EVALUATION OF EFFECT OF INDUCED MUTAGENESIS ON MORPHOLOGICAL AND AGRONOMIC TRAITS OF SELECTED IRISH POTATO ((*Solanum tuberosum L.*) VARIETIES IN KENYA.

BY

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DECLARATION

Declaration by the candidate

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DEDICATION

To the God Almighty who created me in His own Image, I praise and glorify your Name for your Guidance throughout my school life and to all who made my roots to school successful.

ABSTRACT

Potato is ranked fourth most important food crop in the world while in Kenya, it's the second most important food crop after maize. Although Potato has the widest genetic diversity among related wild species than any other cultivated plant, the germplasm cannot all be directly used for breeding due to a combination of ploidy level and endosperm balance number (EBN) incompatibility. Induced mutation, vegetative micro propagation and genetic engineering are a viable alternative method of improvement. The study was aimed in testing effect of induced mutagenesis on morphological and agronomical traits of Kenyan varieties of Irish potato (*Solanum tuberosum L)*. Three Potato varieties: Kenya Mpya, Asante and Sherehekea were tested for induced soma-clonal mutation after irradiation dosages of 0, 3, 6, 10 and 12 Gy. The five levels of mutation dosages were evaluated to determine the suitable dosage level using augmented design. The LSD means separation by dosage at 5% level results indicated that the genetic variability occurred in all the mutagenic treatments and morphological traits under study showed wide range of genetic variability. All dosage levels were significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ for both qualitative and quantitative traits at all environments. Principal component analysis (PCA) allowed reduction of four traits into two variables (principal components), with weight of tubers contributing most in the first PCA and plant height contributing most at second PCA. Principal component analysis of the traits evaluated at both sites (Eldoret and Mau-Narok) showed similarity with weight of tubers contributing most in the first PCA and flowering period contributing most in the second PCA. The traits that form the first and second principal components show the strongest discriminatory power, which diversifies the studied accessions. Plot of first two PCoA axes from the sites separated accessions into clusters showing maximum and minimum similarities in comparison to controls. Selected varieties M81, S34 and A101 demonstrated quality traits in terms of increased yield and late blight resistance. Morphological descriptors after correlation analysis, showed positive correlation among the potato accessions in relation to parental traits magnifying genetic divergence among the accessions.

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LIST OF ACRONYMS

ANOVA:	Analysis of variance
CIP:	International Potato Center
DNA:	Deoxyribonucleic acid
EBN:	Endosperm Balance Number
FAO:	Food Agriculture Organization
GIZ:	Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH
Gy:	Gray
IAEA:	International Atomic Energy Agency
NPCK:	National Potato Council of Kenya
PCA:	Principal Component Analysis
PCoA:	Principal Co-ordinate Analysis

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Cultivated potato *(Solanum tuberosum L.)* originated from the Andean region of South America (Haverkort *et al*., 2008, Park *et al.,* 2008). The Spanish conquest of South America began in 1532, bringing to an end the Inca Empire. As the Spanish extended their control of this vast new land, and people moved to Europe and they are believed to have travelled with potatoes as source of food. Spanish soldiers were the first European to record the existence of the potato, in 1538, in the Upper Cauca valley in what is now Colombia; however, the first recorded mention of the potato in Europe occurred in 1587 when the Austrian botanist Clusius described some tubers he had received from Spain. It was imported from Europe to Africa by missionaries and later by colonial administrators in the 19th Century. The European settlers introduced the crop in Kenya initially in Kiambu, Murang'a and Nyeri districts in the late 19th century primarily for domestic consumption and later, for export. Indigenous Kenyan farmers started potato cultivation in 1920 and entered the export market in 1923. New potato varieties and seed potato production were introduced at the National Agricultural Laboratories, Kabete in 1903 and at Plant Breeding Station, Njoro in 1927 (MoA, 2010)

Potato is one of the most important food crops in the world and is ranked fourth place after wheat, rice and maize (FAO, 2013 and Khan *et al*., 2012). Amongst other root crops, potato is at the top of the list followed by cassava, sweet potato and yams (Park *et al*., 2008). In Kenya, Irish potato is the second most important food crop after

maize (Obare *et al*., 2010). Potato is very productive and nutritious as compared to wheat and rice, it's an important source of starch and contains high quality protein and vitamin C, the potato has an approximately five times higher crop yield per hectare compared to other root crops (Struik and Wiersema 1999). Potato has very diverse purposes and is grown for local consumption, commercials both import and export e.g. for fresh potato consumption, starch production, chips, French fries and crisps.

Irish potato is grown widely under various climatic conditions such as tropical, subtropical, temperate environments and at various altitudes because of its high adaptation ability (Park *et al*., 2008, Doehlonan and Sleper, 1995). In Kenya, potato is cultivated in areas of altitudes between 1,500 and 3,000 metres above sea level, where the country's main staple food, maize, has no comparative advantage. At this altitude, potatoes grow faster than maize and produce more energy and protein per hectare per day. The Potato production areas are found mainly in the highlands of the Central, Eastern, Rift Valley regions, slopes of Mount Kenya and Mount Elgon (Bungoma County) in Western Kenya are prominent production areas (GIZ, Kaguongo *et al*., 2014).

Fig 1: Map showing major potato growing areas in Kenya.

(Source : Google maps, 2015)

Kenya is the fifth biggest potato producer in Sub-Saharan Africa; estimated at 7 to 10 tonnes per hectare (Muthoni *et al*., 2011), compared to a global average yield of 17 tonnes per hectare (FAO STAT, 2011). Potatoes are grown by up to 800,000 farmers, who are mainly smallholders estimated that 83 percent of the land under potato cultivation belongs to smallholders dedicating 0.2 to 0.4 hectares to potato production, while approximately 17 percent of potato farms belong to larger-scale farmers dedicating 2 to 10 hectares to the crop (Janssens *et al*., 2013).

The low yields have been attributed to poor agronomic practices, low use of inputs especially fertilizers, low soil fertility, limited access to good quality seeds and variety, diseases especially bacterial wilt, late blight and viruses (Muthoni and Nyamongo 2009; Kaguongo *et al.,* 2008). Late blight, caused by oomycete pathogen *Phytophthora infestans*, is one of the most severe potato diseases responsible for the European potato famine in the 19th century, which caused starvation deaths of more than one million people in Ireland (Razukas *et al*., 2007; Hansen *et al*., 2005). It's also a major disease in potato growing areas in tropical highlands of Sub-Saharan African causing serious economic consequences often resulting from complete or partial devastation of infected fields; Documentation of late blight occurrence on potato has been reported in various countries of Sub-Saharan Africa by a number of researchers (Olanya *et al*., 2001).

Potato breeding is aimed at improving resistance to diseases and pests, improving taste, cooking qualities, skin color, shape, maturity and yield (Hawkes, 1990).The potato probably has the widest genetic diversity among related wild species than any other cultivated plant, with ploidy levels, varying from monoploid (2n = x = 12) to hexaploid (2n = 6x = 72). Tetraploid being common cultivated potato, the germplasm can not all be directly used for breeding due to a combination of ploidy level and endosperm balance number (EBN) incompatibility (Bisognin 2011, Park *et al*., 2008).

Alternative breeding methods such as induced mutation, vegetative micro propagation and genetic engineering should be incorporated. Induced mutation techniques have been successfully used to improve yield, quality, and disease and pest resistance in many crops including potato (Gnanamurthy *et al*., 2012; Ahloowalia, 2004; Arabi, 2004; Wongyai *et al*., 2001 and Love *et al*., 1993). In Irish potatoes, several induced mutations have been carried out including genetic analysis of somaclonal variants on

gamma induced mutants of potato (*Solanum tuberosum* L.) cv. Diamant using RAPD-PCR technique (Humera *et al*., 2012) and Induction of salt-tolerant potato (*Solanum tuberosum* L.) mutants with gamma irradiation and characterization of genetic variations via RAPD-PCR analysis (Orkun *et al*., 2012).

1.2 Statement of problem

In Kenya, 98% of potato growers are small-scale farmers, producing less than 0.4 ha of potatoes per year per farm (total of two planting seasons) producing 83% of the national production (Janssen's *et al*., 2013). Production remains below target, access to high quality seeds, disease and pest management practices are two areas that directly impact on yields and quality of tubers (MOAF, 2014).

Farmers prefer cultivars for home consumption to be tasty, high yielding and resistant to late blight while the cultivars should have high market demand and be high yielding if they are destined for the market, tuber quality characteristics such as skin color, tuber size, tuber shape and time to maturity are often key factors in cultivar acceptability based on local consumer preferences and criteria for potato processing (Muthoni *et al*., 2013). Efforts towards development of the Irish potato industry in Kenya have focused on development and dissemination of high yielding varieties (Nyogaka *et al*., 2009; MOA, 2006). Current varieties face challenges in production due to diseases like bacterial wilt, viruses and late blight Muthoni and Nyamongo, 2009; Kaguongo *et al*., 2008). Late blight caused by Phytophthora infestans, is one of the most devastating disease, extraordinarily virulent and adaptable pathogen and can completely eliminate the potato crop (Haas *et al*., 2009; Fry, 2008; Junqi song *et al*., 2003; Olanya *et al*., 2001). It's the most important single factor influencing potato production in Kenya and causes significant yield losses (Muthoni *et al*., 2013).

Numerous potato varieties have gone through the breeding programme and have been released to the farmers based on their tolerance to late blight. However, despite cultivation of varieties with resistance to late blight, the disease continues to devastate the crop. At times when the rainfall is high coupled with cool temperatures, the disease can be extremely high even on the most tolerant varieties leading to complete loss of the crop (Wakahiu *et al*., 2007). Mostly it's controlled by the application of chemicals, however, available fungicides tend to be expensive and have potentially adverse environmental effects and some strains of the pathogen are resistant to some fungicides (Champoiseau *et al*., 2010; Kaguongo *et al*., 2010; Riungu, 2011).

1.3 Justification

Overtime, the Irish potato has grown to become the second most important food crop after maize in Kenya. Its importance is attributed to its high nutritive value, good productivity and good processing qualities for starch, flour, bread, soap, alcohol, weaning foods and animal feed (MoA, 2010). Average per capita consumption is estimated at 30 kg per day and is expected to rise due to increases in potato consumption by urban populations (FAO, 2013) and rapid population growth. Present estimates indicate that around 1 to 1.5 million tonnes of potatoes are marketed in Kenya per season. Potato is ideal as a food security crop as it has a short season and provides food within just 2.5 to 3 months, especially when planting fast-maturing varieties. At the same time, farmers are assured of a harvest as the crop is drought resistant and will provide some produce, even with little rain (GIZ, kaguongo *et al*., 2014).

However, potato cultivation faces various biotic constraints of fungal, bacterial and viral origin. Among the diseases, late blight of potato caused by *Phytophthora*

infestans is the most devasting causing 50 – 70 % potato yield loss in the tropics under favorable environmental conditions; it spreads and inflicts damage in epidemic form to leaves, stems, petioles and tubers (Younis *et al*., 2009). The disease is controlled by spraying fungicides, however, given the different brands in the market, the different application regimes and the fact that application is in most cases dependent on the weather conditions, a correct, early timing of the first fungicide application is as important for forestalling disease; however, not all farmers are cognizant of this (Wangombe and Van Dijk, 2013).

Chemical control is expensive, short durational and have health hazard while biological control is in its infancy for this disease. It is well established that usage of fungicides for a long time causes disturbance in ecosystem and might lead to the appearance of fungicide-resistant isolates in addition to their hazardous effects on humans and animals. The most effective and environmental friendly way to prevent widespread devastating late blight is to incorporate natural resistance in potato cultivars (Jiang *et al*., 2006).

Introgression of resistance genes to the cultivated potato in a traditional way takes lots of work and patience, it necessitates application of a more complicated crossing diagram, using so-called 'bridge cross breeding'. It only allows crossing in one resistance gene at a time, unless you have the luck that the wild variant contains several resistance genes and that at the same time those resistance genes are very close together in the DNA of that wild variant. Furthermore, after cross-breeding with wild variants, many back-crossings are needed to arrive back to the desired cultivated potato properties (report from VIB ('Vlaams Instituut voor Biotechnologie'). Many plant breeders now prefer to develop cultivars that have "polygenic" or "field

resistance" to the pathogen with combinations of several "minor" genes, which gives resistance through induced mutation breeding or genetic engineering (Deacon, 2013).

1.4 Objectives

1.4.1 Broad Objectives

1. Improve productivity of Irish potatoes through induced mutation both morphologically and agronomically.

1.4.2 Specific Objectives

1. To determine the diversity of mutants using morphological and agronomic traits.

2. To determine the best irradiation dosage

3. To evaluate selected mutants for adult plant late blight resistance

4. To evaluate yield potential of the selected mutants

1.5 Hypotheses (Null)

1. No significance difference between mutants and parents in morphological and agronomic traits

2. All levels of radiation dosages had no significance difference

3. No mutant depicted adult plant late blight resistance

4. There is no mutant that depicted yield potential and early maturity

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of Irish potato

The cultivated potato (*Solanum tuberosum L.*) originated from the Andean region of southern Peru about 10 000 years ago where the Incas cultivated the plant largely for food and many other wild potato species (Ovchinnikova *et al*., 2011). Following the Spanish conquest of the Inca Empire, the Spanish introduced the potato crop in the second half of the 16th century in Europe where it dominated as staple food (Solomon and Barker, 2001). It was imported from Europe to Africa by missionaries and thereafter by colonial administrators in the 19th Century. The European settler farmers introduced the crop in Kenya initially in Kiambu, Murang'a and Nyeri districts in the late 19th century primarily for domestic consumption and later, for export. Indigenous Kenyan farmers started potato cultivation in 1920 and entered the export market in 1923 (MoA, 2010).

2.2 Taxonomy

The English word potato came from Spanish word Patata. Potato (*Solanum tuberosum L.*) belongs to the family Solanaceae which includes about 90 genera and 2,800 species which includes tomato (*Solanum lycopersicum L.*), eggplant *(Solanum melongena* L.) pepper *(Capsicum annum L.*) and tobacco (*Nicotiana tabacum L.*) (Gillund et al., 2011). The genus solanum where potato belongs consists of about 2,000 species of which 150 are tuber bearing (Word, 1991). The genus is further subdivided into several subsections one of which is potatoe which contains tuber bearing potatoes, Potatoe is divided into tuberosa which contains 54 species both

cultivated and wild potatoes. One of these is solanum tuberosum which is further divided into tuberosum and Indigena. Cultivated irish potato belongs to tuberosum, therefore binomial name Solanum tuberosum L., subspecies Indigena is also cultivated but restricted to central and south America (Nash et al., 2008; Hawkes, 1990).

2.3 Genetics

Potato has complex and variable genetic makeup. There are several ploidy levels, varying from monoploid (2n = x = 12) diploid (2*n* = 2*x* = 24), triploid (2*n* = 3*x* = 36), tetraploid (2*n* = 4*x* = 48) and pentaploid (2*n* = 5*x* = 6) to hexaploid (2n = 6x = 72) with tetraploid ones being the cultivated potatoes. The high degree of ploidy not only makes it genetically diverse, but also makes it difficult to create new cultivars. The cultivated autotetraploid potato exceeds twice that predicted by the inbreeding coefficient. This response has been attributed to a decrease in favorable interactions of second and third order and a frequency reduction of tetragenic and trigenic loci, while triploid and the pentaploid genotypes are often sterile. However, the latter can be maintained through vegetative propagation (Bisognin, 2011; Park *et al*., 2008)

2.4 Agronomy of potato

A potato is herbaceous annual plant that grows up to 100 cm tall; it's a low-growing, branching perennial plant with weak stems that produces a tuber which are botanically thickened stems which is commonly known as potato that is rich in starch. Growth of a potato plant occurs in several stages: sprout development, plant establishment, tuber initiation, tuber bulking, and tuber maturation. Timing of these growth stages varies depending upon environmental factors, such as elevation and temperature, soil type,

availability of moisture, cultivar selected, and geographic location (Dwelle and Love, 1993). As the potato plant grows, its compound leaves manufacture starch that is transferred to the ends of its underground stems (stolons). The stems thicken to form a few or as many as 20 tubers close to the soil surface. The number of tubers that actually reach maturity depends on available moisture and soil nutrients and variety. At the end of the growing season, the plant's leaves and stems die down to the soil level and its new tubers detach from their stolons (FAO, 2008).

The potato is a very accommodating and adaptable plant, and will produce well in a wide range of soil type with high level of acidity of pH 5.5 to 6.9; therefore, medium textured loamy soils with good organic matter are best. Proximity of the site to a water course is also important, ensuring the crop can be irrigated. This allows the crop to pass for washed pre packs which command a higher premium for the grower. A correct rotation is required to avoid the build-up of potato cyst eelworm, disease and volunteers. Potatoes should not be grown more often than one year in four, but in areas of intensive ware production a longer Interval may be required (FAO, 2008)

To prevent the build-up of pathogens in the soil, avoid growing potatoes on the same land from year to year. Instead, potatoes should be grown in rotations of three or more years, alternating with other dissimilar crops, such as maize, beans and alfalfa. Crops susceptible to the same pathogens as the potato (e.g. tomato) are avoided, in order to break the development cycle of potato pests (FAO, 2008). Growth of a potato plant occurs in several stages: sprout development, plant establishment, tuber initiation, tuber bulking, and tuber maturation. Timing of these growth stages varies depending upon environmental factors, such as elevation and temperature, soil type, availability of moisture, cultivar selected, and geographic location (Dwelle and Love, 1993)

High yielding crops of potatoes require adequate quantities of nitrogen (N), potassium (K) and phosphates (P) if they are to be allowed to achieve their potential. Suggested compounds of N, P, K to use are 10:10:20 (N: P: K) or 7:6:17(N: P: K) or a combination to give the required amounts of N, P and K(Anonymous, 2012).Plant seed pieces 10 to 12 inches apart and cover them in a furrow 2 to 3 inches deep. Space the rows 24 to 36 inches apart. The 24-inch spacing is often beneficial because plants will shade the soil and prevent high soil temperatures that inhibit tuber development. Harvest times will vary depending on the growing season and the size of tuber you want. After harvesting, set the tubers out in a dry, well ventilated position for a few hours to dry and cure the skin. Once dry store them in paper or hessian potato sacks in a dark, cool but frost free place. Avoid storing in polythene bags as potatoes will 'sweat' and rot (Thompson and Morgan, 2004).

2.4 Cultivation of Irish potato

Potato is one of the most important food crops in the world and is ranked at the fourth place in world food production after wheat, corn and rice (Muthoni *et al*., 2013; Nyogaka *et al*., 2009; Muthoni and Nyamongo, 2009). Compared with wheat and rice, the potato has an approximately five times higher crop yield per hectare and one and half times more energy production per hectare and day (Park *et al*., 2008).

Potato is grown in more than 100 countries, under temperate, subtropical and tropical conditions. It is essentially a “cool weather crop”, with temperature being the main limiting factor to production: tuber growth is sharply inhibited in temperatures below 10°C and above 30°C; while optimum yields are obtained where mean daily temperatures are in the 18 to 20°C range. For that reason, potato is planted in early spring in temperate zones and late winter in warmer regions, and grown during the

coolest months of the year in hot tropical climates. In some sub-tropical highlands, mild temperatures and high solar radiation allow farmers to grow potatoes throughout the year, and to harvest tubers within 90 days of planting (in temperate climates, such as in northern Europe, that can take up to 150 days).

Kenya is the fifth biggest potato producer in Sub-Saharan Africa, with an output of 790,000 tonnes in 2006 (FAO, 2008). Production of the crop has been variable but with a general increase in area under the crop from 2,400 hectares producing 16,000 metric tonnes in 1939 to 108,000 hectares producing 2.5 million tonnes in 2009. The crop is grown annually by seasons with an average yield of 24 tonnes per hectare (MoA, 2010). Potato is the second most important staple crop after maize and plays a major role in national food and nutritional security. Its ability to grow in high altitude areas where maize does not do well and its high nutritional value makes it an important food crop (Obare *et al*., 2010). The high altitude areas are between 1,500 and 3,000 metres above sea level. The major production areas are found in Rift Valley, Central and Eastern provinces (MoA, 2008). The crop is mainly grown by small scale farmers who account for over 90% of the production. Most of the production is mainly rain fed and carried out in scattered patches of intensive small-scale agriculture (lung'aho *et al*., 2005; Anonymous, 2009).These areas include slopes around Mt. Kenya, such as Meru, Embu, and Kirinyaga; parts of Laikipia and on both sides of the Nyandarua (Aberdare range) that coves parts of Nyeri, Muranga, Kiambu and Nyandarua Districts. They are also grown in the highlands on Mau Escarpment (Mau Narok and Molo), Tinderet, Nandi Escarpment and Cherangani hills. Small acreages are also cultivated in Kericho and Kisii areas and isolated patches near the Coast in the Taita hills (Kirumba *et al*., 2004)

Average production in Kenya is up to two harvests per year. The total production area has increased in recent years and is estimated to have reached 150,000 to 160,000 hectares to date In addition to there being up to 800,000 potato farmers (Kaguongo *et al.*, 2013). At the same time, farmers are assured of a harvest as the crop is drought resistant and will provide some produce, even with little rain (GIZ, Kaguongo *et al*., 2014). Most potato growers are small-scale farmers; it is estimated that 90% of them have land holdings of less than 1 ha, less than 0.05% of potato growers have more than 25 ha of land (Janssen's *et al*., 2013).

2.5 Economic importance of Irish potato globally

In Kenya, the potato is the second most important food crop after maize, which contributes 32% of overall dietary energy consumption and 68% of energy consumption from cereals (MoA 2007; Wang'ombe and Meine, 2013). Potato is the world's leading vegetable crop and is grown in 79% of the world's countries. It is second to maize in terms of the number of producing countries and fourth after wheat, maize and rice in global tonnage (Qasim *et al*., 2013). Therefore, potatoes represent an important source of energy, with a high delivery of energy per unit land, water and time, and are a valuable source of minerals and vitamins for the diet (Anderson et al., 2010). Potato plays multiple and important roles in local food systems and for food security. It is well suited for cultivation in environmental conditions such as tropical, subtropical and temperate because of its high adaptation ability where other crops may fail and its short and flexible vegetative cycle makes it well suited for rotation with other major crops, such as wheat, rice, maize or soybeans (Doehlonan and Sleper, 1995; FAO, 2008; Riungu, 2011; Muthoni *et al*., 2012). Thus, potato helps to

increase the availability of food, contributing to a better land use ratio by raising the aggregate efficiency of agricultural production systems (Gastelo *et al*., 2014).

The fact that potato is grown in regions with high incidences of poverty, under nutrition and food insecurity such as the tropical highlands of Africa, the Andes of South America, or the Indo-Gangetic basin of southern Asia, underlines its particular importance (Bruinsma, 2003; CIP, 2014;). All over the world, potato provides income generating opportunities as a cash crop and generating employment which contributes to alleviating poverty (Scott, Rosegrant, and Ringers, 2000). In kenya, Potato (*Solanum tuberosum* L.) plays a major role in food security in Kenya ,contributes to poverty alleviation through income generation and employment creation as 2.5 million people work in the potato value chain (Kaguongo *et al*., 2013).

The average composition of the potato is about 80% water, 2% protein, and 18% starch. As a food, it is one of the cheapest and easily available sources of carbohydrates and proteins and contains appreciable amount of vitamins B and C as well as some minerals. Moreover, protein of potato is of high biological value. Potato is becoming increasingly important crop, as it is staple food in most of the European countries and is a good and cheap source of food calories and its high starch content can meet the energy requirements of the people living in food deficit countries. Accredited to its short duration, nutritional superiority and high amount of food per unit area and time, potato production in developing countries has been increased by about 25% over the last 4 decades (Qasim *et al*., 2013).Therefore, at the commercial level; potatoes are mainly consumed as chips served in restaurants and take-away facilities in Nairobi and other major towns in Kenya. The white varieties are the most popular potatoes for chips, although a few take-away facilities prefer the red varieties, which they claim takes less oil and gives higher volume of chips than the white

varieties. In homes, especially within the producing areas, potatoes are consumed daily to make stews and mashes, in the local dishes potatoes are mixed with vegetables such as carrots and cabbages and meats then made into stews which are eaten with ugali, chapati or rice. They are also used in traditional dishes in which they are mashed together with maize and beans or peas and other pulses into which some varieties of green vegetables may be added (Karuri, 2000)

The recurrent episodes of famine in periods of drought in recent years, coupled with Kenya's reliance on maize imports to meet its domestic needs suggest that the country has not thus far succeeded in realizing successful food security strategies. The potato has a demonstrated capacity to feed large populations as showed how population and urbanization in Europe and America increased sharply during the eighteenth and nineteenth century following the introduction of the potato as a new food crop. The potato provides more food per hectare than other staples, given its short time to mature (80 to 120 days), which allows two crops per year (Wang'ombe and Meine, 2013). Despite the importance of the crop, potato sector is plagued by numerous problems such as lack of proper pest and disease management, a disorganized marketing system, lack of clear policies on packaging, lack of clean seeds and poor storage facilities (Riungu, 2011; Muthoni *et al*., 2013).

2.6 Constraints in Irish potato production

Average potato yields in North America and Western Europe often reach 40 tonnes per ha, yields in developing countries are usually below 20 tonnes per hectare (KALRO, 2005; Nyogaka *et al*., 2009). The national average potato yields in Kenya have remained low even as more land is devoted to the crop. Over time, some potato cultivars have been rejected and replaced by others in Kenya; low yield and

susceptibility to diseases were cited as the major weaknesses (Muthoni *et al*., 2013).This contrasts with the experience of other regions that have experienced the green revolution innovations that can lead to increased potato yields, clean seeds, fertilizers, chemicals and irrigation. In Africa, however, despite a 4% rise in potato-farmed land, yields remained constant in the same period. This suggests that there is an immense potential for improvement of potato yields in Africa (Wang'ombe and Meine, 2013).

The low potato yields have been attributed to poor agronomic practices, low use of inputs – especially fertilizers and fungicides - low soil fertility, Seasonability, limited access to good quality seeds, poor seed stock, a disorganized marketing system, lack of clear policies on packaging, diseases (Particularly late blight, bacterial wilt, brown rot, and viruses), and insect pests such as the potato tuber moth (Riungu, 2011; Muthoni *et al*., 2013; Janssen's *et al*., 2013; Muthoni *et al*., 2013)

Most farmers produce potatoes twice a year due to bimodal rainfall patterns in most potato growing areas. The long rainy season lasts from March/April to June/July, while the short rainy season lasts from October to December. Off-season potato production is limited to a few areas where irrigation is available. This seasonality of production limits profitability in potato farming as the majority of farmers depends on rainfall leading to gluts and scarce times consecutively. Farm gate prices during the scarce periods are often 2-4 times higher than the price during the glut season.

Another constraint on potato production in the highlands of Kenya is the rapid decline of soil fertility due to continuous cultivation and practicing of intensive cropping systems which replenishes nutrients as fertilizer is mostly applied below the recommended rate (Kaguongo et al., 2008).This has also led to acidity problem compounded by the fact that the soils in the highlands are derived from acidic

volcanic rocks and have been highly leached by high rainfall leading to soil pH of less than 5.5. This severely limits availability of potassium, nitrogen, phosphorus, Sulphur, Calcium and Magnesium while availing excessive levels of Aluminium, Manganese, Boron, Iron and Zinc. It is quite possible that the problem of low soil pH has led to nutrient imbalances that lead to even further decline of potato yields. Soil analysis as a basis for fertilizer application is therefore critical in most potato producing areas.

Shortage of clean planting materials/certified seed, force farmers to plant seeds from informal sources such as farm-saved (self supply), local markets or neighbours which has led to low yields, poor quality produce, and spread of pests and diseases (GIZ-PSDA Kenya, 2011; Riungu, 2011).Brown rot or 'bacterial wilt' is a widespread and important disease contributing to poor yields, high harvest losses and poor quality of farm-saved seeds, Besides late blight that causes huge yield losses. Seed costs amount to between 40 and 50% of the total input cost and even more in case of certified seed, Lack of cash, credits and high interest rates force small-scale farmers to a low input - low output strategy, resulting into product quality and low yields (Janssen’s et al., 2013).

Seed and ware potatoes are stored for a short period of at most 2 to 3 months, most storage facilities are very simple such as pits, in piles or even in house, losses during storage can average up to 20%; due to lack of suitable storage facilities and training on proper seed storage (The Organic Farmer, July 2012). Therefore, when the rains finally come, farmers are forced to plant whatever potato tubers are available, whether well sprouted or not. Planting of unsprouted seed tubers results in plants with one or two stems leading to low yields. Such tubers also take long to emerge in the field and the plants mature late in the season; such a crop suffers from moisture stress and other pests such as aphids (Kabira et al., 2006; Kinyua et al., 2012; Nyongesa et al., 2012).

In addition, climate change has led to low and erratic rainfall, in such uncertain situations, only well sprouted seed tubers have a chance of carrying a potato crop to maturity. Therefore, availability of well sprouted seed tubers at the beginning of each planting season will go a long way in increasing potato productivity and yields in the Kenyan highlands (Nderitu et al., 2014).

2.7 Late blight pathogen on cultivated potato

Late blight is a disease caused by the oomycete pathogen *Phytophtora infestans* (Mont.) de Bary, it's one of the world's most devastating disease of potato and tomato responsible for Irish Great Famine in the 1840s leading to deaths of more than one million people in Ireland (V. Sedlakova *et al*., 2011; Fry *et al*., 2006; Simko *et al*., 2009; Jiang *et al*., 2006). Currently, late blight is responsible for multibillion-euro losses annually in both potato and tomato production if not controlled (Biosafety report, 2011; Razukas *et al*., 2007; Mostafa and Gado 2007).

The pathogen belongs to the family of the *Pythiaceae*, which together with the *Peronosporaceae* and *Albuginaceae*, makes out the order of the *Peronosporales* (Turkensteen *et al*., 2003). *P. infestans* is characterized by high levels of genetic and phenotypic diversity (Grünwald and Flier, 2005) having two mating types, termed A1 and A2, if both mating types are present in a population, they frequently undergoes sexual reproduction and co-occurs with the two closely related species *P. mirabilis* and *P. ipomoeae* as shown in fig 2. (Flier *et al*., 2003; Grünwald and Flier, 2005). It's a hemibiotrophic fungal disease with pathogeneciy mechanisms of filamentous microbes such as oomycetes that initially requires living host cells causing extensive necrosis of host tissue culminating in profuse sporulation (Kamoun and Smart, 2005). The life cycle of *P. infestans* is complex and it follows three basic steps, formation of

mycelium in the host plant, spatial expansion of the affected area lesion in the host plant and formation and dispersal of spores (CIP, 2010). Favorable conditions for late blight are cool nights (50 to 60°F) and warm days (60 to 70°F) accompanied by fog, rain, or long periods of leaf wetness. Conditions must remain moist for 7 to 10 hours for spore production to occur (Barbara J. Christ, 1998)

2.7.1 Disease Cycle and Epidemiology

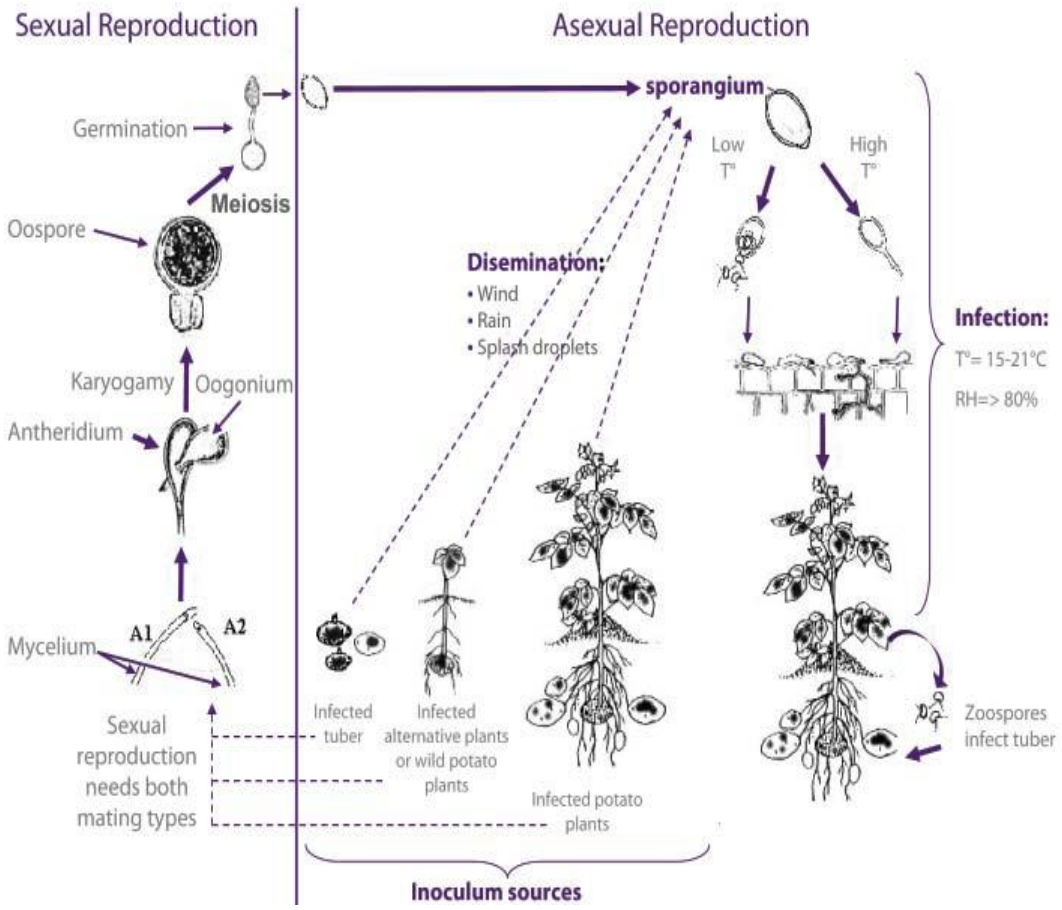

Fig 2: The life cycle of *P. infestans*

(Source: Perez, 1999.)

2.7.2 Symptoms of potato late blight

An infected leaf with symptoms of late blight appear as water soaked pale green irregular leaf lesions, lesions are not limited by veins, they coalesce enlarge rapidly

and turn brown or purplish black, shrivel and dry out blighting and killing the entire leaf within a few days(Plate 1). During periods of high relative humidity and leaf wetness, the underside of the lesions and infected stems are covered with fine white cotton like moldy growth composed of sporangiospores and sporangia. However, infection can occur on both leaves and stems while other times Lesions may occur on petioles or stems only, leaving leaves green and healthy which makes detection difficult. On petioles and stems lesions appear as oily, brown and later turning black with rot and the whole plant may die (plate1) (Akhtar *et al*., 2012).

Plate 1: Late blight infected potato at Mau-Narok in the year 2014.

(Source : Author, 2015)

Plate 2: Initial stages of late blight infected potato at Mau-Narok in the year 2014. (Source : Author, 2014)

2.7.3 Management of potato late blight

Measures to prevent late blight damage are vital since the disease can eliminate a potato crop. Three complementing ways of protecting potato plants against late blight include: Variety resistance, use of fungicides and Planting out of season. There are two types of fungicides commonly used: Contact fungicides, which protect the leaves against the entry of the fungus into the plant also called "protectant" fungicides. The second type of fungicide is systemic which protects the plant from within the plant, and cure the plant from the disease once it gets infected (CIP, MoA, Gtz, KALRO, PRAPACA and ASARECA, 2007). However, high cost of fungicide applications, increasing awareness of health and environmental risks and occurrence of isolates resistant to some modern fungicides world-wide pressures to minimize the use of chemical sprays (Forbes *et al*., 2007; Fry *et al*., 2006).Therefore, breeding for host resistance remains the priority (Park *et al*., 2008).

There are two ways that resistance to P. infestans is expressed in the potato plant, the first one is characterized by triggering a hypersensitivity response (HR) called race-specific resistance, vertical resistance, qualitative resistance, unstable resistance or complete resistance. It is governed by R genes with strong effects that produce products which in turn interact with products of avirulence genes (Avr) of the pathogen. The second type of resistance is governed by minor genes of additive effect and is called general resistance, quantitative resistance, and polygenic resistance, non-specific resistance, and partial resistance, horizontal or field resistance. Inheritance is quantitative and because it is governed by several or many genes, it is theoretically more stable and effective against all the pathogen races (CIP, 2010; Grunwald *et al*., 2001; Simko, 2002).

If no resistant varieties and fungicides are available, the only way to grow potatoes is outside of the main rainy season since the disease does not cause problems in hot or dry weather as environmental conditions conducive for the pathogen results in field resistance (Dejmalova and Dolezal *et al*., 2011). However, potato plant does not do very well in hot dry weather either and other potato pests and diseases prevail (CIP, 2007).

2.7.4 Economic importance of late blight

Phytophthora infestans is an extraordinarily virulent and adaptable pathogen with very many strains due to mutation (Fry, 2008; Haas *et al*., 2009). It's historical and economic significance as the causal agent of the Irish potato famine and still continues to cost modern agriculture billions of dollars annually but also impacts subsistence farming in developing countries (Kamoun and Smart, 2005; Fry, 2008). Spores of the pathogen primarily travel in air, eventually landing on plants where the spores colonize leaves and cause them to die. Spores also can enter the soil, reach potato tubers, and destroy them. The disease is a threat to home gardeners and commercial farmers as the disease can wipe out potato fields within a week

To appreciate the importance of potato late blight in developing countries one needs to understand the role of this crop in the livelihoods of the rural poor people. Production and consumption of potato are declining in industrialized countries, but just the opposite is happening in most developing countries (Forbes *et al*., 2007). Late blight of potato caused by *Phytophthora infestans* is the most important factor influencing potato production in Kenya and causes significant yield losses, Search for a more yielding, resistance to late blight, good agronomic traits variety is the priority in the potato programme. Numerous potato varieties have gone through the breeding

programme and their tolerance to late blight, however, the disease continues to devastate the crop (Lung'aho and Wakahiu *et al*., 2007; Olanya *et al.*, 2001).

Mostly, control of potato late blight is by chemical control which is expensive, short durational and have health hazard while biological control is in its infancy for this disease (Haverkort *et al*., 2008). It is well established that usage of fungicides for a long time causes disturbance in ecosystem and might led to the appearance of fungicide-resistant isolates in addition to their hazardous effects on humans and animals (Mostafa and Gado, 2007; Chaurasia, 2005). Besides, high costs are incurred to purchase the chemicals and most developing countries funds for purchasing fungicides are limited, therefore, late blight can completely eliminate the potato crop (Otipa *et al*., 2003; Asakaviciute *et al*., 2007; Kaguongo *et al*., 2008, 2010; Riungu, 2011; Muthoni *et al*., 2013). Due to such heavy losses, this disease exerts a visible impact on a country's economy in term of pricing system, foreign exchange spending on potato import from foreign countries (Ahmad *et al*., 2009).

Incorporation of resistance in potato cultivars and screening of potato germplasm would considerably reduce costs in regions with intensive potato production, increase yields and make production more environmentally friendly and improve the image of arable farming to enhance food security in developing countries (Khan *et al*., 2003; Ahmad *et al*., 2009; Haverkort *et al*., 2008).

2.8 Breeding methods

Plant breeding is a technology that deals with evolution of crop varieties using principal of various sciences; it involves natural and artificial selection to identify plants with novel and useful characters (Rauf *et al*., 2010). The major goal of plant breeding is to produce crop varieties with superior traits through creation of genetic

variations that conform to agricultural production application. The frequently addressed traits are biotic and a biotic stress tolerant, grain/biomass yield, end use characteristics such as taste and concentration of other biological molecules such as proteins, sugars, lipids, vitamins and fibers and ease of processing of products in terms of harvesting, milling, baking, malting and blending (Arterburn, Jones and Kidwell, 2007).

In many sexually propagated crops, genetic variability is usually done through hybridization (Ahloowalia, 2004), selfing and crosses within and between parents and hybrids are carried out, evaluated and hybrids with promising traits are released as new varieties. However, the process is not suitable for asexually propagated plants due to high heterozygosis and hybrid vigor which make the vegetatively propagated crops very vulnerable to inbreeding depression, thus they are not submitted to self-pollination (Bisognin, 2011). Therefore, availability of biotechnology has become the compliment tool to conventional breeding, several techniques such as recombination breeding, mutation breeding and transgenic breeding, each with unique way of generating variation and of selecting target lines both for sexually and asexually propagated crops have been employed (Ahloowalia, 2004; FAO/IAEA, 2008). Mutation breeding refers the process of treating plant cells with mutagens to facilitate crop Breeding; Mutations are changes in the DNA sequence of a cell's genome caused by radiation, viruses, transposons, mutagenic chemicals, or errors that occur during meiosis or DNA replication. Mutations are naturally occurring, or can be induced ; induced mutation Create new variation not found in nature ,generate novel alleles orders of magnitude faster than occur spontaneously ,generate variation in organisms where traditional introgression is impeded (ex: linkage drag, or asexual propagation) and broadly applicable (FAO/IAEA, 2014)

2.8.1 Conventional breeding methods

Classical breeding refers to crossing of closely or distantly related individuals to produce new crop varieties or lines with desirable characteristics since new traits/genes are transferred. This is done through sexual hybridization which involves transferring pollen from one parent to another through various processes, selection of parent, emasculation, bagging and artificial cross pollination to obtain hybrids. The subsequent generations of hybrids are grown and plants selected, homozygosity of desired variety is arrived at following breeding methods; pedigree, single seed descent or mass bulk method.

However, major limitations lie in use of conventional breeding method in potato breeding. This is because of high degree of ploidy level and low heritability; the cultivated autotetraploid potato exceeds twice that predicted by the inbreeding coefficient. This response has attributed to decrease in favorable interactions of second and third order and a frequency reduction of tetragenic and trigenic loci therefore making potato genetically diverse, and difficult, tedious and time consuming to create new cultivars (Park *et al*., 2009; Bisognin, 2011).

2.8.2 Molecular/Genetic Engineering Breeding

Genetically Modified Organism (GMO) is organism whose genetic material has been altered by genetic engineering techniques generally known as recombinant DNA technology. Genetic engineering has expanded the genes available to breeders to utilize in creating desired variations for new crops. Genetic engineering is being employed in various parts of the world, to create crops with beneficial traits such as disease and pest resistance crops, herbicide –tolerant crops etc.However, genetic engineering of plants has proven to be controversial, issues surrounding food security and environmental impacts have risen regarding GMO practices. For example, GMOs

are questioned by some ecologists and economists concerned with GMO practices such as terminator seeds.However,molecular breeding techniques have advantage over conventional breeding in that they allow addition of specific genes encoding desired traits to a cultivar or advanced breeding line while preserving its intrinsic features. The technique expedite generation of new cultivar from parental clones through cellular techniques such as transformation, regeneration of transformed cells into new plants followed by identification, isolation and modification of specific genes coding for interesting traits (Jongedijk *et al*., 1992)

Molecular and genetic engineering breeding methods emphasize on vertical resistance also known as ‘single gene, gene- for-gene, race specific or qualitative resistance where defence responses are invoked through interactions between specific a virulence (Avr)gene products (effector proteins) produced by the pathogen and single resistance (R) gene products produced by the plant. Disease resistance starts with a recognition of the pathogen Avr factors by plant R proteins, followed by signal transduction leading to a hypersensitive response (HR) and death of the infected cell. If a plant lacks the correct *R* gene to match at least one of the *Avr* genes possessed by an invading pathogen, that plant will be unable to use its *R* genes to detect and stop the pathogen (Biosafety report, 2011/05; Kamoun and Smart, 2005). However, this has been replaced with ‘horizontal resistance’’ also known as multigenic, quantitative or partial resistance, a plant’s defence mechanisms generated via interactions between the products of multiple plant genes. Hence, the plant and the pathogen do not require matching *R* and *Avr* genes for a timely plant defence response to occur (Biosafety report, 2011/05) ; Kamoun and Smart, 2005). Horizontal resistance is believed to be much more durable than vertical resistance due to the interaction of many genes which recognize different races of the same pathogen. Durable or horizontal

resistance is described as being effective during prolonged and widespread use in an environment conducive to the disease (Johnson, 1984). However, horizontal resistance is difficult to breed for due to its polygenic nature and poorly understood mechanisms of action (Jiang *et al*., 2006)

The concept of inducing resistance of plants against disease was introduced vigorously in plant disease management, many successful trials carried out under laboratory and greenhouse conditions to enhance plant disease resistance against fungal diseases (Cohen *et al.*, 1991; Cohen, 1994 and Agostini *et al.*, 2003) bacterial diseases (Buonaurio *et al.*, 2002), viral diseases (Anfoka, 2000) and even nematodal diseases (Oka *et al.,* 1999). Several transgenic potato varieties have been developed resistant to viruses and late blight resistance while other research and findings are ongoing (Kuhl *et al*., 2007; Haverkort *et al*., 2008; Wageningen University and Research Centre in the Netherlands, 2005; Gebhardt *et al*., 2002; Govers *et al*., 1998). Many plant breeders now prefer to develop cultivars that have "polygenic" or "field resistance" to the pathogen with combinations of several "minor" genes, which gives absolute resistance through induced mutation breeding or genetic engineering (Deacon, 2013).

2.8.4 Mutation breeding

Mutation refers to sudden and random change in genetic material of a cell that causes the cell and all cells derived from it differ in appearance or behavior from normal type hence creating variation. Mutation can occur naturally through evolutional and environmental influence known as spontaneous mutation or it can be influenced by man artificially referred to as induced mutations. Cells with mutation are known as Mutant while agents causing mutations are known as mutagens. However, spontaneous mutation happens at a lower rate or may not happen to be able to be

relied on breeding to cater for food security hence induced mutations are advised. Occasionally potato plants produce a spontaneous mutation reported by Darwin (1868) and Carriere (1865) but it doesn't happen often enough to be useful as a breeding method (East 1917) and Folsom (1923). However, it's still responsible for large number of the recent variations used to breed vegetative propagated crops (Owoseni *et al*., 2006).

2.8.5 Induced mutation breeding

Mutagenesis causes desirable changes in crops. Different mutagens will impact differently, depending on the mode of action, as well as the energy/concentration of the mutagen whether physical or chemical e.g x-rays, gamma rays and ethyl-methane sulphonate (EMS). Both mutagens are used *in vivo* treatments and some of them can be used *in vitro*. Physical and/or chemical mutagens cause random changes in the nuclear DNA or cytoplasmic organelles, resulting in gene, chromosomal or genomic mutations. Induced mutagenesis is an established method for plant improvement, whereby plant genes are altered by treating seeds or other plant parts with chemical or physical mutagens. Voluminous work has been done worldwide for the improvement of both seed and vegetatively propagated crops through induced mutation (Dotta, 2009).

Crop improvement through the use of induced mutation began over 70 years ago and continues to be used widely by plant breeders (Arabi, 2004).Use of induced mutation can create new genetic variations within crop varieties where natural genetic variation or hybridization offers limited variability and insufficient (Ahloowalia *et al*., 2004). Additionally, induced mutation offers advantage such as improvement of existing cultivars in a shorter period than it takes to produce new cultivar by conventional breeding. The usefulness of mutagen to produce effective mutagenesis is dependent

upon specific properties of mutagenic employed and specific characteristics of biological part of plant to be treated. Chemical/physical mutagens induce heritable variation available for selection, hybridization and clonal propagation to supplement existing germplasm (Novak, 1990).

Induced mutations by x-ray treatments of potatoes was performed for the first time by Jacobson (1923) who reported considerable increase in yield and larger tubers in two different cultivars. Since then, several induced mutagenesis have been carried out on potatoes for earliness, increased resistance to different diseases and increased starch content of the tuber (solomonko 1962, 1965). X- Irradiation of 55 potato plants, 19 plants depicted increased resistance to phytophthora infestans though the material was tested during one vegetative cycle (Kishore *et al*., 1963). Other induced mutations include; Genetic analysis of somaclonal variants on gamma induced mutants of potato using RAPD-PCR technique (Humera *et al*., 2012) and Induction of salt-tolerant potato (*Solanum tuberosum* L.) with gamma irradiation and characterization of genetic variations of mutants via RAPD-PCR analysis (Orkun *et al*., 2012). Induced mutation techniques have been successfully used to improve yield, quality, and disease and pest resistance in many crops including potato (Gnanamurthy *et al*., 2012; Ahloowalia, 2004; Arabi, 2004; Wongyai *et al*., 2001 and Love *et al*., 1993).

In Kenya, mutation breeding has been adopted, however, few crops have been developed through mutation such as cowpea (Pathak *et al*., 1996) and wheat (Kinyua *et al*., 2000).Other successful induced mutations in other crops are; study of genetic variability in sugarcane induced through mutation breeding (Imtiaz *et al*., 2007), genetic improvement for oil quality through induced mutagenesis in groundnut (*arachis hypogaea* l.) by (Kavera *et al*., 2008) and genetic variability studies in identified mutants of sesame (*sesamum indicum* l.) by (Supriya *et al*., 2007) amongst

others. A combination of *In vitro* technology and radiation/chemical-induced mutagenesis has been recommended to improve cultivars of vegetative propagated crops (Karmarkar *et al*., 2001). The use of *In vitro* cultures in mutation breeding offers several advantages over the *In vivo* techniques including, obtaining explants from pre-existing cultures and recovering mutants and rapidly micro-propagating them under controlled environmental conditions and depicts higher mutation frequencies (Heineken, 1960; Bhagwat and Duncan,1998).

2.8.6 Effects of mutagens

The success of mutation breeding to produce effective mutation mainly depends on the rate/dosage of mutation, the number and part of plant to be used. Both Physical and chemical mutagens penetrate plant tissues and collide with molecules, knocking electrons out of orbits, creating ions, break covalent bonds, including the sugar-phosphate backbone of DNA .Others manifest after many generations of cell replication causing non-stochastic effects while others increase the probability of abnormalities in offspring due to chance of mating of individuals carrying same mutation causing stochastic effects. Higher doses are carcinogenic and lethal bringing about mortality, high pollen and seed sterility. Therefore, radio-sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting material survive) dose before massive irradiation of materials since it varies depending on plants. For example, appropriate/best dosage for Irish potato is still being determined between ranges of 1-50Gy (Orkun and Sema, 2012).

Induced mutations occur randomly in the genome, their target cannot be directed therefore mass/big population is required to create chance of mutation taking place. Only one of the two or more alleles of a locus is affected, inheritance is ever recessive; therefore homozygozity is required for proper expression. Mutation

techniques have produced some promising cytoplasmic male sterile lines which significantly increase seed set, making hybrid seed production more economical. Some new early season lines with better grain quality and disease resistance have also been developed. Generally, mutagenesis has been successfully used to induce genetic variability in many crops with desirable characters of economic importance such as increased yield, earliness (Wongyai *et al*., 2001), modified plant architecture, closed capsules, disease resistance (Cagirgan, 2001),seed retention, larger seed size, desirable seed/tuber color and oil content (Hoballah,2001).

Many new cultivars have been directly or indirectly released in the world through induced mutations. The number of mutants varieties officially released by FAO/IAEA by beginning of 21st century has reached 2252 for crops like cereals, oilseeds, pulses, vegetables, fruits, fibers and ornamental plants (Kharkwal *et al*., 2004). Exposure to gamma rays known for their simple application, good penetration, reproducibility and limited disposal problems (Chahal and Gosal, 2002), therefore, potato may undergo effective mutation that could benefit food security and agriculture.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

Two sites, one located at high altitude, Mau-Narok (ADC- farm)) and another at medium altitude, University of Eldoret (Chepkoilel farm) were used in the study. Mau-Narok site is located at an altitude of 2,900 m above the sea level and lies between latitudes 0°36'S and longitude 36°0'E. The area receives an average annual rainfall of 1,200-1,400mm. The minimum temperatures of 6-14°C and maximum of 22-26°C have been reported (Wanyera *et al*., 2010). University of Eldoret site is located at an altitude of 2,140 m above sea level and lies between longitude 35°18' E and latitude 0° 30'N. The site receives rainfall ranging between 900 to 1,300 mm with an annual average of 1,124 mm. The average annual temperature is 23°C with a minimum of 10°C (Okalebo *et al*., 1999). Glasshouse experiment was carried out at the University of Eldoret biotechnology glasshouse under controlled environment for seed multiplication and increase in size of the micro-tubers to withstand environmental effects.

3.2 Sources of genotypes

Three potato tubers used for study were collected from KISIMA farm while mutagenesis was carried out at seibersdorf laboratories, Vienna, Austria Three potato varieties all developed by KALRO/CIP include, ASANTE: suitable for mid and high altitude region and can yield 35 to 45 tonnes a hectare in 100 to 120 days after planting. It's highly susceptible to late blight (KALRO 1998). KENYA MPYA: suitable for mid and high altitude region and can yield 35 to 45 tonnes a hectare in 90

days, and can be harvested while the plant is still green, has the shortest dormancy period of 1 to 2 months until shoots begin sprouting. It's moderately resistant to late blight (KALRO/CIP 2010). SHEREKEA: the highest yielding variety, capable of yields of between 40 to 50 tonnes that can be harvested 100 to 120 days after planting and fairly resistant to late blight, however, it breaks dormancy in 2 to 3 months (KALRO/CIP, 2010).

3.3 Potato tissue culture and irradiation of the explants

The three varietal tubers were planted in the greenhouse separately according to variety for one month, after which they were ready to be cultured in-vitro. The nodal cuttings with leaves from each plants grouped according to variety grown in the glasshouse were initiated and micro-propagated using media containing full strength MS 5519, 20g sucrose and 1.8g gelrite at a pH 5.8. Some of *in vitro* nodal cuttings (with leaf) cultures were selected and used in radio-sensitivity tests to determine the optimal dose treatment using gamma irradiation for mutation induction. The optimal dosage was determined and ranged between 0 to 50 Gy, therefore, the rest of *in vitro* nodal cuttings (with leaf) were irradiated using dose level of 0, 3, 6, 9, and 12 Gy that gave micro-tuber labelled M_1V_2 followed by in-vitro shoot propagation to dissolve chimeras; After which, in-vitro shoots with leaves was induced for *in- vitro* micro-tuber production using media with full strength MS 5519 and 2mg/L Kinetin. Micro-tuber propagation was performed for each genotype.

3.4 Screening for morphological and agronomic traits

The micro -tubers at vegetative stage two labeled M_1V_2 were planted in the glasshouse at the University of Eldoret to produce new tubers at stage three (M_1V_3). The micro-tubers from the parental material were used as control/checks; no fertilizer was used, weeding and watering was done accordingly. Inoculation was done at the 45^{th} day after planting using hand sprayer on whole plant coverage (Sharma, 1990). Data collected in the glasshouse included, disease score which was recorded after six days after inoculation up to day ten, plant height and tuber properties on harvest no selection was done. The second season, M_1V_3 plants were taken to the field at the two sites (University of Eldoret and ADC farm-Mau Narok). In both sites morphological and agronomical traits of mutant potatoes were recorded which included; plant growth habit, leaf size, leaf color, flower frequency, flower color, flowering period, disease score, plant height, tuber shape, tuber skin color, number of tubers and weight of tubers per plant respectively.

3.5 Experimental design

The experimental design for the trials was augmented design comprising of block, entry, accession and dosage; different levels of dosages, and the variety, acted as treatments. The parent/check was replicated three times in each block; the rest of plants were not replicated therefore forming a single plant experiment. A single plant experiment is performed when there is limited seed supply of test lines in the early stage of breeding hence insufficient seeds are available for a replicated experiment. The basic idea of the augmented design is that control lines are arranged in a standard design; each replication of the control lines is placed in a soil-homogeneous block and the block is augmented to contain more non-replicated test lines. Based on control

lines in a standard design, the block effects can be estimated to adjust the observed values of the test lines, and the error to test the significance of performance differences among lines (You *et al*., 2013).

3.6 Morphological characters scored

Data was collected for each plant of the three varieties (accessions) under different mutation dosages in the glasshouse and the two sites at flowering stage, after flowering and during harvest time. The morphological and agronomic traits done at flowering time were; plant height, leaf outline size, leaf color, flower color, frequency of flower, shape of tuber and skin color of tuber were scored according to UPOV (Table 1) .The disease severity was scored according to Henfling, 1979 late blight score scale (Table 2).The number of tubers per plant was counted manually and weighed using laboratory weigh balance respectively.

Table 1: UPOV Morphological DUS testing of Irish potato Guidelines on variety descriptors

Parameter	**Upov descriptor**
Plant growth habit	3=Upright, 4=Upright to semi-upright 5=semi-upright, 6=semi-upright to spreading, 7=spreading
Leaf outline size	1=Very small, 4=small, 5=medium 6=medium to large, 7=large, 8=very large
Leaf green colour	1=Very light, 3=light, 4=light to medium, 5=medium, 7=dark
Flower colour	1=White, 2=Purple, 3=Red/Blue
Shape of tuber	1=Round, 2=Short oval, 3=oval, 4=Long - oval, 5=Long, 6=Very long
Skin colour	1=Lightbeige, 2=Yellow, 3=Red, 4= Red parti-colored,5=Blue, 6= Blue parti-colored, 7= Reddish brown
Frequency of flowers	1=absent, 7=present

Source: UPOV, 2001

Table 2: Henfling modified disease estimation scale for late blight of potato

GRADE	% Incidence	Nature of Infection (Level of Resistance / Susceptibility)
1	0.000	Not seen on field
2	0.1000	Only few plants affected here and there; up to 1or2 spots in12 yards radius.
3	1.000	Up to 10 spots per plant, or general light spotting
4	5.000	About 50 spots per plant or up to 1 leaflet in 10 attacked
5	25.00	Nearly every leaflet with lesions, plants still retaining normal form; field may smell of blight but looks green, although every plant is affected.
6	50.00	Every plant affected and about 50% of leaf area destroyed by blight; field looks green flecked with brown.
7	75.00	About 75% of leaf area destroyed by blight, field looks neither predominantly brown nor green
8	95.00	Only a few leaves left green but stems green
9	100.0	All leaves dead, stem dead or drying

Source: Henfling (1979).

3.7 Preparation of pure lateblight innoculum

The pure late blight culture was prepared at KALRO-Tigoni pathology laboratoty.

3.7.1 Procedure

Pea agar preparation

Materials and reagents were prepared ready for the experiment, they included; peas, distilled, technical agar, sucrose, antibiotics, jars, sieve and petri-dishes.60 g of fresh peas plus 375 ml of water was grinded/blend for 2min, was later sieved into a jar.7.5 g of technical agar, 3.75 g of sucrose and antibiotics was added, mixed completely under shaker and topped up with distilled water up to 500 ml and then auto cleaved at 121^0c for 15min after which it was poured into sterilized Petri dish to make culture plate and cooled(plate 3).

Method of sample collection

The diseased leaf sample was taken in KALRO-Tigoni fields during occurrence or epidemic of late blight. The leaves with typical symptom of small lesion and a visible white mildew synonymous with late blight symptoms (ZHU *et al*., 2001) were selected. An infected leaf was sampled randomly from different infected plants within a field. Each sample was labeled with place, date of collection and the name of host variety.

Production of P. infestans inoculums

The white colony of late blight pathogen (according to Jie-hua *et al*., 2001) was removed from freshly infected leaves and cultured on pea agar medium in petri-

dishes for 10 – 14 days at 18ºC in darkness until the dishes were fully covered with the pure colony(Fig 2). The sporangium was harvested using cotton swab soaked in 10 mL sterile water and a cotton swab and soaked in a jar containing distilled to dispense the sporangia. The sporangia suspensions was filtered through four layers of cheesecloth to remove mycelia fragments and adjusted to 25, 000 sporangia per milliliter with a hemacytometer. After the sporangia concentration was determined, the suspension was placed at 4ºC until ready to be used (Jie-hua *et al*., 2001).

Plate 3: Stages in inoculums' preparation at pathology lab in Kalro-Tigoni (Source : Author, 2015)

Screening for Foliage Resistance

Whole plant screening was done in the glasshouse having controlled temperature and humidity. Plants were raised in pots in the glass house. Forty days after planting, the pots were covered with black polythene paper to create conducive conditions for late blight. The humidifier was put on for 1-2 hrs, to ensure that leaf surface was completely wet. The sporangia suspension prepared above was sprayed to the plants, the humidifier was put off for a night and the plants were incubated at $18\pm1^{o}C$ with 90% humidity. After 6 days, score of the plants was taken using Henfling modified disease estimation scale for late blight of potato.

3.8 Data Analysis

The data was subjected to statistical analysis using SAS 9.1.3 portable, analysis of variance (ANOVA) was computed and means separated using least significance difference (LSD) where appropriate at 5% level. The other data was subjected to Genstat software to analyze for principal component analysis (PCA) and Principal Co-ordinate analysis (PCoA) using Manhattan distance and similarity matrix.

Model: $X_{ijkl} = \mu + G_i + D_j + GD_{kij} + \mathcal{E}_{ijkl}$

μ: Overall mean

G_i **:** Effects of i^{th} treatment (genotype)

D_j**:** Effects of j^{th} interaction (dosage)

DG_{ij}**:** Effects of genotype and dosage interaction

E_{ijkl}**:** Random error

CHAPTER FOUR

RESULTS

4.1 Qualitative and Quantitative Traits

4.1.1 Different accessions showing varied variation levels per variety. Kenya Mpya variety

Irradiation Dosage manifested various levels variation on both qualitative and quantitative traits of Kenya Mpya variety and its mutants. Dosage 12 Gy was different with yield in terms of weight of tubers higher, disease score and plant height lower than control. Dosage 6Gy followed with yield in terms of weight of tubers slightly higher, plant height slightly lower and no varied disease score level in comparison to control. However, the rest of traits scored decreased drastically across the other dosage levels 10gy and 3gy sequentially (Table 3).

Table 3: Variations of quantitative and qualitative traits of Kenya Mpya variety under different levels of dosage.

BLK	ETY	ACN	DG	GH	LS	LC	F F	FC	FP	PH	TS	SC	NT	WT	DS
1	1	C-1	0	5	4	5	7	1	30	32	1	1	4	116.4	7
1	2	M109	6	5	7	7	7	1	32	28	2	1	2	354.5	6
1	3	M89	6	3	5	5	7	1	30	29	2	1	7	177.4	5
1	4	M5	3	5	5	5	7	1	36	32	2	1	5	48.8	8
1	5	M30	3	5	5	5	1	1	0	0	0	1	0	0	9
1	6	M284	10	5	5	5	7	1	28	26	2	1	4	57.8	7
1	7	M33	10	3	4	3	7	1	30	34	2	1	3	53.1	9
1	8	M80	12	5	4	5	7	1	30	30	2	1	4	357.7	5
1	9	M81	12	5	5	5	7	1	30	24	2	1	5	414.1	3

Block (BLK), Entry (ETY), Accession (ACN), Dosage (DG), Growth habit (GH), Leaf size (LS), Leaf color (LC), Flower frequency (FF), Flower color (FC), Flowering period (FP), Plant height (PH), Tuber size (TS), Skin color (SC), Number of tubers (NT), Weight of tubers (WT), Disease score (DS), Control/check (C-1) and Kenya Mpya (M).

Asante Variety

The dosages showed different across all the traits with dosage 6 Gy contributing more in terms of weight of tubers, lower disease score/severity and plant height being lower than control. Dosage 12 Gy followed with yield in terms of weight of tubers slightly higher, plant height and disease score did not vary in comparison to control. However, the rest of traits scored decreased drastically across the other dosage levels 10 Gy and 3 Gy sequentially (Table 4). Dosage 10 Gy had lower score in terms of disease severity but lowest weight in terms of weight of tubers .Dosage 3 Gy had higher weight of tubers and slightly lower disease severity in comparison to control.

Table 4: Variations of quantitative and qualitative traits of Asante's variety under different levels of dosage.

BLK	ETY	ACN	DG	GH	LS	LC	FF	FC	FP	PH	TS	SC	NT	WT	DS
2	1	C-1	0	7	5	3	7	3	30	28	2	4	T	43.2	8
2	2	A48	3	3	4	5	7	3	30	36	2	3	3	78.4	6
2	3	A77	3	3	4	5	1	3	28	24	1	4	3	88.7	5
2	4	A103	6	5	5	5	7	3	30	20	2	7	4	167.3	4
2	5	A101	6	7	7	7	7	3	28	18	1	4	6	211.5	3
2	6	A61	10	5	5	5	7	3	28	32	1	3	2	28.7	6
2	7	A42	10	3	5	5	7	3	0	32	0	0	0	0	5
2	8	A78	12	7	7	7	7	1	34	28	2	1	4	86.2	8
2	9	A35	12	7	5	5	7	3	26	28	2	4	3	95.5	5

Block (BLK), Entry (ETY), Accession (ACN), Dosage (DG), Growth habit (GH), Leaf size (LS), Leaf color (LC), Flower frequency (FF), Flower color (FC), Flowering period (FP), Plant height (PH), Tuber size (TS), Skin color (SC), Number of tubers (NT), Weight of tubers (WT), Disease score (DS), Control/check (C-1) and Kenya Asante (A).

Sherehekea variety

Results on dosage levels were different across all the traits with dosage 10 Gy contributing more in terms of weight of tubers, lower disease score/severity and plant height being lower than control. Dosage 6 Gy and 12 Gy followed with yield in terms of weight of tubers slightly higher, plant height and disease score did not vary in comparison to control. However, dosage 3 Gy contributed least across all traits compared to controls, disease severity was more prevalence (Table 4).

Table 5: Variations of quantitative and qualitative traits of sherehekea variety under different levels of dosage.

BLK	ETY	ACN	DG	GH	LS	LC	FF	FC	FP	PH	TS	SC	NT	WT	DS
3	1	C-1	0	7	5	7	7	3	28	32	1	3	8	98.2	7
3	2	S100	3	3	4	5	7	3	30	28	1	3	3	48.9	10
3	3	S1	3	3	4	5	7	3	30	28	1	3	3	8.9	10
3	4	S98	6	7	5	7	7	3	30	28	1	3	5	88.5	7
3	5	S30	6	7	5	7	7	3	30	28	1	3	5	98.5	7
3	6	S15	10	7	5	7	7	3	30	33	1	3	7	109.8	5
3	7	S34	10	7	5	7	7	3	32	29	1	3	7	111.3	5
3	8	S17	12	7	7	7	7	3	32	28	1	3	4	56.8	7
3	9	S114	12	7	7	7	7	3	32	28	1	3	4	86.8	7

Block (BLK), Entry (ETY), Accession (ACN), Dosage (DG), Growth habit (GH), Leaf size (LS), Leaf color (LC), Flower frequency (FF), Flower color (FC), Flowering period (FP), Plant height (PH), Tuber size (TS), Skin color (SC), Number of tubers (NT), Weight of tubers (WT), Disease score (DS), Control/check (C-1) and Kenya Sherehekea(S).

4.2 Qualitative and Quantitative Traits

4.2.1 Glasshouse experiment

Cumulative means separation on dosage levels of 3 quantitative traits recorded at the glasshouse showed significant difference ($p \leq 0.001$) for weight of tubers, number of tubers and disease severity per plant. Dosage 6 Gy was significantly different with yield in terms of weight of tubers and number of tubers higher than control; dosage 10 Gy was significantly different from control with yield in terms of weight of tubers being lower; number of tubers decreased drastically in dosage 3 Gy, 10 Gy and 12 Gy. The level of disease severity/score was also lower at 6 Gy and 3 Gy as compared to control (Table 6).

Table 6: Means of cumulative quantitative traits of Irish potato accessions separated in terms of dosage level grown in the glasshouse in the year 2015

Dosage/Traits	WT	HT	NT	DS
0	10.84b	40.66a	3.02b	8.29a
3	9.04b	40.74a	2.05c	6.79bc
6	32.66a	40.29a	3.89a	6.79bc
10	6.28c	39.15a	1.85c	7.49abc
12	9.82b	40.51a	1.79c	7.63ab
MEAN	10.18	39.83	2.20	7.35
EMS	69.36***	62.36	2.05***	7.89***
CV%	81.79	19.83	64.98	38.25

***=** significant at P≤0.05, **=significant at P≤0.01, ***=significant at P≤0.001. Weight of tubers (WT), height (HT), and number of tuber (NT), Disease score (DS). Means having the same letter are not significantly different at the 5% level of significance according to LSD.

4.2.2 Mau-Narok

Cumulative Means separation by dosages showed significant difference (P≤0.05) for weight of tubers, skin color and number of tubers; (P≤0.01) for leaf size and flower frequency ;(P≤0.001) for growth habit, leaf color, flower color, tuber shape and disease score. Dosage of 12 Gy contributed more in terms of weight of tubers which was higher than the control; dosage 3 Gy and 6 Gy were significantly different from the control in terms of growth habit; dosage 12 Gy was significantly different from control in terms of leaf size; dosage 6 Gy was significantly different from control in flower frequency; dosage 3 Gy,6 Gy and 12 Gy were significantly different from control in flower color; dosage 10 Gy was significantly different from control in flowering period; dosage 12 Gy was significantly different from control in tuber

shape; dosage 6 Gy and 10 Gy were significantly different from control in skin color; dosage 3 Gy,6 Gy,10 Gy and 12 Gy were significantly different from control in number of tubers and dosage 6 Gy and 12 Gy were significantly different from control in disease severity/score by having lower susceptibility (Table 7).

Table 7: Means of cumulative quantitative traits of Irish potato accessions separated in terms of dosage level grown at Mau-Narok in the year 2015

Dosage/ Traits	WT	GH	LS	LC	FF	FC	FP	HT	TS	SC	NT	DS
0	94.26ab	5.86a	4.86bc	4.43b	7.00a	2.14a	30.09ab	30.76a	1.29b	2.43ab	4.62a	6.81ab
3	57.29b	4.20c	4.57c	4.57b	6.20ab	1.53b	28.17ab	27.87a	1.43b	1.80ab	2.93b	7.23a
6	77.90ab	4.75bc	5.08ab	5.05b	5.95b	1.4b	29.28ab	28.63a	1.38b	1.50b	3.15b	5.80b
10	62.68b	5.28ab	4.89bc	4.82b	6.79ab	2.48a	26.67b	27.47a	1.39b	2.64a	3.06b	7.10a
12	117.56a	5.71a	5.53a	6.00a	7.00a	1.43b	31.43a	28.78a	2.43a	1.79ab	4.436ab	5.14c
MEAN	74.2	5.06	4.91	4.88	6.52	1.91	28.44	28.36	1.47	2.1	3.37	6.6
EMS	5470.6*	1.99***	0.69***	1.49***	2.55***	0.78***	72.82	45.91	0.52***	3.14*	6.79*	4.82***
CV%	**94.77**	**29.65**	**17.64**	**29.24**	**21.66**	**46.69**	**33.02**	**31.67**	**53.64**	**79.46**	**79.46**	**84.28**

***=** significant at P≤0.05, **=significant at P≤0.01, ***=significant at P≤0.001. Weight of tubers (WT), growth habit (GH), leaf size (LS), leaf color (LC), flower frequency (FF), flower color (FC), flowering period (FP), height (HT), tuber shape (TS), skin color (SC), number of tuber (NT), Disease score (DS) at 2 and 3 months. Error Mean Square (EMS), Coefficient of Variation (CV). Dosage and sites means having the same letter are not significantly different at the 5% level of significance according to LSD.

4.2.3 Eldoret experiment

The dosages showed significant difference (P≤0.05) for weight of tuber ;(P≤0.01) for plant height ;(P≤0.001) for leaf size, flower color, tuber shape and disease score. Growth habit, leaf color, flower frequency, flowering period, skin color and number of tuber were not significant at all. Dosage 6 Gy contributed more in terms of weight

of tubers higher than the control dosage 0 Gy; dosage 3 Gy and 10 Gy were significantly different from the controls in leaf size, dosage 3 Gy was significantly different from control in flower frequency; dosage 3 Gy and 6 Gy were significantly different from control in flower color; dosage 10 Gy was significantly different from control in plant height; dosage 6 Gy was significantly different from parent in skin color (Table 8).

Table 8: Means of Cumulative quantitative traits of Irish potato accessions separated in terms of dosage level grown at Eldoret in the year 2015

Dosage/ Traits	**WT**	**GH**	**LS**	**LC**	**FF**	**FC**	**FP**	**HT**	**TS**	**SC**	**NT**
0	53.03b	5.48a	4.81a	4.48a	7.00a	2.14ab	30.57a	34.00a	1.38a	2.43a	3.76a
3	46.09b	5.00a	4.52c	4.44a	6.11b	1.44c	30.59a	31.63ab	1.52a	1.59ab	4.89a
6	95.89a	5.04a	5.37ab	4.30a	6.61ab	1.35c	28.65a	31.24ab	1.37a	1.28b	4.69a
10	72.16ab	5.38a	4.93bc	4.52a	6.69ab	2.45a	27.52a	26.19b	1.5a	2.45a	3.47a
12	82.75ab	4.64a	5.45a	4.27a	7.00a	1.73bc	27.64a	31.64ab	1.82a	2.00ab	3.55a
Mean	72.79	5.18	5.01	4.42	6.97	1.88	28.75	29.89	1.47	1.94	4.09
EMS	4758.3*	2.36	0.78***	1.67	2.06	0.77***	90.12	89.70**	0.62***	2.39	11.89
CV%	**94.77**	**29.65**	**17.64**	**29.24**	**21.66**	**46.69**	**33.02**	**31.67**	**53.64**	**79.46**	**84.28**

***=** significant at P≤0.05, **=significant at P≤0.01, ***=significant at P≤0.001.Weight of tubers (WT), growth habit (GH), leaf size (LS), leaf color (LC), flower frequency (FF), flower color (FC), flowering period (FP), height (HT), tuber shape (TS), skin color (SC), number of tuber (NT), Disease score (DS) at 2nd and 3rd months. Error Mean Square (EMS), Coefficient of Variation (CV).Dosage and sites means having the same letter are not significantly different at the 5% level of significance according to LSD

4.2.4 Mau-Narok experiment

In Table 9, disease score/severity, number of tubers, tuber shape and flowering period were positively correlated to weight of tubers at (P≤0.001); plant height and leaf size were positively correlated to weight of tuber at (P≤0.05) and flower frequency was

positively correlated to weight of tuber at (P≤0.01).Number of tubers, skin color, flower color and flower frequency were positively correlated to growth habit at (0.01);Flowering period and leaf color were positively correlated to growth habit at (P≤ 0.05);Leaf size was positively correlated to Growth habit at (P≤0.001).Disease score/severity was negatively correlated to number of tubers at (P≤0.001); negatively correlated to tuber shape and flowering period at (P≤0.01).Number of tubers and tuber shape were positively correlated to leaf size at (P≤0.05);Flower frequency was positively correlated to leaf size at (P≤0.01);Leaf color was positively correlated to leaf size at (0.001).Number of tubers and plant height were positively correlated to leaf color at (P≤0.01);Flower frequency was positively correlated leaf color at (P≤0.05).Number of tubers, tuber shape, plant height and Flower period were positively correlated to flower frequency at (P≤0.001).Disease score was positively correlated to flower color at (P≤0.01);Skin color was positively correlated to flower color at (P≤0.001);Plant height was negatively correlated to flower color at (P≤0.001);Flowering period was negatively correlated to flower color at (P≤0.05).Number of tuber and tuber shape were positively correlated to flowering period at (P≤0.001).Disease score was negatively correlated to flowering period at (P≤0.01);Plant height was positively correlated to flowering period at (P≤0.01);Number of tuber was positively correlated to plant height at (P≤0.01);Skin color was negatively correlated to plant height at (P≤0.001).Disease score was negatively correlated to tuber shape at (P≤0.01);Number of tuber was correlated to tuber shape at (P≤0.001);Skin color was correlated to tuber shape at (0.01).Disease score/severity was negatively correlated to number of tubers at (P≤0.001) (Table 9).

Table 9: Correlation among quantitative/qualitative traits of Irish potato accessions in Mau-Narok site year 2015

	WT	GH	LS	LC	FF	FC	FP	HT	TS	SC	NT
WT											
GH	0.117										
LS	0.143*	0.286***									
LC	0.088	0.166*	0.254***								
FF	0.206**	0.194**	0.184**	0.170*							
FC	0.048	0.210**	0.107	0.104	0.050						
FP	0.275***	0.135*	0.023	0.040	0.222***	0.149*					
HT	0.157*	0.080	0.075	0.187**	0.318***	0.248***	0.169**				
TS	0.322***	0.082	0.156*	0.019	0.299***	0.127	0.516***	0.076			
SC	0.098	0.184**	0.114	0.046	0.021	0.710***	0.269	0.331***	0.185**		
NT	0.695***	0.159**	0.133*	0.177**	0.222***	0.044	0.345***	0.175**	0.297***	0.153*	
DS	**0.582*** **	**0.112**	**0.066**	**0.003**	**0.038**	**0.165****	**0.201****	**0.094**	**0.187****	**0.002**	**0.371*** **

***=** significant at P≤0.05, **=significant at P≤0.01, ***= significant at P≤0.001.Weight of tubers (WT), growth habit (GH), leaf size (LS), leaf color (LC), flower frequency (FF), flower color (FC), flowering period (FP), height (HT), tuber shape (TS), skin color (SC), number of tuber (NT), Disease score (DS) at 2 and 3 months.

4.2.5 Eldoret experiment

In Table 10, number of tubers, skin color, tuber shape, and plant height and flower period were positively correlated to weight of tubers at (P≤0.001); flower frequency was negatively correlated to weight of tubers at (P≤0.01).Plant growth habit was positively correlated to skin color, flower color and leaf size at (p≤0.001).Skin color, flower color and leaf size were correlated to plant growth habit at (P≤0.001);Plant height was negatively correlated and leaf color was positively correlated at (P≤0.05).Flower color and leaf color were positively correlated to leaf size at (P≤0.001);flower frequency was positively correlated to leaf size at (P≤0.01).Number of tubers and skin color were positively correlated to leaf color at (P≤0.05);Flower color was correlated to leaf color at (P≤0.001).Skin color was positively correlated to flower color at (P≤0.001).Number of tubers, skin color and tuber shape were positively correlated to flowering period at (P≤0.001).Number of tuber was positively correlated to plant height at (P≤0.001).Number of tuber and skin color were positively correlated to tuber shape at (P≤0.001).

Table 10: Correlation among quantitative/qualitative traits of Irish potato accessions in Eldoret site year 2015

	WT	GH	LS	LC	FF	FC	FP	HT	TS	SC	NT
WT											
GH	**-0.103**										
LS	**0.101**	**0.346*** **									
LC	**0.088**	**0.136***	**0.378*** **								
FF	**-0.178** **	**0.064**	**0.196** **	**0.025**							
FC	**0.111**	**0.474*** **	**0.222*** **	**0.286*** **	**-0.081**						
FP	**0.273*** **	**-0.055**	**-0.001**	**0.019**	**0.047**	**-0.056**					
HT	**0.203*** **	**-0.139***	**-0.061**	**0.023**	**0.015**	**-0.303*** **	**0.112**				
TS	**0.407***	**-0.011**	**-0.055**	**0.089**	**-0.009**	**-0.047**	**0.502*** **	**0.042**			
SC	**0.327*** **	**0.313*** **	**0.112**	**0.152***	**-0.120**	**0.692*** **	**0.326*** **	**-0.197** **	**0.385*** **		
NT	**0.641*** **	**-0.101**	**0.104**	**0.142***	**-0.059**	**-0.067**	**0.259*** **	**0.316*** **	**0.337*** **	**0.107**	

***=** significant at P≤0.05, **=significant at P≤0.01, ***=significant at P≤0.001.Weight of tubers (WT), growth habit (GH), leaf size (LS), leaf color (LC), flower frequency (FF), flower color (FC), flowering period (FP), height (HT), tuber shape (TS), skin color (SC), number of tuber (NT).

4.3 Factor and Principal Component Analysis (PCA)

Principal component analysis at glass house showed that the first two principal components were important and accounted for 95.42% of the total variation. The first component (PCA1) accounted for 65.62% of the variation with major contribution from weight of tubers followed by plant height, then number of tubers and consequently disease score/severity. PCA2 accounted for 29.80% of the variation with greater contribution from plant height, disease score, number of tubers and weight of tubers sequentially (Table 11).

Table 11: Principal component analysis of 581 Irish potato accessions in the glasshouse showing their contribution to quantitative traits variation

Quantitative traits		
Variables	**PCA 1**	**PCA 2**
Dscore	0.01020	0.04815
HT	0.15001	0.98728
NT	0.09140	0.00519
WT	0.98440	-0.15143
% variation	**65.62**	**29.80**

Disease score (Dscore), Plant height (HT), Number of tubers (NT), Weight of tubers (WT).

Principal component at medium altitude environment (Eldoret) showed that all components were important and both qualitative and quantitative traits contributed to a total of 98.09% PCA1 accounted for 96.38% of variation with weight of tubers

contributing the greatest, followed by Flowering period, plant height, number of tubers, skin color, tuber shape, leaf color, flower color, leaf size, plant growth habit and lastly flowering frequency sequentially. PCA2 accounted for 1.71% of the total variation with flowering period contributing most, followed by plant height, number of tuber, tuber shape, flower frequency, leaf color, skin color, leaf size, plant growth habit and lastly flower color (Table12).

Table 12: Principal component analysis of 147 Irish potato accessions in Eldoret showing their contribution to both quantitative and qualitative traits variation

Qualitative/quantitative traits		
Variables	**PCA 1**	**PCA 2**
FC	0.00121	-0.02959
FF	-0.00364	0.01681
FP	0.03743	0.75986
GH	-0.00231	-0.01828
HT	0.03695	0.64258
LC	0.00147	0.00138
LS	0.00109	-0.00487
NT	0.03263	0.06740
SC	0.00700	-0.00003
TS	0.00439	0.02570
WT	0.99804	-0.05455
%Variation	**96.38**	**1.71**

Flower color (FC), Flower frequency (FF), Flowering period (FP), Plant Growth habit (GH), Plant height (HT), Leaf color (LC), Leaf size (LS), Number of tubers (NT), Skin color (SC), Tuber shape (TS), Weight of tubers (WT).

Principal component Analysis at Mau-Narok (High altitude area) showed that the components were important with total contribution of both qualitative and quantitative traits at 98.97% of the total variation. PCA1 accounted for 97.72% of the variation with major contribution from weight of tubers, flowering period, number of tubers,

plant height, flower frequency, tuber shape, skin color, plant growth habit, leaf size, leaf color, flower color and disease score/severity. PCA2 accounted for 1.25% of the variation with flowering period contributing most, plant height, number of tubers, flower frequency, tuber shape, skin color, plant growth habit, leaf color, leaf size, disease score, flower color and weight of tubers systematically (Table 13).

Table 13: Principal component analysis of 147 Irish potato accessions in Mau-Narok showing their contribution to both quantitative and qualitative traits variation

	Qualitative/Quantitative traits	
Variables	**PCA1**	**PCA 2**
Dscore	-0.01795	-0.01128
FC	-0.00044	-0.02392
FF	0.00436	0.04450
FP	0.03160	0.95793
GH	0.00236	0.01621
HT	0.01259	0.26889
LC	0.00147	0.01131
LS	0.00176	-0.00448
NT	0.02392	0.05403
WT	0.99895	-0.03569
SC	0.00286	0.03413
TS	0.00366	0.04026
%Variation	**97.72**	**1.25**

Disease score (DScore), Flower color (FC), Flower frequency (FF), Flower period (FP), Plant Growth habit (GH), Plant height (HT), Leaf color (LC), Leaf size (LS), Number of tubers (NT), Weight of tubers (WT), Skin color (SC) and Tuber shape (TS).

4.4 Analysis of Similarity and Distance Matrix Using Principal Co-Ordinate Analysis (PCoA)

4.4.1 PCoA in glasshouse

Plot of first two PCoA axes in the glasshouse showed accession 89, 113, 368, 109, 284 were distinguished from the other accessions. Accession 89, 109 and 113 are Kenya Mpya varieties at 6 Gy, accession 368, is Asante variety at 6 Gy and accession 284 is Kenya Mpya at 10 Gy. Accession 109 from glasshouse had higher yield in terms of weight of tubers, plant height of 46cm and moderately resistant to late blight with every plant affected and about 50% of leaf area destroyed by blight (Fig 3).

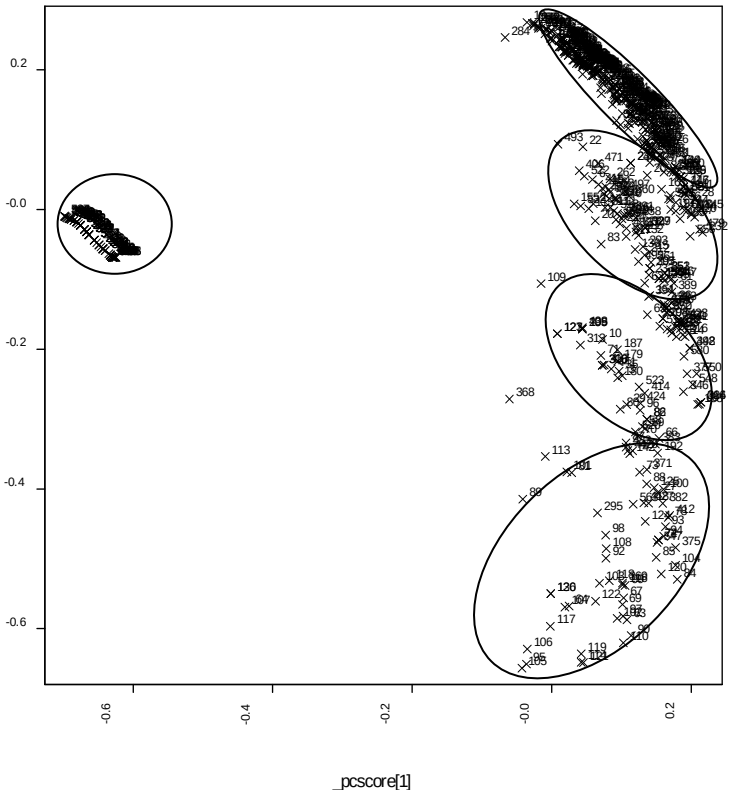

Fig 3: Scatter plot diagram of the first two Principal co-ordinate analyses depicting genetic diversity amongst 581 accessions grown in glasshouse based on morphological traits.

4.4.2 PCoA at Eldoret field

Plot of first two PCOA axes in Eldoret field indicates Accession 89 was completely different from the other accessions and accession 101,97,103,91,55,30,34,33 and 92 were distinguished from the other accessions considering the scores of first two PCOA. The circle grouped accession(119,105,113,100,124,107,116,98,114) together

and they were different from parents/controls. Similar circle also grouped accession (48, 35, 78, 77, 61, 42, 48, 16, 1 and 41) together and they were different from parents/controls. Accession 97, 91, 89 and 92 are Asante at 6 Gy; accession 101 and 103 are Asante at 10 Gy; accession 55 and 33 are Kenya Mpya at 6 Gy and lastly accession 34 is Sherehekea at 6 Gy. Accession 101 from Eldoret proved the best with higher yield/weight of tubers, days to flowering period of 36days, and plant height of 29cm (Fig 4).

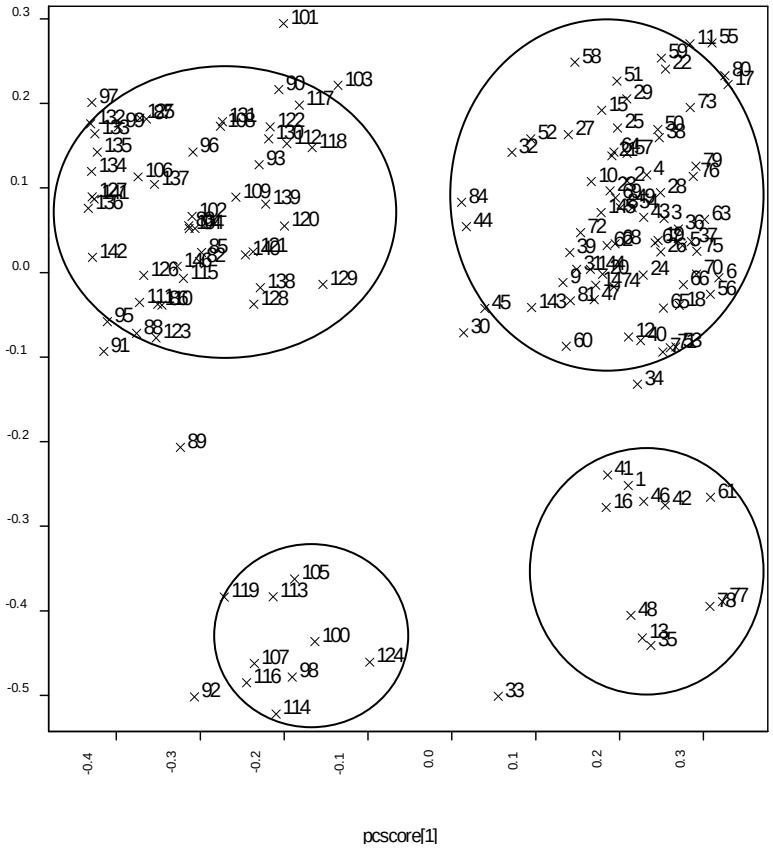

Fig 4: Scatter plot diagram of the first two Principal co-ordinate analyses depicting genetic diversity amongst 146 accessions grown in Eldoret based on morphological traits.

4.4.3 PCoA at Mau-Narok field

Plot of first two PCOA axes in Mau-Narok field indicates accession 91, 81, 80, 146, 90, 10, 5, 63, 43 and 92 were distinguished from the other accessions. Accession 90, 91 and 92, are Asante at 6Gy; accesssion10, 63, and 43 are Kenya Mpya at 6Gy;

accession 5 is Kenya Mpya at 3Gy and accession 81 and 80 were Kenya Mpya at 12Gy. Accession 81 had higher yield/weight of tubers and plant height of 32 and days to flowering period of 30days and highly resistant to late blight Up to 10 spots per plant, or general light spotting (1% infection) (Fig 5).

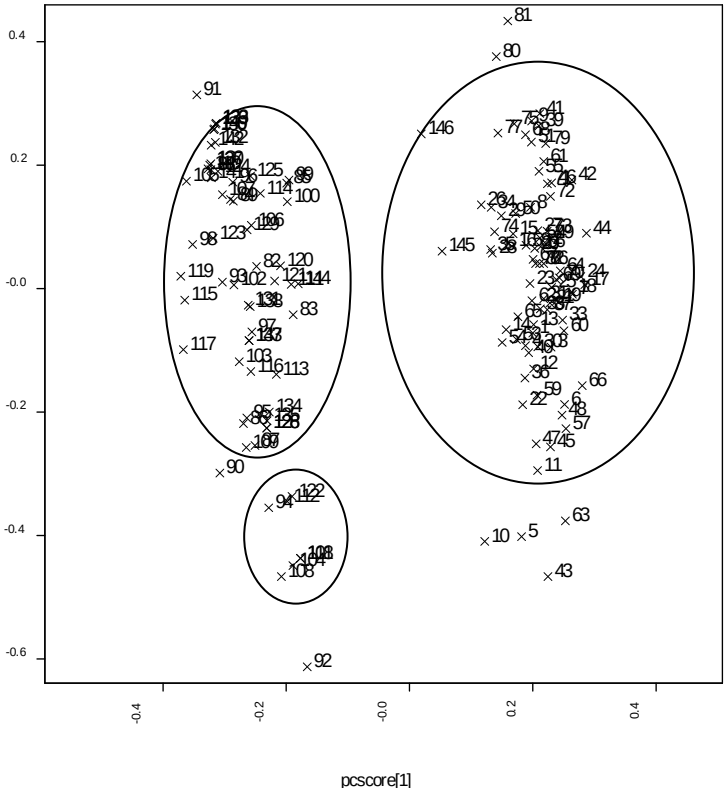

Fig 5: Scatter plot diagram of the first two Principal co-ordinate analyses depicting genetic diversity amongst 146 accessions grown in Mau - Narok based on morphological traits.

CHAPTER FIVE

DISCUSSION

5.1 Effects of mutagenesis on potatoes performance in controlled and field experiments.

Due to their genotypic differences, plants respond differently to irradiation dosages. Higher doses of radiation cause chromosomal damage in plant meristematic cells, deceleration of the cell cycle, and delay of mitosis, which significantly affect overall plant regeneration and development, however an increase in radiation dosages boosts mutation frequency (Orkun and Sema, 2012;Wongyai *et al*., 2001).

In this study, five levels of mutation dosages inclusive of control (0 Gy, 3 Gy, 6 Gy, 10 Gy and 12 Gy) were evaluated to determine the suitable dosage level. The LSD means separation by dosage results at 5% level indicate that the genetic variability occurred in all the mutagenic treatments and morphological and agronomic traits under study showed wide range of genetic variability. All dosage levels were significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ for both qualitative and quantitative traits at all sites. Analysis of Anova showed that variability across all levels of dosages varied with dosage 6 Gy contributing highest cumulative percentage/positive variability amongst all other dosages, followed by 3 Gy, 10 Gy and 12 Gy compared to parents/control (0 Gy). Thus, within a group of the same type of plant irradiated with a given dosage, the formation of different genotypic and phenotypic characters was observed in relation to variety.

Dosage 12 Gy was effective to Kenya Mpya variety, accession M81 was high yielding disease score lower than control (Table 3). Therefore, it was highly resistant to late blight Up to 10 spots per plant (1% infection). Dosage 6 Gy was effective to Asante's

variety contributing more in terms of weight of tubers; A101 was high yielding and disease score/severity lower than control (Table 4). Dosage 10 Gy was effective to Sherehekea variety with Accession S34 contributing more in terms of weight of tubers, lower disease score/severity than control (Table 5). It was moderately resistant to late blight with every plant affected and about 50% of leaf area destroyed by blight. The highly positive correlations suggested that all these traits provided the similar information about the variations among the genotypes and they all were tending to discriminate the genotypes in similar fashion (Aghaei *et al*., 2010). The positive and significant association of these traits can provide plant breeders an understanding of phenotypic traits and their degree of association to be able to plan breeding schemes and managements of plant germplasm and new accessions.

The analysis of diversity through Principal component analysis (PCA) in regard to quality traits allowed their grouping according to the similarity hierarchy (Gregorczyk *et al*., 2008). Traits evaluated at both sites (Eldoret and Mau-Narok) showed similarity with weight of tubers contributing most in the first PCA and flowering period contributing most in the second PCA. The traits that form the first and second principal components show the strongest discriminatory power that diversifies the studied accessions (Rymuza *et al*., 2012). The strongest discriminatory power, regardless of the growth system, was shown by weight of tubers, plant height and flowering period. This traits are important in potato breeding since they contribute to economic yield of potatoes (Arterburn *et al.*, 2007).

PCoA explores for similarities between items, by analyzing distance matrix, such that similar cases are close together. In this study, Plot of first two PCoA axes in glasshouse separated accessions into five clusters showing maximum similarity and minimum similarities in all three genotype. However, accessions 89, 113, 368, 109 treated at 6 Gy and 284 treated at 10

Gy were completely different from the other mutants and were distinguished from similarity coefficients that reflected the genetic diversity between the accessions. Accession 89, 368 and 284 had plant height playing a major role in the first axis of differentiation compared to parents. Plot of first two PCoA axes plotted from the Eldoret site separated accessions into four clusters with two clusters constructed on the basis of the similarity matrix showing that the mutants were genetically close to each other: circle (119, 105, 113, 100, 124, 107, 116, 98, 114) and circle (48, 35, 78, 77, 61, 42, 48, 16, 1 and 41). Maximum similarity and minimum similarity was recorded across all the three genotypes. However, accession 89 was completely different from the other accessions and accession 101, 97, 103, 91, 55, 30, 34, 33 and 92 treated at 6 Gy and accession 103 and 101 treated at 10 Gy were completely different from the other genotypes and were distinguished from similarity coefficients that reflected the genetic diversity between the accessions. Plot of first two PCOA axes from Mau-Narok site separated accessions into three clusters showing maximum similarity and minimum similarity in all three genotype. However, accession 90, 91, 92, 10, 63, and 43 treated at 6 Gy; accession 5 treated at 3 Gy and accession 81 and 80 treated at 10 Gy were completely different from the other genotypes and were distinguished from similarity coefficients that reflected the genetic diversity between the accessions.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study was conducted to evaluate effect of induced mutagenesis on morphological and Agronomical traits of potato mutants majoring yield, maturity time and late blight resistance. The results indicate that mutation was effective and it created sufficient genetic variations among the potato accessions tested hence can be exploited for breeding and selection of improved genotypes of Irish potato production.

Cumulative means separation of level of dosages and Analysis of Variance showed that variability occurred across all levels of dosages varied with dosage 6 Gy contributing highest total of both quantitative and qualitative traits. However, all dosages behaved differently in regard to potato variety showing the higher the dosage level the higher the percentage/positive variability.

Morphological descriptors after correlation analysis, principal component analysis (PCA) and principal co-ordinate analysis (PCOA) grouped the potato accessions into groups in relation to parental traits magnifying genetic divergence among the accessions. The main goal of potato breeding is to develop potential varieties that ensure the highest and stable production in a range of environments. For this reason, there is need to collect, characterize potato genotypes and cluster analysis groups genotypes on the basis of similarity and thus provides a hierarchical classification (Arslanoglu *et al*., 2011).

Selection of various accessions with higher yield in terms of weight of tubers, low levels of late blight infection was discovered which were M81 which was Kenya

Mpya irradiated at dosage 12 Gy, S34 which was Sherehekea irradiated at dosage 6 Gy and A101 which was irradiated at dosage 10 Gy. Therefore, specific objective 3 and 4 were successfully achieved and induced mutation have been successfully used to improve yield, quality, and disease and pest resistance in many crops including potato (Gnanamurthy *et al*., 2012)

6.2 Recommendation

1. The accessions that performed well in terms of yield (Number of tubers and weight of tubers) and late blight resistance in both sites are recommended for further testing and inclusion in the Irish potato breeding and improvement programme: Selection, plant development and characterization of Gene sources.
2. Other dosage levels can be evaluated for other positive traits and since effective mutation occurred with increased dosage level, further evaluations can be done on dosage level above 15 Gy.

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APPENDICES

Appendix 1: Software and experimental design description

Augmented design

It's a single plant experiment (no replication), it's used in segregating population with limited seed supply of test lines in the early stage of breeding scheme, and therefore, insufficient seeds are available for a replicated experiment. In addition, for large numbers of test lines, it is difficult to arrange them in one block because of environmental heterogeneity in the field. Thus the augmented design with only one replication for test lines is proposed (You et al., 2013; Federer, 1956; Federer et al., 1975; Federer and Raghavarao, 1975). The basic idea of the augmented design is:

(1) Control lines are arranged in a standard design

(2) Each replication of the control lines is placed in a soil-homogeneous block

(3) The block is augmented to contain more non-replicated test lines.

Based on control lines in a standard design, the block effects can be estimated to adjust the observed values of the test lines, and the error to test the significance of performance differences among lines (You et al., 2013).

SAS PROGRAMME FOR AUGMENTED DESIGN

PROC GLM and **PROC MIXED** data is arranged in design 1-CVS" with inputs Accession, Block, Entry followed by other qualitative and quantitative traits inclusive.

Appendix 2: Potato Catalogue Kenya 2013 as Per National Potato Council of Kenya (NPCK)

NATIONAL IRISH POTATO VARIETY DESCRIPTION USED IN THE STUDY: solanum tuberosum L.

Variety name/code	**Year of Release**	**Owner(s)**	**Maintainer and seed source**	**Optimal production altitude range(masl)**	**Duration to maturity(months)**	**Tuber yield(t ha`1)**	**Special attributes**
Asante	1998	KALRO	KALRO-Tigoni	1800-2600	3-4	35-45	Round with pink smooth skin stems upright to semi-upright light green leaves Good chipping, boiling & mashing quality Fairly Tolerant to late blight
Kenya Mpya	2010	KALRO/CIP	KALRO-Tigoni/PQS	1400-3000	3.0-3.5	35-45	Oval/round tubers tall potato plant with a height of about 1 meter strong semi erect stems light green

							medium sized leaves white flowers regular flowering habit Oval shape with shallow eyes Cream-colored skin with pink eyes and cream-colored flesh Early tuberization, large size tubers cream white skin color with pink eyes shallow eye depth cream white flesh color Resistant to late blight

							Good storability Short dormancy Good for table, chips and mashing Wide adaptability
Sherekea	2010	KALRO/CIP	KALRO-Tigoni/PQS	1800-3000	3.5-4.0	40-50	oblong/round tubers medium sized potato plant (slightly below1 meter in height) Strong semi erect-stems dark green small to medium sized leaves very abundant light purple flowers Regular flowering

							habit. High number of tubers per plant red skin color Medium eyes depth Cream flesh color High Resistant to late blight and viruses Good storability Intermediate dormancy Good for table, crisp and mashing