 66.0 |
| **A28** | 49.0 | 65.0 | 34.0 | 53.0 | 12.0 | 43.0 | 18.0 |
| **A29** | 25.0 | 16.0 | 33.0 | 14.0 | 60.0 | 37.0 | 36.0 |
| **A3** | 11.0 | 8.0 | 17.0 | 54.0 | 58.0 | 49.0 | 49.0 |
| **A30** | 18.0 | 40.0 | 15.0 | 18.0 | 59.0 | 70.0 | 24.0 |
| **A31** | 58.0 | 16.0 | 60.0 | 31.0 | 62.0 | 72.0 | 34.0 |
| **A32** | 43.0 | 71.0 | 1.0 | 5.0 | 68.0 | 73.0 | 31.0 |
| **A33** | 11.0 | 16.0 | 73.0 | 66.0 | 48.0 | 60.0 | 33.0 |
| **A34** | 4.0 | 8.0 | 58.0 | 35.0 | 70.0 | 63.0 | 45.0 |
| **A35** | 33.0 | 27.0 | 37.0 | 34.0 | 28.0 | 27.0 | 60.0 |
| **A36** | 49.0 | 27.0 | 22.0 | 10.0 | 8.0 | 8.0 | 47.0 |
| **A37** | 11.0 | 53.0 | 43.0 | 33.0 | 22.0 | 24.0 | 30.0 |
| **A38** | 68.0 | 27.0 | 71.0 | 72.0 | 23.0 | 47.0 | 60.0 |
| **A40** | 7.0 | 27.0 | 10.0 | 11.0 | 2.0 | 1.0 | 52.0 |
| **A4** | 49.0 | 32.0 | 68.0 | 70.0 | 73.0 | 67.0 | 6.0 |
| **A39** | 49.0 | 32.0 | 28.0 | 19.0 | 50.0 | 9.0 | 55.0 |
| **A41** | 25.0 | 27.0 | 49.0 | 45.0 | 52.0 | 68.0 | 63.0 |
| **A42** | 25.0 | 32.0 | 24.0 | 57.0 | 17.0 | 40.0 | 53.0 |

**DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha-1); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t $ha^{-1}$); TTN=Total tuber number per ha; TTW= Total tuber weight (t/ha).**

**Asante potato mutants ranked based on some agronomic and bacterial wilt resistance parameters CONTINUED……..**

| Clones | DTOW | AUDPC | TTN | TTW | PSTTN | PSTTW | PWTTW |
|---|---|---|---|---|---|---|---|
| **A43** | 58.0 | 40.0 | 12.0 | 29.0 | 6.0 | 14.0 | 27.0 |
| **A44** | 25.0 | 16.0 | 53.0 | 17.0 | 31.0 | 16.0 | 10.0 |
| **A45** | 18.0 | 53.0 | 18.0 | 67.0 | 29.0 | 71.0 | 1.0 |
| **A46** | 25.0 | 32.0 | 19.0 | 28.0 | 64.0 | 49.0 | 47.0 |
| **A47** | 58.0 | 40.0 | 52.0 | 49.0 | 34.0 | 37.0 | 9.0 |
| **A48** | 33.0 | 40.0 | 47.0 | 64.0 | 32.0 | 22.0 | 26.0 |
| **A49** | 49.0 | 16.0 | 28.0 | 51.0 | 25.0 | 19.0 | 20.0 |
| **A5** | 43.0 | 40.0 | 19.0 | 48.0 | 54.0 | 35.0 | 5.0 |
| **A50** | 64.0 | 60.0 | 4.0 | 2.0 | 7.0 | 5.0 | 19.0 |
| **A51** | 25.0 | 16.0 | 55.0 | 49.0 | 47.0 | 63.0 | 14.0 |
| **A52** | 18.0 | 32.0 | 26.0 | 6.0 | 37.0 | 30.0 | 39.0 |
| **A53** | 64.0 | 65.0 | 54.0 | 38.0 | 13.0 | 6.0 | 4.0 |
| **A54** | 11.0 | 32.0 | 11.0 | 21.0 | 35.0 | 54.0 | 55.0 |
| **A55** | 4.0 | 12.0 | 22.0 | 7.0 | 41.0 | 32.0 | 58.0 |
| **A56** | 11.0 | 53.0 | 19.0 | 32.0 | 3.0 | 13.0 | 43.0 |
| **A57** | 1.0 | 2.0 | 5.0 | 9.0 | 11.0 | 28.0 | 16.0 |
| **A58** | 18.0 | 40.0 | 3.0 | 8.0 | 9.0 | 25.0 | 50.0 |
| **A59** | 3.0 | 7.0 | 14.0 | 47.0 | 9.0 | 32.0 | 7.0 |
| **A6** | 58.0 | 53.0 | 38.0 | 57.0 | 57.0 | 46.0 | 71.0 |
| **A60** | 33.0 | 16.0 | 6.0 | 4.0 | 24.0 | 41.0 | 67.0 |
| **A61** | 43.0 | 71.0 | 9.0 | 27.0 | 20.0 | 60.0 | 25.0 |
| **A62** | 7.0 | 16.0 | 34.0 | 44.0 | 45.0 | 57.0 | 69.0 |
| **A63** | 2.0 | 4.0 | 28.0 | 22.0 | 30.0 | 32.0 | 44.0 |
| **A64** | 33.0 | 60.0 | 66.0 | 71.0 | 61.0 | 56.0 | 32.0 |
| **A65** | 18.0 | 16.0 | 61.0 | 63.0 | 51.0 | 49.0 | 15.0 |
| **A66** | 33.0 | 40.0 | 41.0 | 24.0 | 15.0 | 21.0 | 40.0 |
| **A67** | 18.0 | 3.0 | 2.0 | 1.0 | 1.0 | 3.0 | 40.0 |
| **A68** | 11.0 | 40.0 | 6.0 | 16.0 | 46.0 | 42.0 | 12.0 |
| **A69** | 33.0 | 16.0 | 64.0 | 59.0 | 71.0 | 57.0 | 64.0 |
| **A7** | 58.0 | 60.0 | 13.0 | 25.0 | 40.0 | 14.0 | 35.0 |
| **A70** | 73.0 | 1.0 | 16.0 | 52.0 | 35.0 | 30.0 | 72.0 |
| **A71** | 25.0 | 40.0 | 8.0 | 3.0 | 44.0 | 29.0 | 45.0 |
| **A72** | 4.0 | 4.0 | 41.0 | 39.0 | 32.0 | 39.0 | 37.0 |
| **A8** | 58.0 | 53.0 | 50.0 | 36.0 | 16.0 | 17.0 | 62.0 |
| **A9** | 68.0 | 65.0 | 44.0 | 55.0 | 3.0 | 12.0 | 10.0 |
| **AP** | 49.0 | 40.0 | 40.0 | 46.0 | 19.0 | 26.0 | 3.0 |

**DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha-1); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t $ha^{-1}$); TTN=Total tuber number per ha; TTW= Total tuber weight (t/ha).**

**Appendix IV: Kenya Mpya potato mutants ranked based on some agronomic and bacterial wilt resistance parameters**

| Clones | DTOW | AUDPC | TTN | TTW | PSTTN | PSTTW | PWTTW |
|---|---|---|---|---|---|---|---|
| M1 | 11.0 | 18.0 | 12.0 | 4.0 | 6.0 | 5.0 | 31.0 |
| M10 | 17.0 | 31.0 | 18.0 | 15.0 | 10.0 | 24.0 | 18.0 |
| M11 | 17.0 | 35.0 | 13.0 | 20.0 | 19.0 | 4.0 | 34.0 |
| M12 | 8.0 | 6.0 | 22.0 | 40.0 | 40.0 | 43.0 | 12.0 |
| M13 | 30.0 | 35.0 | 26.0 | 22.0 | 3.0 | 3.0 | 43.0 |
| M14 | 38.0 | 18.0 | 21.0 | 6.0 | 35.0 | 26.0 | 2.0 |
| M15 | 27.0 | 2.0 | 32.0 | 12.0 | 26.0 | 10.0 | 25.0 |
| M16 | 30.0 | 18.0 | 15.0 | 23.0 | 22.0 | 39.0 | 26.0 |
| M17 | 42.0 | 12.0 | 41.0 | 39.0 | 8.0 | 32.0 | 37.0 |
| M18 | 30.0 | 31.0 | 24.0 | 23.0 | 15.0 | 6.0 | 41.0 |
| M19 | 5.0 | 8.0 | 10.0 | 29.0 | 42.0 | 26.0 | 5.0 |
| M2 | 38.0 | 42.0 | 25.0 | 28.0 | 7.0 | 14.0 | 7.0 |
| M20 | 11.0 | 8.0 | 35.0 | 19.0 | 32.0 | 15.0 | 20.0 |
| M21 | 5.0 | 12.0 | 33.0 | 31.0 | 23.0 | 32.0 | 7.0 |
| M22 | 42.0 | 18.0 | 30.0 | 21.0 | 37.0 | 31.0 | 37.0 |
| M23 | 30.0 | 31.0 | 40.0 | 27.0 | 39.0 | 39.0 | 42.0 |
| M24 | 30.0 | 35.0 | 6.0 | 35.0 | 1.0 | 12.0 | 4.0 |
| M25 | 23.0 | 18.0 | 4.0 | 8.0 | 2.0 | 9.0 | 19.0 |
| M26 | 8.0 | 18.0 | 34.0 | 43.0 | 28.0 | 26.0 | 15.0 |
| M27 | 11.0 | 25.0 | 43.0 | 41.0 | 43.0 | 41.0 | 1.0 |
| M28 | 38.0 | 25.0 | 36.0 | 36.0 | 20.0 | 15.0 | 35.0 |
| M29 | 11.0 | 25.0 | 39.0 | 34.0 | 38.0 | 36.0 | 35.0 |
| M3 | 23.0 | 12.0 | 8.0 | 9.0 | 27.0 | 38.0 | 15.0 |
| M30 | 4.0 | 8.0 | 5.0 | 3.0 | 12.0 | 19.0 | 27.0 |
| M31 | 17.0 | 25.0 | 17.0 | 18.0 | 15.0 | 20.0 | 10.0 |
| M32 | 27.0 | 18.0 | 19.0 | 32.0 | 14.0 | 29.0 | 6.0 |
| M33 | 23.0 | 35.0 | 22.0 | 36.0 | 11.0 | 6.0 | 32.0 |
| M34 | 8.0 | 6.0 | 28.0 | 33.0 | 33.0 | 23.0 | 28.0 |
| M35 | 17.0 | 12.0 | 27.0 | 26.0 | 21.0 | 17.0 | 30.0 |
| M36 | 2.0 | 2.0 | 42.0 | 42.0 | 9.0 | 21.0 | 9.0 |
| M37 | 30.0 | 25.0 | 20.0 | 30.0 | 30.0 | 42.0 | 29.0 |
| M38 | 29.0 | 35.0 | 10.0 | 7.0 | 24.0 | 35.0 | 33.0 |
| M39 | 17.0 | 25.0 | 2.0 | 1.0 | 3.0 | 2.0 | 22.0 |
| M4 | 1.0 | 1.0 | 9.0 | 10.0 | 18.0 | 25.0 | 22.0 |
| M40 | 30.0 | 40.0 | 7.0 | 14.0 | 41.0 | 11.0 | 10.0 |
| M41 | 11.0 | 12.0 | 38.0 | 38.0 | 29.0 | 22.0 | 17.0 |
| M42 | 38.0 | 8.0 | 16.0 | 12.0 | 36.0 | 18.0 | 13.0 |
| M5 | 11.0 | 40.0 | 30.0 | 5.0 | 13.0 | 6.0 | 40.0 |
| M6 | 5.0 | 12.0 | 14.0 | 17.0 | 5.0 | 1.0 | 3.0 |
| M7 | 30.0 | 42.0 | 3.0 | 11.0 | 31.0 | 36.0 | 39.0 |
| M8 | 17.0 | 2.0 | 29.0 | 16.0 | 17.0 | 12.0 | 24.0 |
| M9 | 23.0 | 31.0 | 36.0 | 25.0 | 34.0 | 34.0 | 20.0 |
| MP | 2.0 | 2.0 | 1.0 | 2.0 | 25.0 | 30.0 | 14.0 |

**DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha-1); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t $ha^{-1}$); TTN=Total tuber number per ha; TTW= Total tuber weight (t/ha).**

**Appendix V: Kenya Sherekea potato mutants ranked based on some agronomic and bacterial wilt resistance parameters**

| Clones | DTOW | AUDPC | TTN | TTW | PSTTN | PSTTW | PWTTW |
|---|---|---|---|---|---|---|---|
| **S1** | 2.0 | 31.0 | 8.0 | 22.0 | 31.0 | 6.0 | 1.0 |
| **S10** | 21.0 | 12.0 | 17.0 | 8.0 | 30.0 | 29.0 | 18.0 |
| **S11** | 11.0 | 24.0 | 43.0 | 39.0 | 39.0 | 33.0 | 10.0 |
| **S12** | 25.0 | 24.0 | 35.0 | 33.0 | 11.0 | 13.0 | 42.0 |
| **S13** | 8.0 | 7.0 | 25.0 | 30.0 | 32.0 | 46.0 | 47.0 |
| **S14** | 29.0 | 4.0 | 4.0 | 12.0 | 5.0 | 19.0 | 3.0 |
| **S15** | 2.0 | 2.0 | 15.0 | 28.0 | 47.0 | 40.0 | 33.0 |
| **S16** | 36.0 | 46.0 | 23.0 | 40.0 | 13.0 | 20.0 | 5.0 |
| **S17** | 5.0 | 7.0 | 42.0 | 42.0 | 35.0 | 43.0 | 5.0 |
| **S18** | 21.0 | 12.0 | 43.0 | 29.0 | 43.0 | 45.0 | 14.0 |
| **S19** | 43.0 | 35.0 | 19.0 | 16.0 | 3.0 | 3.0 | 29.0 |
| **S2** | 43.0 | 38.0 | 41.0 | 41.0 | 41.0 | 25.0 | 35.0 |
| **S20** | 11.0 | 12.0 | 1.0 | 1.0 | 8.0 | 9.0 | 2.0 |
| **S21** | 11.0 | 12.0 | 2.0 | 3.0 | 21.0 | 28.0 | 9.0 |
| **S22** | 11.0 | 12.0 | 8.0 | 6.0 | 35.0 | 17.0 | 36.0 |
| **S23** | 36.0 | 12.0 | 12.0 | 23.0 | 17.0 | 36.0 | 13.0 |
| **S24** | 29.0 | 38.0 | 16.0 | 18.0 | 16.0 | 15.0 | 38.0 |
| **S25** | 11.0 | 24.0 | 7.0 | 43.0 | 40.0 | 24.0 | 11.0 |
| **S26** | 25.0 | 31.0 | 37.0 | 26.0 | 29.0 | 38.0 | 15.0 |
| **S27** | 29.0 | 35.0 | 20.0 | 10.0 | 1.0 | 1.0 | 42.0 |
| **S28** | 29.0 | 12.0 | 17.0 | 20.0 | 7.0 | 20.0 | 16.0 |
| **S29** | 36.0 | 12.0 | 2.0 | 5.0 | 3.0 | 2.0 | 27.0 |
| **S3** | 29.0 | 24.0 | 31.0 | 25.0 | 9.0 | 5.0 | 12.0 |
| **S30** | 36.0 | 38.0 | 13.0 | 34.0 | 15.0 | 15.0 | 44.0 |
| **S31** | 4.0 | 4.0 | 40.0 | 7.0 | 27.0 | 25.0 | 8.0 |
| **S32** | 21.0 | 45.0 | 45.0 | 46.0 | 20.0 | 29.0 | 31.0 |
| **S33** | 8.0 | 31.0 | 36.0 | 44.0 | 32.0 | 23.0 | 29.0 |
| **S34** | 1.0 | 2.0 | 29.0 | 15.0 | 6.0 | 20.0 | 24.0 |
| **S35** | 11.0 | 4.0 | 21.0 | 37.0 | 2.0 | 29.0 | 22.0 |
| **S36** | 5.0 | 7.0 | 30.0 | 35.0 | 25.0 | 41.0 | 24.0 |
| **S37** | 29.0 | 38.0 | 14.0 | 17.0 | 22.0 | 7.0 | 37.0 |
| **S38** | 45.0 | 12.0 | 33.0 | 31.0 | 9.0 | 36.0 | 28.0 |
| **S39** | 29.0 | 35.0 | 46.0 | 4.0 | 26.0 | 44.0 | 21.0 |
| **S4** | 46.0 | 24.0 | 37.0 | 37.0 | 45.0 | 11.0 | 19.0 |
| **S40** | 25.0 | 12.0 | 31.0 | 21.0 | 22.0 | 39.0 | 4.0 |
| **S41** | 21.0 | 38.0 | 47.0 | 2.0 | 24.0 | 18.0 | 45.0 |
| **S42** | 11.0 | 7.0 | 39.0 | 47.0 | 17.0 | 4.0 | 39.0 |
| **S43** | 11.0 | 12.0 | 6.0 | 9.0 | 27.0 | 13.0 | 22.0 |
| **S44** | 5.0 | 24.0 | 25.0 | 24.0 | 38.0 | 11.0 | 41.0 |
| **S45** | 8.0 | 31.0 | 8.0 | 27.0 | 19.0 | 25.0 | 26.0 |
| **S46** | 11.0 | 12.0 | 8.0 | 45.0 | 42.0 | 33.0 | 20.0 |
| **S5** | 11.0 | 38.0 | 25.0 | 36.0 | 34.0 | 42.0 | 34.0 |
| **S6** | 25.0 | 7.0 | 33.0 | 19.0 | 46.0 | 32.0 | 32.0 |
| **S7** | 46.0 | 1.0 | 24.0 | 13.0 | 37.0 | 35.0 | 46.0 |
| **S8** | 36.0 | 46.0 | 5.0 | 11.0 | 14.0 | 9.0 | 7.0 |
| **S9** | 36.0 | 38.0 | 25.0 | 14.0 | 44.0 | 47.0 | 40.0 |
| **SP** | 36.0 | 24.0 | 22.0 | 32.0 | 11.0 | 8.0 | 16.0 |

**DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha-1); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t $ha^{-1}$); TTN=Total tuber number per ha; TTW= Total tuber weight (t/ha).**

**Appendix VI: The summary of statistical analysis regarding genetic diversity of Asante potato mutant clones based on 20 microsatellite loci**

| Marker | Allele Frequency | Allele Number | Gene Diversity | Heterozygosity | PIC |
|---|---|---|---|---|---|
| **STG0016** | 0.89 | 4 | 0.20 | 0.03 | 0.20 |
| **STM5114** | 0.51 | 2 | 0.01 | 0.04 | 0.14 |
| **STM1053** | 0.92 | 3 | 0.15 | 0.06 | 0.15 |
| **STI0012** | 0.93 | 2 | 0.13 | 0.05 | 0.12 |
| **STI0032** | 0.58 | 3 | 0.10 | 0.01 | 0.06 |
| **STI0004** | 0.93 | 5 | 0.13 | 0.10 | 0.12 |
| **STM0031** | 0.66 | 3 | 0.05 | 0.10 | 0.05 |
| **STM1104** | 0.63 | 3 | 0.02 | 0.04 | 0.36 |
| **STM1052** | 0.42 | 2 | 0.01 | 0.08 | 0.24 |
| **STM1106** | 0.90 | 3 | 0.18 | 0.02 | 0.16 |
| **STM0037** | 0.45 | 4 | 0.11 | 0.07 | 0.34 |
| **STI0030** | 0.45 | 5 | 0.60 | 0.01 | 0.51 |
| **STM2013** | 0.92 | 2 | 0.15 | 0.03 | 0.14 |
| **STM1049** | 0.32 | 6 | 0.74 | 0.26 | 0.70 |
| **STM5127** | 0.41 | 7 | 0.71 | 0.24 | 0.66 |
| **STI046** | 0.34 | 4 | 0.24 | 0.04 | 0.02 |
| **STI0036** | 0.99 | 2 | 0.03 | 0.01 | 0.03 |
| **STI0023** | 0.53 | 5 | 0.64 | 0.02 | 0.60 |
| **STWAX-2** | 0.97 | 2 | 0.05 | 0.04 | 0.05 |
| **STPoAc58** | 0.95 | 2 | 0.10 | 0.02 | 0.10 |
| **Mean** | 0.68 | 3 | 0.22 | 0.06 | 0.24 |

**Appendix VII: The summary of statistical analysis regarding genetic diversity of Kenya Mpya potato mutant clones based on 20 microsatellite loci**

| Marker | Allele Frequency | Allele Number | Gene Diversity | Heterozygosity | PIC |
|---|---|---|---|---|---|
| **STG0016** | 0.80 | 3 | 0.34 | 0.02 | 0.32 |
| **STM5114** | 0.77 | 4 | 0.38 | 0.06 | 0.33 |
| **STM1053** | 0.96 | 1 | 0.01 | 0.01 | 0.01 |
| **STI0012** | 0.95 | 2 | 0.09 | 0.02 | 0.08 |
| **STI0032** | 0.91 | 4 | 0.08 | 0.20 | 0.04 |
| **STI0004** | 0.94 | 4 | 0.05 | 0.34 | 0.10 |
| **STM0031** | 0.95 | 3 | 0.09 | 0.07 | 0.08 |
| **STM1104** | 0.87 | 4 | 0.02 | 0.24 | 0.04 |
| **STM1052** | 0.54 | 4 | 4.00 | 0.08 | 0.06 |
| **STM1106** | 0.86 | 1 | 0.32 | 0.36 | 0.05 |
| **STM0037** | 0.92 | 3 | 0.20 | 0.10 | 0.20 |
| **STI0030** | 0.82 | 4 | 0.24 | 0.03 | 0.01 |
| **STM2013** | 0.72 | 4 | 0.24 | 0.04 | 0.10 |
| **STM1049** | 0.28 | 5 | 0.78 | 0.06 | 0.50 |
| **STM5127** | 0.21 | 7 | 0.84 | 0.01 | 0.82 |
| **STI046** | 0.67 | 5 | 0.44 | 0.08 | 0.34 |
| **STI0036** | 0.98 | 5 | 0.05 | 0.10 | 0.04 |
| **STI0023** | 0.56 | 5 | 0.62 | 0.30 | 0.58 |
| **STWAX-2** | 0.98 | 2 | 0.05 | 0.01 | 0.04 |
| **STPoAc58** | 0.95 | 5 | 0.09 | 0.08 | 0.08 |
| **Mean** | 0.78 | 4 | 0.45 | 0.11 | 0.19 |

**Appendix VIII: The summary of statistical analysis regarding genetic diversity of Kenya Sherekea potato mutant clones based on 20 microsatellite loci**

| Marker | Allele Frequency | Allele Number | Gene Diversity | Heterozygosity | PIC |
|---|---|---|---|---|---|
| **STG0016** | 0.86 | 5 | 0.25 | 0.06 | 0.25 |
| **STM5114** | 0.81 | 3 | 0.01 | 0.03 | 0.01 |
| **STM1053** | 0.96 | 3 | 0.08 | 0.24 | 0.08 |
| **STI0012** | 0.89 | 5 | 0.20 | 0.14 | 0.19 |
| **STI0032** | 0.94 | 3 | 0.21 | 0.42 | 0.12 |
| **STI0004** | 0.96 | 3 | 0.02 | 0.01 | 0.01 |
| **STM0031** | 0.87 | 2 | 0.16 | 0.42 | 0.04 |
| **STM1104** | 0.34 | 4 | 0.42 | 0.21 | 0.04 |
| **STM1052** | 0.92 | 5 | 0.23 | 0.06 | 0.01 |
| **STM1106** | 0.87 | 2 | 0.04 | 0.02 | 0.04 |
| **STM0037** | 0.91 | 2 | 0.16 | 0.18 | 0.14 |
| **STI0030** | 0.96 | 3 | 0.08 | 0.04 | 0.08 |
| **STM2013** | 0.98 | 2 | 0.04 | 0.47 | 0.04 |
| **STM1049** | 0.28 | 6 | 0.78 | 0.02 | 0.74 |
| **STM5127** | 0.23 | 5 | 0.84 | 0.13 | 0.82 |
| **STI046** | 0.96 | 2 | 0.15 | 0.08 | 0.08 |
| **STI0036** | 0.96 | 2 | 0.08 | 0.01 | 0.08 |
| **STI0023** | 0.38 | 6 | 0.75 | 0.24 | 0.72 |
| **STWAX-2** | 0.96 | 2 | 0.05 | 0.32 | 0.08 |
| **STPoAc58** | 0.96 | 2 | 0.08 | 0.03 | 0.08 |
| **Mean** | 0.80 | 3 | 0.23 | 0.16 | 0.18 |