UNIVERSITY OF CAPE COAST

DEVELOPMENT OF MAIZE STREAK DISEASE RESISTANT LINES USING GAMMA RADIATION

YAYRA AFRAM

2020

Yayra Afram

University of Cape Coast

UNIVERSITY OF CAPE COAST

DEVELOPMENT OF MAIZE STREAK DISEASE RESISTANT LINES USING GAMMA RADIATION

BY

YAYRA AFRAM

Thesis submitted to the Department of Crop Science of the College of Agriculture and Natural Science, University of Cape Coast, in partial fulfilment of the requirement for the award of Doctor of Philosophy degree in Crop Science

March, 2020

DECLARATION

Candidate's Declaration

I thereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature …… Date ……

Name: Yayra Afram

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature …… Date ….....

Name: Prof. Elvis Asare-Bediako

Co-Supervisor's Signature ……. Date ……

Name: Dr. Godwin Amenorpe

ABSTRACT

Maize streak disease (MSD) is the most important viral disease affecting maize production in Ghana. Management options employed to mitigate the effect of the disease has not been successful. Breeding for disease resistant is the most sustainable option. Mutation breeding was used in this study to develop maize mutants' lines that are resistant MSD. Radiosensitivity was first carried out to determine the optimum dose for mutation induction in six cultivars of maize grown in the study region. Four maize genotypes namely Obatanpa, Honampa, Pann 53 and Dapango were selected based on the radiosensitivity test results and acutely irradiated at 288.5 Gy, using a cobalt 60 (^{60}Co) source delivering at a dose rate of 300 Gy hr^{-1}. The irradiated seeds were planted with controls. Separate field trials for the selected maize genotypes were conducted from M_1 to M_4 generations to screen for MSV resistance and improved agronomic traits. Fourteen putative mutants were selected across the four maize genotypes at the end of the M_4 generation based on disease severity score and yield indices. The selected mutants were planted in a screen house and challenged with MSV to confirm resistance by polymerase chain reaction (PCR) and visual symptomology. The results indicate that, four (TZS/KPO/140001 TZS/KPO/140003 TZS/KPO/140004 and TZS/KPO/140005) out of the 14 mutants selected were resistant to MSV while the remaining ten (10) were susceptible. The four mutants were all mutated from the Obatanpa maize genotype. This study was therefore conducted to induce host plant resistant to MSD.

KEY WORDS

Disease resistance

Maize streak disease

Mutant

Polymerase chain reaction (PCR)

Radiosensitivity

Symptomology.

ACKNOWLEDGEMENTS

I am grateful, first and foremost to God of Israel, for His Divine Grace, favour and sustenance to complete this work. I would like to express my deep and sincerest gratitude and appreciation to my supervisors, Professor Elvis Asare-Bediako, Dean School of Agriculture, University of Cape Coast and Dr. Godwin Amenorpe, Centre Manager, Nuclear Agriculture Research Centre of the Ghana Atomic Energy Commission (GAEC). I also wish to express my profound gratitude, to Dr. Grace van der Puije, Head of Department of Crop Science, UCC and Professor Harry M. Amoatey Head of Department of Nuclear Agriculture and Radiation Processing of the Graduate School of Nuclear and Allied Sciences, University of Ghana (Atomic campus) for their valuable contributions, guidance, patience, encouragement and constructive suggestions towards the success of this thesis. My next thanks go to the following institutions and research centers; Radiation Technology Center of Ghana Atomic Energy Commission, Soil and Irrigation Research center of University of Ghana, Nkwanta Municipal Agricultural Research Station, Maize Breeding and Entomology Division of Crops Research Intitute, Kwadaso – Kumasi, Staff and Students of Kwadaso Agricultural College and last but not the least, Crop Science Department of Kwame Nkrumah University of Science, and Technology, Kumasi. Special thanks to the entire staff of the Molecular Biology Laboratory of the Cocoa Research Institute of Ghana through Kirkhouse Trust Laboratory.

I am finally indebted to all my benefactors who contributed both materials and financial resources to my course not forgetting Prof. Elvis Asare-Bediako, Dr. Godwin Amenorpe, Iddrisu Abdulai, Amekli Alice, Obeng Java Vida, Kessie Gustav, Kaledzi Dotse, Pastor Philip Bayor, my former teachers, students and my Prayer warriors all over my beloved country Ghana.

DEDICATION

This whole thesis is dedicated to the Graduate School of Nuclear and Allied Sciences, University of Ghana and Ghana Atomic Energy Commission and all Public Universities in Ghana notably, University for Development Studies, University of Ghana, Kwame Nkrumah University of Science and Technology and University of Cape Coast.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures **Page**

LIST OF ABBREVIATIONS AND ACRONYMS

AAP	Acquisition Access Period
AAS	Atomic Absorption Spectrometry
AGDP	Agricultural Gross Domestic Product
ANOVA	Analysis of variance
ASI	Anthesis to Silking Interval
CIMMYT	International Maize and Wheat Improvement centre
CV%	Coefficient of variation
DAE	Days After Emergence
DNA	Deoxyribonucleic acid
DS	Disease severity
dsDNA	Double stranded DNA
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immuno-absorbent assay xii
et al	Etcetera
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization of the United Nations
G x E	Genotype by environment interactions
g	grammes
GAEC	Ghana Atomic Energy Commission
GMO	Genetically modified food
Gy	Gray
HR	Highly resistant
HS	Highly Susceptible
IAP	Inoculation Access Period

kDa	kiloDaltons
Kg	Kilogram
LD_{50}	Lethal dose killing 50% of target
Lsd	Least significant difference
M_1, M_2, M_3 and M_4	Mutant generations, one, two, three and four.
MAbs	Monoclonal antibodies
mg	Milligrammes
ml	milliliter
Mld	Mild
mM	Millimolar
MoFA	Ministry of Food and Agriculture
MSV	Maize Streak Virus
MSD	Maize Streak Virus Disease
NDSilk	Number of Days to 50% Silking
NDTassel	Nunmber of Days to 50% Tasselling
NS	Not Significant
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RCA	Rolling circle amplification
RD_{50}	Refers to the dose which reduces growth rate and seed production by 50%.
RDP	Recombination detection program
Rep	Replicase
RFLP	Restriction fragment length polymorphism

RNA	Ribonucleic acid
S	Significant at p=0.05;
ssDNA	Single stranded DNA
T ha^{-1}	Tonne per hectare
TNA	Total nucleic acids
UCC	University of Cape Coast
µg	Microgram
µl	Microlitre
µm	Micrometre

CHAPTER ONE

INTRODUCTION

Maize streak disease (MSD) is a viral disease transmitted by insect vector. The virus belongs to a genus Mastrevirus of the family Geminiviridae that is endemic in sub-Saharan Africa and neighbouring Indian Ocean islands. The disease is epidemics throughout the maize growing regions of Africa (Harkins et al., 2009; Darren & Shepherd, 2009). The development of conventionally resistant maize varieties has been a priority since the 1950s in affected regions, with a good deal of success: however, there are several genes associated with resistance, and conventional breeding is complex (Harkins et al., 2009). Genetic engineering offered some hope but consumers are skeptical about the safety of the living modified organisms (Shepherd et al., 2007). Mutagenesis is simple, cost effective and also a faster method of developing resistant lines in other crops reported except maize (IAEA, 2011). Mutagenesis was used in this study to develop resistant lines for maize streak disease. The MSD resistant line is important to boost productivity of maize in Ghana since demand for maize from the feed industry, breweries, small livestock producers and local consumers has increased remarkebly as a result of population pressure (MOFA, S.R.I.D, 2011).

Production and consumption of maize

Maize (*Zea mays* L.) originated from Central Mexico 7000 years ago and was brought to Africa by the European merchants in the 16th Century (Smith, 2001). It was introduced to West Africa by the Portuguese merchants who brought it to Ghana through Sao Tome and Principle (Gorter, 1953; Fajemisin and Shoyinka, 1976; Effron et al., 1989). Its cultivation gradually spread from the coast through the rainforest to the Sahel part of the entire sub-region (Gibson and Benson, 2002). It

eventually displaced native food crops such as sorghum (*Sorgum bicolor*) pearl millet (*Pennisetum glaucum*) and fonio (*Digitaria* spp.) (Gibson and Benson, 2002; Doebley, 2004).

Globally 140 million hectares of land is used to grow maize with developing countries cultivating approximately 96 million hectares. Four countries account for more than half (53.6%) of the developing world's land area of maize cultivation with China accounting for 26 million hectares; Brazil, 12 million hectares; Mexico, 7.5 million hectares; and India, 6 million hectares. Although 68% of global maize area is in the developing world, only 46% of the world's maize production of 785 million tons is grown there (USDA, 2014). Maize production in Africa has not seen much improvement over the last few decades with an average production of 51.0 million tons on 18 million hectares of land. The bulk of the production of approximately 12.0 million tons of maize grain comes from South Africa on a 3.1 million hectares of land (Shiferaw et al., 2011). Ghana produces a total of 2.2 million tons of maize on 865 00 hectares of land across the ten regions of the country with Brong Ahafo and Ashanti regions being the two leading producers (MOFA, S.R.I.D, 2011). Maize production provides employment to several people in both rural and urban areas of Ghana, and hence source of income and livelihhod.

Maize is the major staple food in Ghana and is used to prepare a variety of diets including porridge, *banku* and *kenkey*. A small percentage is also used to formulate animal feed which is mainly used by the poultry industry. Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 kcal/100 g (Mopelola et al., 2016). Maize provides many of the vitamins B and essential minerals along with fibre, but lacks some other nutrients, such as vitamin

B12 and vitamin C, and is, in general, a poor source of calcium, folate and iron (Nuss & Tanumihardjo, 2010).

Statement of the Problem

In spite of the immense economic importance of maize, its production in Sub-Saharan Africa (SSA) is hindered by abiotic and biotic constraints leading to low yields that fail to meet demand. Yield losses of up to 100% due to MSD have been experienced in many countries of West Africa and in East Africa (Fajemisin et al., 1986). Martin and Shepherd (2009) reported losses to the tune of US$120-480 million per year due to MSV in Africa in terms of lost income and higher maize prices. Current average yield of maize in Ghana is 2.2 Mt Ha^{-1}, far below the potential yield of 5.5 Mt Ha^{-1} (MoFA, 2017). This yield is generally low compared to global average of 4-5 t ha^{-1} and over 11 t ha^{-1} in the United States of America (FAOSTAT, 2016). At least half of such loss could be potentially recovered with the effective control of MSV (Martin and Shepherd, 2009).

Control strategies such as good farm management practices, application of chemical insecticides, and the planting of MSD tolerant maize varieties has not been able to prevent the disease. The main reason for this is the inherent unpredictability of MSD epidemics which makes it difficult for farmers to decide where and when to apply appropriate interventions (Shepherd et al., 2007a). One of the sustainable ways of managing the MSV disease is breeding for host plants resistant (Lagat et al., 2008). Coordinated attempts have been made over the past 40 years to produce MSV-resistant maize using traditional conventional selective breeding method that only resulted in limited tolerant varieties to MSV such as CIMMYT's OSU23I, CIRAD's C390 and IITA's Tzi3 (Efron et al., 1989), which does not guarantee complete resistance.

Collaborative efforts to incorporate streak resistance as an integral part of maize improvement programme is progressive at various maize breeding research insttutes (Bosque-Perez et al., 1998). The difficulty in breeding a completely resistance maize variety through conventional breeding is the lenghty time required. Before resistant lines are fully developed, new strains of the MSV emerges and negate the objective of the conventional breeding method (Efron et al., 1989). Although there are some successes in the release of a number of MSD tolerant maize varieties such as Akposoe, Mamaba, Etubi Obatanpa and Dorke in Ghana (Twumasi-Afriyie et al. 2012), recent survey by Asare-Bediako et al., (2017) reported all the varieties to be susceptible.

Maize streak disease resistant variety developed through genetic engineering was also reported in South Africa (Shepherd et al., 2007b). Genetic engineering could provide direct solution but consumers are apprehensive about the future implications of genetically modified organisms (Harkins et al., 2007). Besides, most African countries lack research funding to support such expensive venture (Lomonossoff, 1995). Mutagenesis is simple, cost effective and also a faster method of developing resistant lines in other crops reported except maize (IAEA, 2011). Mutation breeding is exploited as an alternative approach to conventional breeding for faster development of useful traits and lunching beyond hybridisation (Amenorpe et al, 2010), Physical and chemical mutagenesis has been proven to be effective in inducement of useful mutants with novel gene alleles and for increasing genetic diversity for enrichment of germplasm resources in crops (Shu, 2009; Waugh et al., 2006). The main precaution of using mutagen is to avoid the annealation of planting material because radio-sensitivity of genotypes are biotype and species dependent (Broertjes & Van Harten, 2013).

Research Questions:

What dose of gamma irradiation would be optimum and effective to induce mutation?

What maize genotypes could be developed into maize streak disease resistant mutant?

Will specific molecular primer be useful in mutant confirmation?

Significance of the Study

Improvement of crop production regarding pest and disease management is one of the main goals in plant breeding. It is estimated that by the year 2030, demand for maize in developing countries will surpass the demand for both wheat and rice (Shiferaw et al., 2011). This shift will be reflected in a 50% increase in global maize demand from its 1995 level of 558 million tons to 900 million tons by 2030. Maize requirements in the developing world alone will increase from 282 million tons in 1995 to 504 million tons in 2030 (Pingali, 2001; Pinstrup-Anderson et al., 1999). The challenge of meeting this unprecedented demand for maize will be to bridge the potential yield gap of maize production by breeding maize streak disease resistant genotypes which has been reported to cause significant yield loss. Yield losses of up to 100% due to MSD have been experienced in many countries (Fajemisin et al., 1986) to the tune of US$120-480 million per year (Martin and Shepherd, 2009) and at least such amount could have been recovered by developing MSD resistant mutants.

Genetic engineering using pathogen derived resistance (PDR) has shown particularly exciting promise as a technique for producing MSV resistant maize (Shepherd et al., 2007a, b). In spite of the enormous potential of genetic engineering, like all other MSD control options, this technology has its own drawbacks. It is an expensive, labour intensive and time-consuming process to push a genetically

modified crop variety along the development pipeline from the initial research phases, through greenhouse and field trials, and finally on to commercialisation. Besides these development costs, genetically modified organisms (GMOs) face stiff opposition from individuals and organizations who raises reservations about the future health complications of GMOs. Breeding for disease resistance through conventional breeding of cross-pollinated species such as maize, also requires development of pure lines by self-pollinating each year, till the seventh year or more. This is not only expensive but time consuming and requires a lot of labour to achieve (Hallauer and Miranda, 1988).

The clearly define available option to control the menace of MSD is therefore to explore the crops own genetic resource through mutation breeding. Mutation breeding unlike conventional breeding is faster, more precise and has been used to improve a host of cereals such as rice (*Oryza sativa*) pearl millet (*Pennisetum* spp.) sorghum (*Sorghum bicolor*) wheat (*Triticum aestivum*), oat (*Avena sativa*), barley (*Hordeum vulgare*) (IAEA, 2011). Maize has been a vital model organism for basic research for nearly a century. As a model organism, maize is the subject of extensive biological investigations such as plant domestication, genome evolution, developmental physiology, epigenetics, pest resistance, heterosis, quantitative inheritance, and comparative genomics (Strable & Scanlon, 1999). The effects of physical and chemical mutagens are well characterized and are very similar to the spontaneous mutation occurring in nature that generates genetic variability for crop improvement (Rai et al., 2011; Suprasanna et al., 2012).

The main advantage of mutation breeding is genetic modification of one or more characters without changing the plant's genome (Wani and Anis, 2008; Amenorpe, 2010). Mutation breeding technique has been utilized to develop diseases resistant

lines in several crops except maize. Barley powdery mildew disease resistance (Mejlhede et al., 2006), cassava mosaic disease resistance (Amenorpe et al., 2010) are few examples. There is therefore the need to employ mutagenesis to develop resistant lines for maize streak disease.

Delimitation

Mutation breeding involves the use of chemical or physical mutagen or the combination of the two. This study however explored only physical form of mutation induction from cobalt 60 gamma radiation source on six (6) varieties (Dapango, Domambin, Dzinu eve, Keta 60, Obatanpa, Pan 53) of maize.

Limitations

The study centre was the northern part of the Volta Region, Nkwanta which is lying between latitudes 7 30° and 8 45° North and longitude 0 10° and 0 45° East, has a relatively short raining season which impedes the late season trial and monitoring. The late season or the minor season is the period with the highest prevalence of the virus transmitting insects (leafhopper) with concomitant high rate of MSV infection. However, the short raining season did not affect the selection process as maize plants showing resistance on the field were tagged, watered up to physiological maturity.

Objective of the study

The overall objective of this study was to use nuclear technique to induce and develop maize lines that are resistant to maize streak disease.

The specific objectives were to:

i. Determine the Lethal dose (LD_{50}) and the optimum dose which reduces. growth rate and seed production by 50% (RD_{50}) in six maize genotypes.

ii. Induce variability for selection of maize streak disease resistant putative mutants by applying an optimum dose of radiation.

iii. Confirm the MSV resistance status of the maize mutants using PCR with MSV specific primer following artificial inoculation.

Organization of the Study

Chapter One	General introduction
Chapter Two	Literature review
Chapter Three	Radiosensitivity test
Chapter Four	Genetic induction for variability in four maize genotypes and selection of useful mutants
Chapter Five	Artificial inoculation of mutants with maize streak virus (MSV) and molecular confirmation for disease resistant
Chapter Six	Summary, conclusions and recommendations

Chapter Summary

Induced mutations in plant breeding have played a significant role in meeting challenges related to world food and nutritional security by way of mutant germplasm enhancement and their utilization for the development of new mutant varieties. A wide range of genetic variability has been induced by mutagenic treatments for use in most economically important plant species. Mutation breeding therefore offers both hope and prospects for breeding against the maize streak viral disease that threatens the much-anticipated increase in productivity of maize in Ghana. Maize streak disease currently threatens food security and livelihoods of the affected communities in Sub

Saharan Africa, where maize is the staple food. Despite the availability of MSD control strategies, ranging from good and timely cultural practices to the use of chemical insecticides and the planting of MSD tolerant cultivars, the disease still remains endemic. The main reason for this is the inherent unpredictability of MSD epidemics which makes it difficult for farmers to decide where and when to apply appropriate control strategies. A novel approach to control the menace of the MSD is mutation breeding which explores the plant's own genetic resources. Unlike conventional breeding and hybridization, mutation breeding is faster, cost effective, transferrable, non-hazardous and environmentally friendly. It is based on selfing mutants until the induced character has a stable expression in the advanced mutant generations.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter has been compiled to cover relevant research advances in maize production, importance and utilisation of maize around the world, the epidemiology of maize streak disease (MSD) effect and interventions as well as the prospects of mutation breeding in crop improvement programs and molecular approaches to disease detection. Latest publications and databases available online as well as manuals and conference proceedings available through library resources were accessed. Review papers, books and book chapters on different aspects of MSD and mutation breeding were also consulted.

Origin, Botany and Classification of Maize

Origin of maize

Archaeological evidence suggests Central Mexico as the origin of maize from a wild grass teosinte, where some small corn cobs, estimated at more than 5000 years old, were found in caves (Piperno and Flannery, 2001). Maize is generally considered a man-made crop as native Americans transformed it over a period of 1000 years through natural and artificial selection to a plant with larger cobs and more rows of kernels, making it a better source of food. Improved maize provided enough food for the bulk of their diet for an entire year, allowing people to live in one location for much longer period of time. The spread of maize from its centre of origin in Mexico to various parts of the world has been remarkable and rapid with respect to its evolution as a cultivated plant and as variety of food products. The inhabitants of several indigenous tribes in Central America and Mexico brought the plant to other

regions of Latin America, the Caribbean, and then to the United States and Canada. European explorers took maize to Europe and later traders brought maize to Asia and Africa (Vollbrecht and Sigmon, 2005; Gibson and Benson, 2002).

Botany and Classification of Maize

Linnaeus included the name as species epithet in the botanical classification Zea (*Zea mays* L.). It is generally agreed that teosinte (*Z. mexicana*) is an ancestor of maize, although opinions vary as to whether maize is a domesticated version of teosinte, (Goodman & Galinat, 1988). Maize is diploid with 20 chromosomes (2n = 2x = 20), Zea is a genus of the family Poaceae, commonly known as the grass family. It is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous leaf blades. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 cm long, on a thickened, almost woody axis (cob). The whole ear is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as silky threads (Hitchcock and Chase, 1971).

Pollen is produced entirely in the staminate inflorescence (tassel) and ovules, entirely in the pistillate inflorescence (ear). Maize could be self-or-cross-or both self- and cross- pollinated. Wind, insects and birds are among a host of pollinators. Maize is naturally suited for outcrossing because the tassels mature faster than the silk, a condition referred to as protandry (Chapman and Peat, 1992; Purseglove 1988). Many forms of maize are used for food, sometimes classified as various subspecies related to the amount of starch each has:

Flour corn —*Zea mays var. amylacea*

Popcorn —*Zea mays var. everta*

Dent corn —*Zea mays var. indentata*

Flint corn — *Zea mays var. indurata*

Sweet corn — *Zea mays var. saccharata* and *Zea mays var. rugose*

Waxy corn —*Zea mays var. ceratina*

Amylomaize — *Zea mays*

Pod corn —*Zea mays var. tunicata* Larrañaga ex A. St. Hil.

Striped maize —*Zea mays var. japonica*

Economic importance

Maize has a host range of economic value more than any other cereal. Virtually every part of the maize plant has a value, including grain, leaves, stalks, tassels and stovers. Maize is a staple in the African region where the consumption ranges from 52 to 328 g/person/day and the region of the Americas where the highest consumption was 267 g/person/day in Mexico (Ranum et al., 2014). It is often grown as a food crop for home consumption as well as for the market. Increasingly it is also used for animal feed. In the developed countries, maize is used primarily as animal feed and secondarily for production of food and industrial products such as starch, sweeteners, and ethanol (Alene et al., 2009).

World Production

The adaptability of maize has made it the world's most widely grown cereal. It is grown in extremely cool, moderate, and very hot climates, under moisture regimes ranging from extreme wet to semiarid, on flat terrain as well as steep hillsides, in many different types of soil, and using a host of production technologies (Pingali, 2001). Estimated world production of maize as at 2014 was 875.3 million tons, with

the United States of America, China and Brazil harvesting 31%, 24%, and 8% of the total production respectively (FAOSTAT, 2016). Africa's contribution to the global output is consistently low with an average production of 51.0 million tons on 18 million ha of land. The bulk of the production of approximately 12.0 million tons of maize grain comes from South Africa on a 3.1 million ha of land followed by Nigeria with estimated production of 8.0 million tons. Major importers of maize include Japan, South Korea, Mexico and Africa (USDA, 2014).

Maize production and utilisation in Ghana

Maize production in Ghana is mainly on subsistence level and is dated as far back as pre- colonial period. The total land area dedicated to the cultivation of maize is approximately 1 million ha with an estimated total production of 2.2 million tons (MOFA, S.R.I.D, 2011) The huge potential gap necessitated a marginal import of 20000 mt to meet domestic demand. Maize is cultivated all over the country with two planting seasons in agro ecological zones having bimodal rainfall and one planting season for the guinea and sudan savanna zones which have unimodal rainfall. The leading maize producing areas in Ghana are Wenchi, Ejura, Techiman and Tamale (MOFA, S.R.I.D, 2017).

Maize is the main staple food in Ghana and it is milled and processed into flour or dough and used to prepare a variety of dishes. The average consumption of maize products is 53 g/person/day. Quite substantial amount of maize is also processed into feed and fed to farm animals, mainly the poultry birds (FAOSTAT, 2016).

Maize production constraints

Although maize is increasingly becoming such an important cereal and staple food crop, the average yield in most developing countries particularly in Africa is still the lowest in the world. World-wide average maize yield is about 4.5 metric tons per hectare while Africa's estimate is as low as 1.7 t ha^{-1} (FAOSTAT, 2005). Unless this situation is urgently addressed, food security in most part of the continent will be under threat. Both socio-economic and biophysical factors are the main contributors to the persistent low yields on farmers' fields. DeVries and Toenniessen (2001) summarised these factors in five categories which include: a) the range and intensity of biophysical constraints; b) large agro-ecological variation; c) the under developed state of seed sectors in most developing countries; d) the absence of policies which encourage crop improvement; and e) very low and declining soil fertility in much of Africa.

Biotic and abiotic constraints

Maize is attacked by an array of biotic and abiotic factors that curtail productivity. These abiotic constraints include drought, declining soil fertility, high acidic soils, soil erosion and high temperatures (Pingali, 2001). Biotic factors are primarily related to tropical insects and diseases and weeds. According to Pingali (2001), the dominant diseases that cause significant yield loss and yield gap between potential and actual yields in Sub-Saharan Africa are downy mildew (*Peronosclerospora sorghi*), turcicum leaf blight (*Exserohilum turcicum*), maize streak virus (MSV), grey leaf spot (*Cercospora zeaemaydis*) and various species of stem borers. Globally MSD is regarded as the third most serious disease of maize after northern corn leafblight (NCLB) and grey leaf spot (GLS) (Pratt and Gordon, 2006). In Africa, MSD is a bigger problem than both NCLB and GLS (Pingali and

Pandey 2001) and is therefore arguably responsible for more human suffering than any other plant disease.

Maize streak virus (MSV)

Maize streak virus belongs to the genus Mastrevirus and family Geminiviridae. Geminiviruses are plant- infecting viruses with a monopartite or bipartite single-stranded DNA (ssDNA) encapsidated in twinned icosahedral particles (Brown et al., 2012). The virus is obligately transmitted by as many as six leaf hopper species in the genus Cicadulina, and mainly by C. *mbila* and C. *storeyi.* Maize streak virus epidemiology is related to environmental influences on the vector species, leading to erratic epidemics in every 3–10 years (Martin and Shepherd 2009). The virus has circular DNA with approximately 2700 base pairs and is reported to have 11 strains of which, MSV-A is the most common and the cause for severe forms of the disease (Martin et al. 2001).

Transmission of MSD geminivirus

Cereal geminiviruses are transmitted only by leafhoppers (Storey, 1928; Harrison, 1985; Jonker and Flett, 1997). Grass geminiviruses are transmitted by a wide range of genera and species in the Cicadellidae. Maize streak virus is one of the seven viruses that attack maize crops in Africa although worldwide there are 32 viruses reported in maize (Rose, 1978). Maize streak virus is believed to have evolved with native grasses and is indigenous to Africa and the adjacent Islands (Storey, 1936; Bock, 1974; Thottappilly et al., 1993). Cicadulina species vary in their ability to transmit maize streak disease. However, the transmission is inherited, dominant and sex linked (Storey, 1928, 1936; ISAAA, 2001) with males being heterozygous (ISAAA, 2001). The DNA of the virus is mechanically transmitted (ISAAA, 2001).

The viral sequence or genome, however, can be introduced into the plant by inoculation techniques via Agrobacterium tumiefaciens. Agrobacterium transmission mechanism can be a useful substitute in inoculation of plants with MSV where there are limited fascilities in mass rearing of leafhoppers (Hammond, 1994).

Maize streak virus disease symptoms

Maize streak disease symptoms are characterized by broken to almost continuous chlorotic stripes centered on the tertiary leaf veins (Briddon et al., 1990). They first manifest as minute pale circular spots on the lowest exposed part of the leaf. Only new leaves develop the symptoms of virus infection while leaves below the point of infection remain healthy (Hill and Waller, 1988). The spots develop into discontinuous paleyellow streaks, up to several millimetres in length, along the blades, parallel to the veins or broken chlorotic streaks on the veins being less affected than the secondary and tertiary veins. The longitudinal chlorotic streaking causes a reduction in photosynthetic area, growth and yield of the plant. The streak often fuses laterally to give narrow broken chlorotic stripes which usually extend over the entire length of fully affected leaves (Lazarowitz et al., 1989).

In highly susceptible genotypes, chlorotic streaks tend to coalesce to form a uniform chlorosis. The chlorosis is as a result of reduced photosynthesis and increased respiration leading to reduction in leaf length and plant height (Damsteegt, 1983). Thus, maize plants infected within the first three weeks after emergence become severely stunted producing little or no ear. If infection occurs eight weeks after emergence the virus does not cause any significant economic loss (Page et al., 1999). Figure 2.1 shows a typical MSV symptoms on maize plant.

Figure 2.1: Maize plant infected by maize streak virus. Photograph by Yayra Afram.

Economic importance of MSD

The MSD infection of maize starts from emergence to tasseling and often infection at seedling stage results in no ear formation (Magenya et al., 2008) but this varies with the level of resistance from different cultivars (Bua & Chelimo, 2010). Infection at the six to eight weeks stage after planting has a little effect on the vigour of the plant but eventually results in undersized and poorly filled ears (Fajemisin, 2003). In susceptible varieties, yield reductions often exceed 70 % depending on the stage of plant maturity when infection occurs (Magenya et al., 2008). Viral infection significantly reduced the plant height and leaf area index of the varieties used (Bua & Chelimo, 2010). The extent of yield loss due to MSD is dependent on weather, vector population densities, percent carry over inoculums, growth stage of the crop when infection occurred and time of infection (Bjarnason, 1986). Severe outbreaks

are often associated with late plantings or second season cropping (Efron et al., 1989). Small-holder farmers are the most affected of significant yield losses because they hold on to landraces in spite of the substantial progresses made to develop varieties that are resistance to the MSD (Martin and Shepherd, 2009).

Genetic basis of MSV resistance

Genetic control of resistance in MSD-tolerant line Tzi 4 conducted by Kyetere et al. (1999), reported a single major gene (designated MSD1) controlling the resistance. However, the mode of inheritance studies or genetic control of resistance to MSD was controlled by two or three gene pairs (Kim et al., 1989). Conventional selective breeding method only produce limited tolerant varieties to MSV such as CIMMYT's OSU23I, CIRAD's C390 and IITA's Tzi3 (Efron et al., 1989), which does not guarantee complete resistance. Other lines documented were IB32 (IITA (1980), TZ-Y and "La Revolution" (Soto et al., (1982), PANNAR's A076, and KARI's Embu 11 (ISAAA, 2001) were also tolerant. CML 202 which was a major QTL developd through genetic engineering was reported to be resistance (Welz et al., 1998, Pernet et al., 1999). None of these were developeded through mutagenesis. Shepherd et al., (2014) also reported MSV-resistant transgenic maize lines constitutively expressing "dominant negative mutant" versions of the MSV Rep. It was later observed that resistance-conferring transgenes are expressed only in MSV-infected cells. However, most known inducible transgene expression systems in genetic engineering are hampered by background or "leaky" expression in the absence of the inducer (Shepherd et al., 2014).

Conventional Breeding of MSD Resistant Lines

Conventional breeding has achieved some level of success in producing important genetic gains in maize breeding. Through conventional selection, resistant cultivars have been developed and have continued to play a major role in reducing the threat posed by MSD. For example, many high-yielding maize cultivars have been developed and released to farmers in sub- Saharan Africa (Timothy et al., 1988). Efforts to improve resistance to MSD in maize germplasm have produced significant selection gains. Conversion of susceptible, but high yielding cultivars and landraces in various countries in Africa, into MSD-tolerant ones at IITA have been made (Efron et al., 1989). The Pannar Seed Company of South Africa has also developed and released MSD-resistant hybrids in several African countries (Barrow, 1992). The pedigree method is often used for developing inbred lines in maize. Lines expressing complete resistance to MSD were developed from five cycles of inbreeding and selection (Rodier et al., 1995). Selections in maize with inbreeding from F1 to F3 generations have resulted in open pollinated cultivars (OPVs) that were 16% improved for MSD resistance (Pixley et al., 2006).

Population improvement selection methods have also been successful in improving MSD resistance. Tang and Bjarnason (1993) reported that both modified full-sib recurrent selection and backcrossing were highly effective in improving MSD resistance without sacrificing yield. They suggested that simple backcross and backcrossing with selfing were equally efficient in converting susceptible cultivars to MSD resistant ones. The limitations faced with the use of conventional methods usually arise from difficulties in working with some traits. Most quantitatively inherited traits have low heritability and are subject to genotype by environment interactions. (Obilana and Hallauer, 1974). The process of selection may also take

long and can be laborious. Dreher et al., (2000) mentioned that phenotypic selection can be difficult to use to efficiently select the best allelic combinations for linked target regions. Mutants with novel gene alleles and increased genetic diversity will be a useful option in the long quest in breeding against the MSD (Shu and Lagoda., 2007).

Heritability, genetic advance, path coefficient and correlation studies of maize

It is imperative for every breeder to know the fraction of phenotypic variation of a trait that is heritable (Kearsey and Pooni, 1996) since the efficiency of a selection programme primarily depends on the degree of genetic variation and heritability of a trait. Heritability presumes that genotypes which are very much related have the tendency to look like one another than the distantly related ones (Falconer and Mackay, 1996) and its estimate helps breeders to allocate resources required to efficiently select for preferred traits and to realize the highest genetic gain within a short time (Smalley et al., 2004). It can be estimated as narrow or broad sense subject to the genetic variance used or on an individual plant basis and on a progeny mean basis depending on the generation used (Hallauer et al., 2010). Njoroge and Gichuru, (2013); Vivek et al., (2010) found out the fraction of phenotypic variation due to genes is high for most foliar diseases ranging from 70 to 85 %.

Information on combining abilities, heterosis and heterotic grouping are important components for the successful development or breeding of new high yielding hybrids in any breeding programme (Legesse et al., 2009). Such information can show the type of gene action involved in controlling quantitative characters, thereby assisting breeders in selecting suitable parents (Hallauer and Miranda, 1988). Significant values of general combining ability (GCA) and specific combining ability (SCA) may be interpreted as indicating the performance of additive and non-additive

gene action, respectively (Sprague and Tatum, 1942). General combining ability helps breeders to exploit existing variability in breeding materials to identitify individual genotype(s) conferring desirable attributes and to distinguish relatedness among genotypes (Vacaro et al., 2002). Specific combining ability helps breeders to determine heterotic patterns among populations or inbred lines, to identify promising single cross hybrids and to assign them into heterotic groups (Vasal et al., 1992). The estimation of additive and non-additive gene action through this technique is useful in determining the possibility of commercial exploitation of heterosis and isolation of pure lines among the progenies of the good hybrids (Stuber, 1994).

Correlations are measures of the intensity of association between genetic traits (Steel and Torrie, 1984). Selection for a single character may increase chances for all traits that are positively correlated but declines for characters that are negatively correlated. According to Hallauer and Miranda (1988) the correlation estimated by the specific coefficient is important in plant breeding because it quantifies the degree of genetic and non-genetic association between two or more traits, allowing the indirect selection. Cruz and Regazzi (1997) also highlighted the importance of correlations, stating that these associations quantify the possibility of indirect selection gains in correlated traits.

Mutation breeding

Mutation breeding or mutagenesis is the purposeful application of mutations in plant breeding. Unlike hybridization and selection, mutation breeding has the advantage of improving a defect in an otherwise elite cultivar, without losing its agronomic and quality characteristics (van Harten, 1998). Induced mutations have played an influential role in increasing the world food security, since new food crop varieties embedded with various induced mutations have contributed to the significant

increase of crop production at locations people could directly access (Jain and Maluszynski, 2004; Mba, 2013).

Naturally, the frequency of spontaneous mutations is very low and cannot be used in plant breeding for developing new improved varieties. Therefore, the rate of mutations needs to be enhanced by physical and chemical mutagenic treatments (Till et al., 2004). Among the various groups of mutagens, gamma rays have proved to be more economical and effective because of their easy availability and power of penetration (Moussa, 2006). The application of mutagen to biological system activates a number of physical and chemical steps between the initial absorption of energy and the final biological injury. One of the most important targets is the water molecule, which is omnipresent in organisms. The ionized water molecule (H_3O^+) and the radicals H^+ and OH^- are produced due to the primary reactions of excitation and ionization. In biological tissues, these ionizations are induced all along the radiation path, leading to chain reactions that produce secondary free radicals as a result of hydrogen ions becoming trapped (Esnault et al., 2010). These radicals can damage or modify important components of plant cells and affect certain physiological and biochemical processes that might be vital for organism survival. Previous studies (Al-Salhi et al., 2004; Hameed et al., 2008) have revealed that seed exposure to high doses of gamma rays disturbs the protein synthesis, hormone balance, leaf gas exchange, and enzyme activity. The morphological, structural, and functional changes depend on the strength and type of mutagen applied. Although mutagenesis has become a short cut to conversional breeding, it is without some limitations. Large doses of mutagens are harmful to organism and a breeder may easily loose a genetic material due to radiation exposure (Dresselhaus et al., 2001). Mutation in nature also occurs in low frequencies and this may lead to screening large

population to detect and select the desirable mutant. It is a long-standing observation that most mutations are recessive. That is, they do not lead to visible phenotypic effects when in heterozygous combination with the dominant allele. This makes most mutation breeding programmes tedious and un attractive as its success is largely based on probability (Çelik & Atak, 2017). Mutagenesis may also lead to apomitic plants with male sterility (Dresselhaus et al., 2001).

Mutation induction

In 1901, Hugo de Vries coined the word "mutation" for sudden hereditary changes in different characters in the evening prime rose (*Oenothera lamarckiana*) (Hugo de Vries, 1901). But later it was found that most of these changes were not gene mutations in the sense of the modern term, but due to polyploidy, polysomy or rare combination of existing genes occurring in a peculiar genome. However, the term "mutation" was retained to define sudden hereditary changes in the genotypes of any organism, excluding the trivial ones which are due to recombination at meiosis or due to somatic crossing over (Auerbach, 1962). Gager (1908) reported the artificial induction of genetic changes by using rays of radiation on plants. Later on, the genetical proof of Mendelian inheritance of artificially induced mutants through X-rays irradiation was demonstrated by Muller (1929) in Drosophilla, Stadler, (1928) in maize, Goodspeed (1929) in barley and Auerbach (1962) in tobacco. The success in the irradiations marked the beginning of new era in both fundamental and applied mutation research.

Mutagenic Agents and Their Use in Mutation Breeding

Mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level. Physical mutagen was used exclusively in mutation research until the effects of certain chemicals on the DNA were published in the mid-

1940s (Auerbach, 1962; Rapoport, 1946). In the beginning, X-rays were used, but gamma rays from radioactive sources such as ^{60}Co and ^{137}Cs became popular because they were made available to many developing countries through the IAEA. Fast neutrons from nuclear reactors can also be used, particularly as this irradiation service is available through the Joint FAO/IAEA Division in Vienna (FAO/IAEA, 2014).

Ionizing radiation results in chromosomal breakages, allowing cross-linking of DNA strands. More deleterious effects, therefore, can be expected than from chemical mutagens that result in minor changes in the DNA such as base-pair substitution. Among the physical mutagens, ultraviolet (UV) rays are non-ionizing (Table 2.1). They therefore have low penetration and are effective in producing purine or pyrimidine dimers, resulting in point mutations. UV rays can be effectively used to irradiate pollen in the late or early uninucleate stages (van Harten, 1998; IAEA, 2011) Treatment conditions and doses are experimentally derived through a process known as a radiosensitivity. An ideal dose for mutation induction has often been recommended as the one that reduces growth and morphogenic performance to 50% (LD_{50}) targeted seed/plant (IAEA, 2014, Amenorpe, 2010).

The discovery and application of chemical mutagens came as an alternative for inducing mutations and also for controlling high rates of chromosome aberrations resulting from ionizing radiation and the accompanied detrimental effect on plant genome (Medina et al., 2004). The use of chemical mutagens is also very simple and can be done in any biological laboratory with basic equipment. However, it should be kept in mind that most chemical mutagens are also strong carcinogens. For this reason, all protocols of mutagenic treatment should be carried out wearing gloves and under a biohazard flow-hood. These safety conditions are not necessary for treatment with sodium azide (NaN_3) which is a very powerful mutagen, but only for a limited

number of species, including barley, rice, maize, oat, sorghum, sesame, jute and soybean (Nilan, 1981). Numerous chemical mutagens have been successfully used for crop improvement. Other widely used chemical mutagens are alkylating agents, with ethyl methane sulphonate (EMS) being the most widely used because of its effectiveness and ease of handling, especially its detoxification through hydrolysis for disposal. Nitroso Compounds are the other alkylating agents also in use, but they are light-sensitive and more precautions need to be taken because of their higher volatility (FAO/IAEA, 2009).

Chemical mutagens are also popular in *in vitro* mutation induction, although irradiation can also be applied at low doses (Jain, 2006). Ethyl Methane Sulphonate (EMS) has thus become the mutagen of choice for developing mutant populations for high throughput screening such as in developing targeting induced local lesions in genomes (TILLING) populations (McCallum et al., 2000; IAEA, 2011). Table 2.1 shows commonly applied physical and chemical mutagens and their sources.

Table 2.3: Commonly applied physical and chemical mutagens and their sources.

Physical mutagen	Source	Chemical mutagen	Source
Gamma (γ) radiation	Radioisotopes	Ethyleneimine (EI)	Alkylating
Alpha (α) particles	Radioisotopes	Dimethyl sulphate (DMS)	Alkylating
Beta (β) particles	Radioisotopes	Diethyl sulfatedES (DES)	Alkylating
Neutrons	Nuclear reactors	Ethylmethanesulphonate (EMS)	Alkylating
Ion beam	Particle accelerators	N-ethyl-N-nitrosourea ENU (ENH)	Alkylating
X-rays	Electrons	N-methyl-N-nitrosourea MNU (MNH)	Alkylating
Cosmic rays	Outer space	N-methyl-N-nitro-N-nitroguanidine MNNG	Alkylating
Ultra violet (UV) light	UV light	Sodium azide NaN3	–
		Streptomycin	Antibiotic

Source: FAO/IAEA (2009)

Macro and Micro mutations

Observable change in plant morphology such as leaf colour are known as macro mutations. Macro mutations produces a large phenotypic effect easily recognizable on individual plant basis. They are oligogenic in nature with observable effects such as alternation of shape and size of leaves and pods etc. Micro mutations produce small phenotypic effect, cannot be recognizable on individual plant basis, detected only in group of plants and need treatment of statistical data. It is polygenic in nature and selection delays till M_3. It is mutations with invisible phenotypic changes. (Hansson et al., 2007). Viable morphological and chlorophyll macro mutations also known as chlrosis are produced in M_2 and M_3 generations. Chlorosis are the most dependable index to judge the effectiveness of mutagen at the phenotypic level and are considered as the major groups of macro mutations. Chlorophyll mutations are classified into albina, xantha, chlorine, viridis, albo-viridis, virido-albina, albo-xantha, striata etc. (Gustafsson, 1940; Gaul, 1964). For crop improvement point of view chlorophyll mutations are considered to be of less breeding value but they are reported in most mutation research studies in various spectrum and frequencies depending upon the dose and type of mutagen.

Applications of Mutation Breeding and Impact of Mutant Cultivars

Officially about 3,218 mutant varieties have been catalogued on the FAO/IAEA mutant database (http://mvgs.iaea.org). Many induced mutants were released directly as new varieties while others were used indirectly as parents to derive new varieties. Mutation induction with gamma-ray radiation was the most frequently used method to develop direct mutant varieties (89%) (FAO/IAEA, 2009). Mutation techniques have played a significant role in increasing rice production in the Asia-Pacific Region. By 2000, 434 mutant varieties of rice were released with

improved characters such as semi-dwarf height, early maturity, improved grain yield, disease- and cold-tolerance, and improved grain quality (Jain and Maluszynski, 2004). During 2000 to 2014, a total of 62 released mutant rice varieties showed abiotic stress tolerance in addition to other agronomic characters (FAO/IAEA, 2014)

Asia tops the worldwide release of mutant cultivars (1142), Europe (847) and North America (160). This list included many important crops, including rice, wheat, cotton, oilseed rape, sunflower, sesame and grapefruit. Of the 2 252 cultivars, 75 percent (1700) are in crops and the rest (552) in ornamental and decorative plants. In sexually propagated crops, with 1603 mutant cultivars released, cereals (1072) dominate, followed by legumes (311), industrial crops (81), vegetables (66), oil crops (59) and others (111). Nearly 25% (815 varieties) of the total registered mutants are developed by China alone. The global monetary value derived from commercial mutant cultivars from combined cereals, cotton and fruit alone, is over $20 billion (Ahloowalia et al., 2004).

Mutation Breeding of Cereals

Cereal species have most often been the subject of improvement through the use of mutation techniques. Cereals such as rice, barley, wheat and maize, other species, including some exotic ones, have also been the subject of mutagenic treatment in several countries (Table 2.2). The majority of more than 1000 mutant cultivars were obtained after radiation, especially gamma ray treatment, and directly released (Rutger, 2006). Development of cereal varieties with waxy starch, composed almost entirely of amylopectine with little or no amylose, has been one of the most important objectives of commercial plant breeding. Waxy starch wheat has broad potential commercial uses in the food, paper and adhesive industries for making better quality products. Despite many breeding efforts over the last two decades, there are no wheat varieties with fully waxy starch. Using the TILLING approach for mutation

generation and discovery, a research team from Anavah Inc., USA, induced and identified 246 mutated waxy alleles in two elite wheat varieties (Dong et al., 2009).

Table 2.4: Some important officially released mutant cereal

Cereal	Scientific name	No. of released mutant cultivars		
		Direct	Crosses	Total
Wheat	*Triticum aestivum*	148	49	197
Rice	*Oryza sativa*	291	143	434
Maize	*Zea mays*	12	56	68
Pearl millet	*Pennisetum*s pp.	3	2	5
Sorghum	*Sorghum bicolor*	12	1	13
Oat	*Avenasativa*	5	16	21
Barley	*Hordeum vulgare*	53	216	269
Rye	*Secale cereal*	4	–	4

Source: (FAO/IAEA Mutant Varieties Database, 2014)

Mutation breeding of maize

There has been series of mutation breeding of maize to determine the effect of gamma radiation on germination, growth, photosynthesis and induced free radicals (Marcu et al., 2013). A site-specific mutagenesis was used in maize to create mutations within the gene encoding large subunit of the endosperm enzyme Adenosine Diphosphoglucose Pyrophosphorylase (AGP) and the result led to an 11−18% increase in seed weight (Giroux, et al., 1996). Plant breeders in Padjadjaran University, Indonesia applied varied doses of gamma radiation to increase the phenotypic variation and diversity of plant height, leaf number, stem diameter, ear weight, days to tasselling and physiological maturity. These traits were screened and developed into a module and used in breeding of maize for anticipating global climate change (Syafii et al., 2015). From the FAO/IAEA Mutant Varieties Database, about 26 varieties of maize from Bulgaria have been directly released through mutation breeding for abiotic stress tolerant (Tomlekova, 2010).

Mutation breeding for disease resistance

The possibility of using induced mutations for disease resistance has been of much interest in recent years. Mode of inheritance to diseases and gene behaviour are important in mutation breeding for disease resistant (Wang et al., 1994). In a related experiment, Rao (1990) treated okra seeds with various doses of gamma rays and chemical mutagents and found some mutants having disease resistance. Different kinds of disease resistant have been catloqed in induced fuits, dwarf and early mutant, cup shaped leaf mutant, tall mutant with long stout fruits and bold seeds and mutant with small fruit and bristles. The indirect use of ionizing radiations has proved to be useful in a few cases for the transfer of resistance genes from some species and even genera to others, to severe linkages, to rebuild karyotypes and for any other way to facilitate gene recombination (Haq et al., 2001). At least 32 independent mutations in the mlo locus have been reported and, of these, most have been induced by chemical mutagens, five with radiation and one (mlo11) occurs naturally (Mejlhede et al., 2006).

Mutation rates, detection and confirmation

Random nature of mutational events applies not only to natural mutations but also to those induced. Thus, there is little control over the magnitude or kind of genetic change expected. However, there are reasonable estimates on the frequency of mutations. Provided an effective treatment is given, a particular gene could be expected to mutate once in about 10 000 treated cells (Yonezawa and Yamagata, 1977). Mutation rates were reported for following crops: *Arabidopsis* has mutation frequency of 14 base mutations per 1.5 kb fragment length out of 3000 induced plants (Till et al., 2003). Mutation rates for hexaploid wheat has been estimated at 1 per 25

kb (Slade et al., 2005). In *Brassica rapa* 1 per 60 kb and for maize and barley the rates were as low as 1 per 500 kb (Stephenson et al., 2010; Till et al., 2004).

Although genome sequencing is considered the 'gold standard' for mutation detection as it can reveal the exact location of a mutation and its type, applying this standard to large populations is costly and is rarely used in practical mutation breeding. Advances in plant genomics in recent times especially large-scale genome sequencing, have opened new possibilities for application of mutation induction techniques in crop improvement. Reverse genetic strategies such as TILLING (Targeting Induced Local Lesions in Genomes) and next generation sequencing are used in development and confirmation of mutants (McCallum et al., 2000). New molecular biological tools such as simple sequence repeat (SSR) have been developed that can screen mutants at the gene level for the presence or absence of Maise streak viral DNA (Caldwell et al., 2004; Till et al., 2003)

In mutation breeding, any deviation from parental lines are considered as "putative mutants", which means they are not necessarily "true mutants". Deceptive mutants are the case for many traits including disease resistance because non-infection may simply be the result of the absence of the pathogen. There are therefore standard procedures for confirming mutant; these procedures are referred to as mutant verification. Putative mutant in seed propagated plants which are cross pollinated takes time to developed. In most cases they are usually evaluated at the M_3 to M_4 stage to identify rare mutant individuals that meet the selection criterion based on the breeding objective (FAO/IAEA, 2009).

Chapter Summary

The genetic potential yield of maize is greatly hampered by maize streak virus (MSV) which belongs to the genus *Mastrevirus*, and in the family *Geminiviridae*. Maize streak disease is widely distributed throughout sub-Saharan Africa and remains a threat to Africa's subsistence farmers as many control measures have not yet brought the desired relief. Maize streak virus is transmitted to maize plants by virus-infected leafhoppers which cause 30 to 100% yield loss. Mutation breeding which is the purposeful application of mutagens in plant breeding, is an ideal approach to broaden the genetic variation for developing useful plant varieties. With 3211 registered mutant varieties in more than 170 different plant species, mutation breeding has proven flexible, workable and ready to use on any crop if objectives and selection methods are clearly defined. Gamma rays are mostly used mutagen for their simple application, good penetration, reproducibility and high mutation frequency. It can shorten breeding time and inherent constraint.

CHAPTER THREE

RADIOSENSITIVITY TEST

Introduction

The first step in mutation breeding is to determine the most appropriate dose to apply to a mass sample. The process involves radiosensitivity, which is the determination of radiation dose that will cause 50% of viable seed not to germinate (LD_{50}) or the dose that leads to a 50% reduction of vegetative growth (RD_{50}) (Kamaruddin et al., 2016). Radiosensitivity is a prerequisite in mutagenesis because genotypes of the same species respond differently to radiation exposure due epigenetic factors (Tabasum et al., 2011).

Gamma rays are electromagnetic radiations which are widely used mutagen due to their simple application, good penetration, reproducibility and high mutation frequency (Chahal and Gosal, 2002; Ramya et al., 2013). Ionizing radiation have major impact on biochemical pathways such as photosynthetic and carotenoids pathways (Teramura et al., 1994). Radiations reduce growth regulators such as cytokinins by breaking them down or not synthesizing, thereby increasing plant sensitivity (Chahal and Gosal, 2002). The level of mutagenic effect can be estimated on the basis of various parameters including delay in seed germination; level of disturbances in the cell cycle; frequency of chromosomal aberrations in meristematic tissues; reduced seedling emergence; reduced seedling and plant growth; appearance of chlorophyll defects; and reduced fertility and plant survival (Esnault, & Chenal, 2010).

The term ‘reduced’ indicates change in expression of a particular character in relation to the control, usually the parent cultivar or the breeding line whose seeds were treated with the mutagen (Shah and Sharif, 1994).

Low doses (doses below LD_{50}) decrease the rate at which mutations occur and may have stimulatory effect on shoot development, shoot height and root length a phenomenon known as hormesis (Kim et al., 2000). A high dose (doses above LD_{50}) is not only injurious to the ultra structural organelles, but also affects the phenotype of the plant (Amenorpe, 2004; Kiong et al., 2008,). Uncontrolled increasing gamma radiation will eventually kill seeds or plants. Before mass irradiation, there is a need to determine the optimum dose of radiation that will induce useful mutants. The optimum dose is pre-determined to serve as a guide for effective mutation induction so as to prevent mass destruction of planting materials during mutagenesis (Marcu et al., 2013).

Objective of the Study

The main objective of the study was to determine the sensitivity of six (6) maize genotypes to radiation and the lethal dose (LD_{50}) for mutation induction.

The specific objectives were to:

i. irradiates at different doses and plant irradiated seeds and controls
ii. Determine the LD_{50} and RD_{50} using germination and growth data.

The null hypothesis tested:

- Maize genotypes are not sensitive to gamma radiation.

Materials and methods

Study area

The radiosensitivity experiment was conducted at the Nuclear Agriculture Centre, Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC). The experimental site (05°40′ N, 0°

13′ W, 76 m above sea level), is located within the Coastal Savannah agro-ecological zone in the Greater Accra region of Ghana and receives less than 1000 mm annual precipitation (Morris et al., 1999). The soil at the site is the Nyigbenya-Haatso series, which is a typically well-drained savannah ochrosol (ferric acrisol) derived from quartzite schist (FAO/UNESCO, 1998). The vegetation of the site is mainly grassland with clusters of shrubs and trees.

Source of material

Six genotypes of maize namely Obatanpa, Pann 53, Dapango, Dormabin, Dzinueve and Keta 60 were collected from farmers, research institution and markets. Plant characteristics of the genotypes selected for irradiation are given in Table 3.1. Obatanpa, Pann 53, and Dapango are registered commercial varieties while Dormabin, Dzinueve and Keta 60 are local varieties.

Table 3.4: Characteristics of Maize Cultivars Chosen for Irradiation

Maize cultivar	Maturity period (days)	MSD Status	Grain yield (t ha^{1})	Registered as variety in
Obatanpa	105 - 110	Susceptible	7.5	1990
Pann 53	90 - 100	Susceptible	8.0	2011
Dapango	95 - 105	Susceptible	8.0	1995
Dormabin	100 - 120	Susceptible	5.5	Unknown
Dzinueve	60 - 90	Susceptible	5.2	Unknown
Keta 60	60 - 90	Susceptible	5.2	Unknown

Source: CSIR-Crops Research Institute, (2014)

Irradiating of the maize seeds

Two hundred (200) seeds were packed in each of the sixteen medical polyethylene bags of dimensions 0.1mm thick, 5 cm wide and 11cm long and zip sealed for the radiosensitivity test of all six cultivars. The first to sixteenth packs were respectively irradiated at 0 (control), 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700 and 750 Gy in aluminum tote boxes of dimensions 1m x 0.5m x 0.5m using the cobalt 60 (^{60}Co) category IV wet storage gamma irradiation source (Board of Radiation and Isotope Company Ltd, Hungary) at the dose rate of 300 Gy/hr located at the Radiation Technology Centre (RTC) of Ghana Atomic Energy Commission (GAEC) Accra, Ghana. The quantity of absorbed dose (Gy) is defined as the amount of energy absorbed per unit mass of the matter at the point of interest. The moisture content of the seeds at the time of mutagenic treatments was between 12 to 13%.

Experimental plot and design

Total land size of 1 acre (100 x 10 m^2) was used in a randomised complete block design (RCBD) with three replicates consisting of 200 plants. The planting distances were 0.4 m x 0.8 m between plants and withing rows respectively. Each block was separated by a 2 m path.

Planting of irradiated seeds

Dry seeds of maize with moisture content of 12-14 % approx. were irradiated at 50, 100, 150, 200 up to 750 Gy of gamma rays from 60Co source. There were total of 96 treatment combinations including control. Untreated dry seeds were used as control. Treated and control seeds were immediately on a clayey loam soil belonging to Nyigbenya – Haatso series located at the Nuclear Agriculture Research

Centre of Biotechnology and Nuclear Agriculture Research Institute (BNARI) in Ghana Atomic Energy Commission (GAEC) in September, 2014.

Agronomic Practices

Planting of the plants was done manually. Weed control was done manually by hoe. No fertilizers or pesticides were applied. The experiment was carried out under rain fed conditions.

Data collection

Determination of LD_{50}

The LD_{50} was determined following Marcu & Darban, (2013).

i. Collection of germination percentage data of genotypes (y) (control and induced plant):

$$\text{Germination} = \frac{Number\ of\ Total\ Germinated\ Seed\ (control)}{Total\ Number\ of\ Seed\ Irradiated} \times 100$$

ii. Plotting of the germination percent data against doses applied (x).

iii. Replacing the "y" value of the regression equation with half of the "control genotype" value to obtain the "x "value which is the LD_{50}.

Determination of RD_{50} for seedling height

The reduction in seedling height of induced plants is calculated relative to the control following FAO/IAEA (2018). RD_{50} of seedling height is calculated using the following three steps:

i. $$\text{Emergence reduction (\%)} = 100 - \frac{(Average\ emergence\ of\ induce \times 100)}{Average\ emergence\ of\ control}$$

ii. Mean emergence reduction of a genotypes (y) (control and induced plant) is ploted against doses applied (x)

iii. Extrapolation of RD_{50} is traced from 50% Emergence reduction of the control genotypes (y) value.

Collection of Morphological and agronomic characters

Morphological characters of 150 M_1 plants from each of the six cultivars were scored using standard descriptors of maize described by IBPGR, (1991). A total of 10 characters were evaluated for each radiation dose (Table 3.2) and data taken 21 days.

Table 3.5: Morphological and agronomic characters used for evaluation 900 M_1 plants (dose range of (0 – 500 Gy).

Characters scored	Procedure
plant height (cm)	height of plants (cm) from the base of the plant to the tip of the flag leaf
number of leaves	by counting all the green leaves of the plant
leave area (cm^2)	leaf length × breadth × 0.75
leaf colour	visual observation
root length (cm)	From the base of the plant to the tip of the logest root
chlorophyll content	chlorophyll fluorometer

Source: IBPRG, (1991)

Determination of chlorosis frequency (CF)

The frequency of chlorosis is calculated following modied Gustafsson (1941) method as follows:

$$CF = \frac{(Number\ of\ \text{chlorotic plants}\ (albina + xantha + viridis) \times 100)}{Total\ number\ of\ induced\ plants}$$

Data analyses

Data on the percentage germination, and all the morphological parameters were subjected to two-way analysis of variance (ANOVA). Significant difference between means were separated with the Least Significance Difference (LSD) at $p \leq 0.05$ (Mead, 2017). Linear regression analysis from GenStat version 12 statistical package was used to estimate the optimum dose causing 50 percent lethality (LD_{50}) for the different maize genotypes using percentage survival in the field as a standard measure of physical effect.

Results

ANOVA for Morphological and agronomic characters

The six genotypes were highly significantly ($P \leq 0.01$) different from each other on basis of germination and significantly ($P \leq 0.05$) different on the bases of plant height, root length and number of leaves. The doses applied were highly significant ($P \leq 0.01$) on the bases of germination percentage, plant height and root length but were were significant ($P \leq 0.05$) for leave area and photosynthesis. Genotype by dose interaction was also highly significant ($P \leq 0.01$) for germination but significant ($P \leq 0.05$) for plant height, root length, leave area and photosynthesis except number of leaves (Table 3.3).

Table 3.6: Analysis of variance for the various morphological traits measured (mean squared)

Source of variation	Df	Germination percentage	Plant height	Root length	Number of leaves	Leave area	Photosynthesis
Rep	2	20.025	1.042	2.69	0.21	307.7	4.31
Genotype	5	2254.51**	118.710*	44.86*	4.57^{ns}	1530.1^{ns}	180.16^{ns}
Dose	8	19800.59**	583.91**	140.89**	19.84^{ns}	18722.5*	84.50*
Genotype × Dose	40	271.689**	13.07*	1.96*	1.02^{ns}	191.6*	51.09*
Error	106	5.79	2.87	0.65	0.18	233	4.32

*Ns = not significant, *: ** significant at 5 and 1 percent respectively*

Source: Field data, Afram (2018)

Germination

Seed germination percentage after gamma irradiation (0 - 400 Gy) revealed that the maximum germination (98%) was observed in Pann control (un-irradiated). Germination percentage decreased with increasing gamma doses for all the cultivars. The mean percentage reduction at every 100 Gy was 7.3% at 100 Gy, 26% at 200 Gy 41% at 300 Gy and 91% at 400 Gy. The lowest germination percentage was recorded at 400 Gy after which additional doses showed lethality for most of the cultivars. Dzinueve was the most sensitive to the gamma radiation as it could not record any germination beyond 350 Gy (Table 3.4).

Table 3.4 Gamma radiation effect on mean germination (%) of six maize genotypes

Dose_rate (Gy)	Dapango	Dormabin	Dzinueve	Keta 60	Obatanpa	Pan 53	means dose rate (Gy)
0	98.4	97.2	93.0	96.2	96.4	93.0	95.7[a]
50	95.4	95.0	90.4	90.8	86.4	88.0	91.0[b]
100	91.2	91.8	88.6	86.0	81.0	90.2	88.1[c]
150	84.8	90.0	81.2	81.4	69.4	90.2	82.8[d]
200	71.6	87.2	55.6	61.2	55.0	89.2	70.0[e]
250	66.2	81.6	25.0	40.2	40.6	85.2	56.6[f]
300	41.0	60.4	3.4	34.6	33.8	56.8	38.3[g]
350	24.4	27.0	6.0	11.8	10.2	11.4	15.1[h]
400	14.8	17.2	0.4	5.8	2.4	9.2	8.3[i]
means	65.3[c]	71.9[a]	49.3[f]	56.4[d]	52.8[e]	68.1[b]	

Means with the same letter are not significantly different ($P \leq 0.05$); Lsd: Dose rate =1.2; Genotype = 1.0

Sensitivity of six maize genotypes to gamma radiation

The graph showing the sensitivity of six maize genotypes to gamma irradiation and mean plant height reduction of a genotypes (y) gainst doses applied (x) are represented in Figures 3.1 and 3.2, respectively. Since LD_{50} is measured at 50% of the target control population, therefore a horizontal line at 50% of control would intercept the regression line of the most sensitive genotype first and the least sensitive genotype last. The most sensitive genotype is Dzinueve, followed by Obatanpa, Keta 60, Pan 53, Dapango and Dormabin being the least. The point at which the horizontal line at 50% of the control population intercept the regression line of the target population has a ccorresponding dose called the LD_{50} for sensitivity of six maize genotypes to gamma grapgh and RD_{50} for the plant height reduction of a genotypes graph.

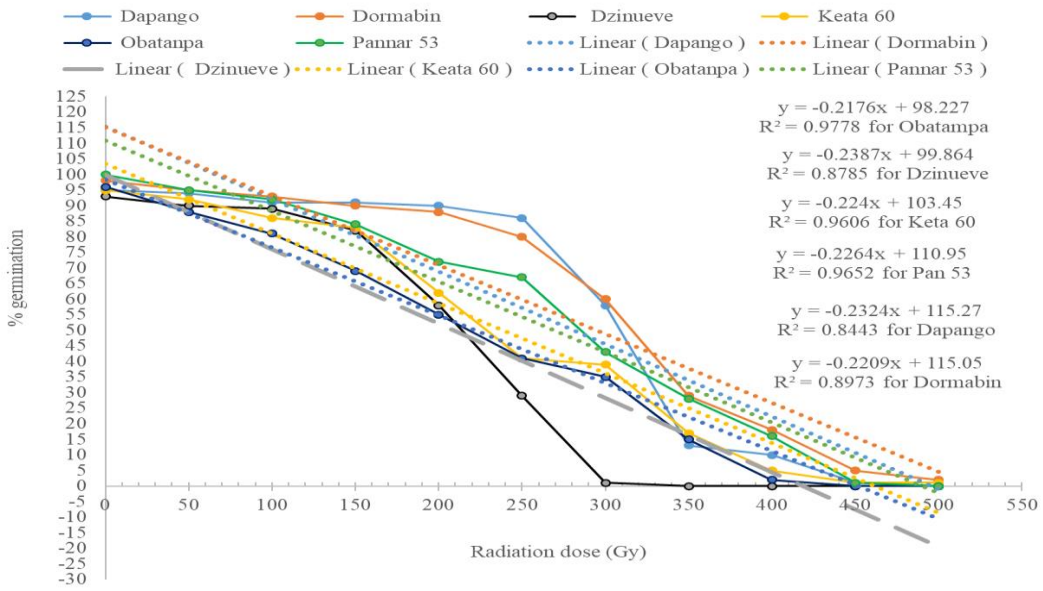

Figure 3.1: Sensitivity of six maize genotypes to gamma irradiation based on germination

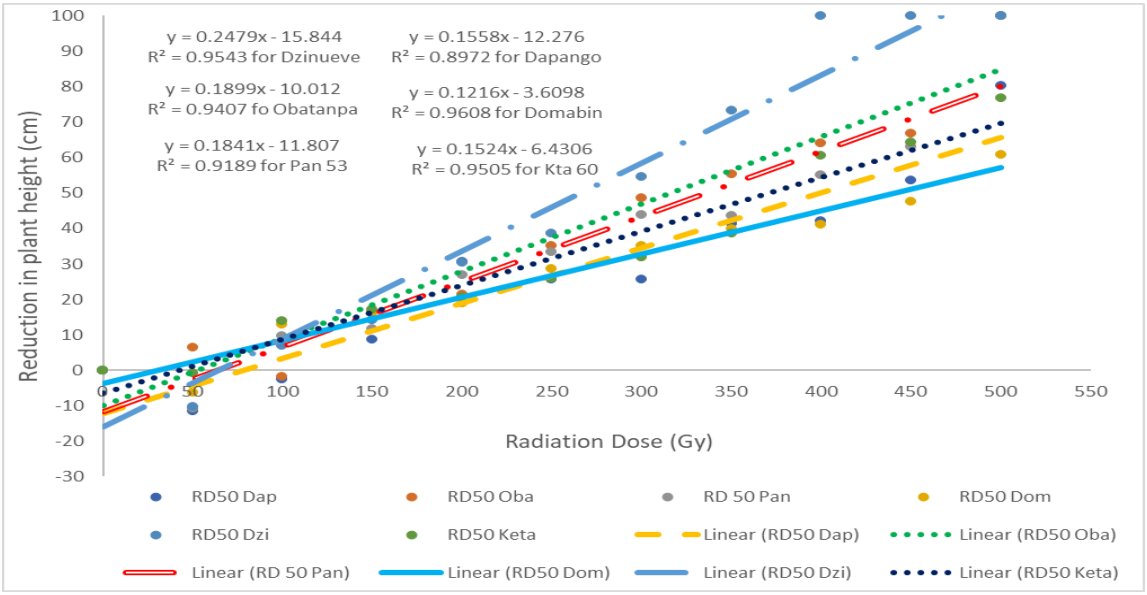

Figure 3.2 Mean plant height reduction of a genotypes (y) (control and induced plant) gainst doses applied (x)

LD_{50} and RD_{50} for six maize genotypes

The LD_{50} and RD_{50} for germination and plant height ranged from 221 – 299 Gy and 280 – 440 Gy, respectively. Dzinueve with the lowest LD_{50} and RD_{50} is more sensitive to gamma radiation than the rest while Dormabin with the highest LD_{50} and RD_{50} is least sensitive to gamma radiation. The mean LD_{50} and RD_{50} for germination and plant height reduction were 258.5 Gy and 345.83 Gy, respectively. The mean LD_{50} value of 258.5 Gy for germination was therefore used as optimal dose for acute irradiation of the actual samples (Table 3.5).

Table 3.5. LD_{50} and RD_{50} for six maize genotypes

Genotypes	Germination LD_{50}	Plant height RD_{50} (Gy)
Dapango	281	340
Dormabin	299	440
Dzinueve	221	280
Keta 60	250	380
Obatanpa	231	300
Pannar 53	269	335
Mean	258.5	345.83
St Dev.	29.97	57.66

Source: Field data, Afram (2018)

Plant height

Low radiation (50 Gy) caused an average increase of 2.4 cm in plant height among the six genotypes. Cultivar Dormabin had the highest stimulatory effect (2.8 cm) of the low dose followed by Obatanpa (2.6 cm) as compared to their respective controls. However, there were significant ($P \leq 0.05$) decrease in plant height at 100 Gy and beyond. There was average reduction in plant height at increasing doses and beyond 400 Gy there was no survival of plants (Table 3.6 and Figure 3.3).

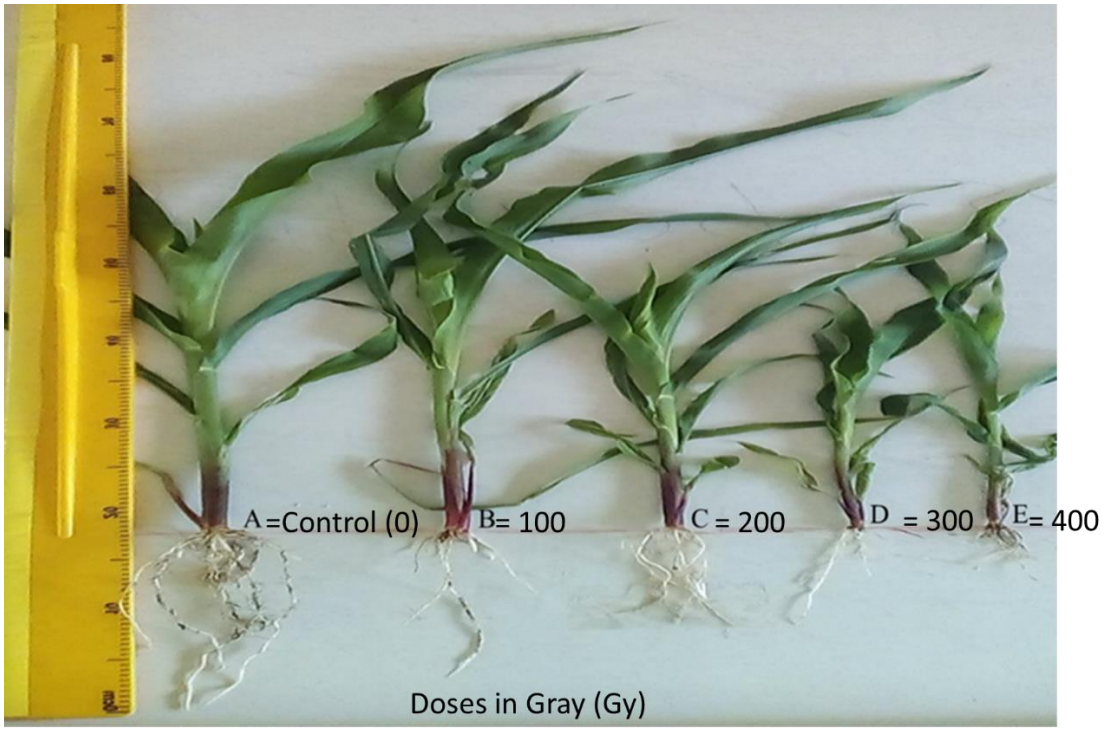

Figure 3.3: The effect of radiation on plant height and root length at 21 days after sowing. Seedling height and root length decreased with an increase in dose of gamma rays.

Source: Field survey, Afram (2018)

Table 3.6 - Gamma radiation effect on mean seedling height of 6 maize genotypes

	Genotypes						
Doses (Gy)	Dapango	Dormabin	Dzinueve	Keta 60	Obatanpa	Pan 53	Mean Dose rate 1.169
0	26.56	29.1	25	27.15	28.44	27.28	27.25[a]
50	26.66	27.61	25.59	24.4	26.64	27.26	26.36[a]
100	24.27	28.04	23.28	23.38	24.96	24.64	24.76[b]
150	24.25	26.39	21.46	22.49	23.75	24.08	23.74[b]
200	18.41	25.63	17.41	21.56	22.37	19.93	20.89[c]
250	19.77	22.12	15.38	20.13	18.45	18.15	19.00[d]
300	14.78	18.85	11.37	18.51	14.65	15.35	15.59[e]
350	15.6	17.5	6.68	16.7	12.74	15.39	14.10[f]
400	15.39	17.16	0	10.74	10.24	12.24	10.96[g]
Mean	20.63b	23.6[a]	16.24[c]	20.56[b]	20.25[b]	20.48[b]	
Genotypes							
0.955							

Means with the same letter are not significantly different ($P \leq 0.05$); Lsd: Dose rate =1.2; Genotype = 1.0

Source: Field data, Afram (2018)

Root length

Root length decreased with increasing gamma radiation of plants in reference to the control. Root length decreased from increasing gamma doses from 100 Gy to 400 Gy. There was an increase of 0.8 cm at 50 Gy dose rate for all the six genotypes. At 100 Gy, Dapango cultivar decreased from 13.10 cm (control) to 9.01cm while Obatanpa decreased from 12.59 cm to 10.69 cm. At 200 Gy, Dormabin cultivar reduced from 12.79 cm to 10.69 cm. Percentage reduction of plant root was highest at 400 Gy and beyond 400 Gy there was no plant survival. (Figure 3.4).

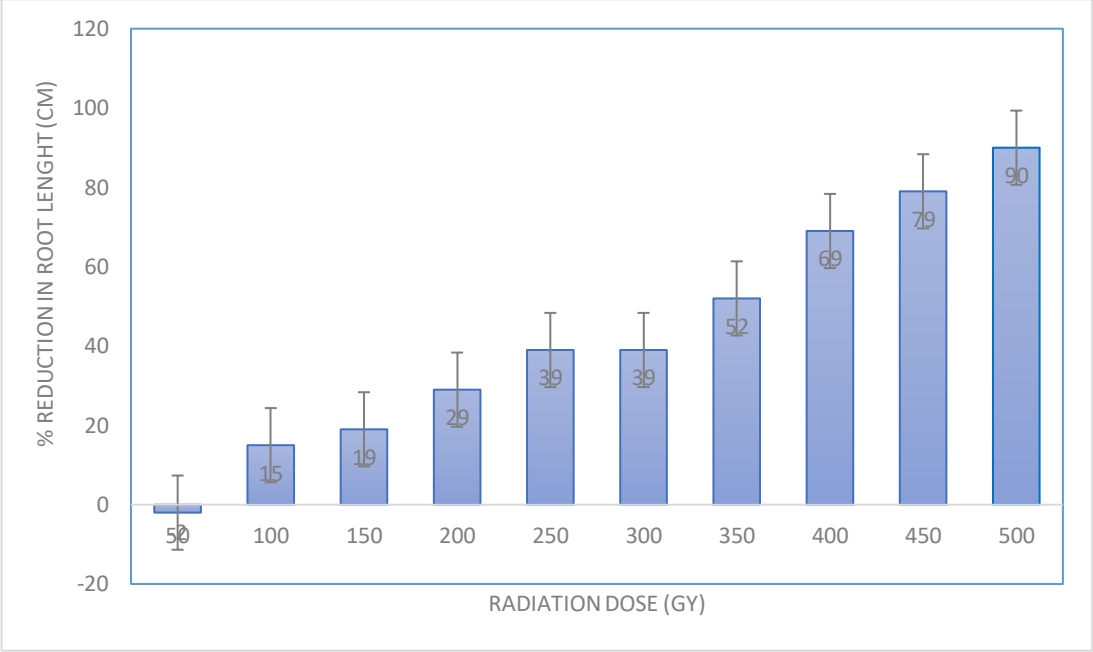

Figure 3.2: Mean effect of radiation dose on percentage reduction in root length 21 DAE.

Effect of radiation dose on plant height and root length

The effect of radiation on both plant height and root length varies for each genotype. Low dose of 50 Gy caused (1cm) increased in plant height and (0.5cm) elongation of root length for all the genotypes. Increasing dose above 100 Gy caused a significant reduction in both plant height and root length. Whereas radiation dose of 100 Gy caused seedling reduction from 27 cm to 24 cm that of root length shortened from 12.3 cm to 10.5 cm. At 200 Gy seedling height shrunk from 27 cm control to 20 cm whiles the root length reduced from 12 cm control to 8.6 cm. At 400 Gy the reduction was 11 cm for plant height and 4 cm for root length (Figure 3.5).

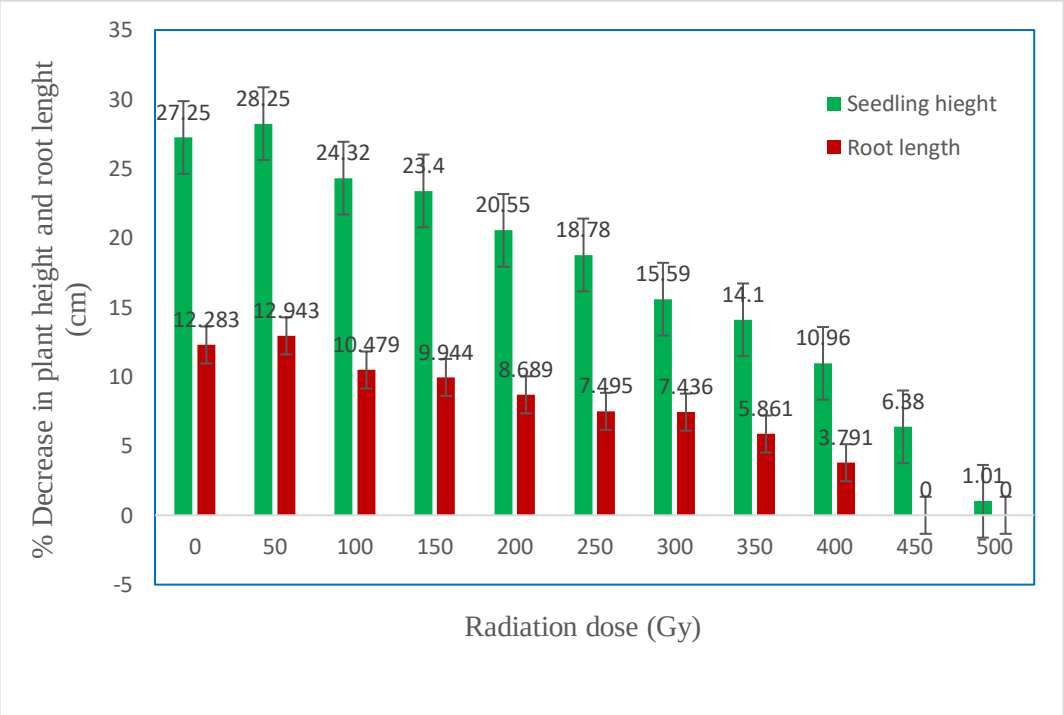

Figure 3.3: Rate of decrease of seedling height and root length in response to gamma radiation.

Leaf area and chlorophil content

There was a significant ($P \leq 0.05$) decrease in leaf area of the irradiated plants compared to their respective controls. Dapango genotype had the greates effect of radiation on leaf area of 78.08 cm^3 as compared to the control 125 $cm^{3,}$ this was followed by Obatanpa from 121 cm^3 to 73.80 cm^3 (Table 3.7).

Data analyses of chlorophyll fluorescence showed chlorophyll concentrations to be higher in local cultivars, namely Dormabin and Dzinueve than in the improved genotypes. The mean values of chlophyll content in the six genotypes ranged from 26.24 to 25.90 for Dapango and 35.84 to 32.77 for Dormabin (Table 3.7). Generally, gamma irradiation negatively affected the photosynthetic rate of the six maize genotypes.

Table 3.7: Gamma radiation effect on mean leaf area and chlorophyll content of 6 maize genotypes

	Leaf area cm3							Chlorophyll content						
Dose (Gy)	Dapango	Dormabin	Dzinueve	Keta 60	Obatanpa	Pan 53	Mean dose (Gy)	Dapango	Dormabin	Dzinueve	Keta 60	Obatanpa	Pan 53	Mean dose (Gy)
0	120.22	96.41	101.76	113.25	96.72	86.28	102.44^{a}	26.01	35.84	33.29	25.01	26.32	30.06	29.42^{c}
50	128.58	94.27	96.02	107.64	100.93	106.59	105.67^{a}	22.10	32.58	34.38	30.54	27.11	30.74	29.58^{c}
100	91.60	93.97	85.22	96.84	93.16	102.88	93.95$_{b}$	23.91	30.73	35.79	34.01	28.92	26.63	30.00^{c}
150	117.66	89.21	78.42	87.51	73.15	90.38	89.39^{b}	24.20	34.82	37.97	28.98	27.71	33.22	31.15^{b}
200	73.25	66.24	54.73	81.12	74.99	88.59	73.16^{c}	23.00	35.05	38.94	29.78	32.63	32.77	32.03^{b}
250	67.71	58.13	43.67	65.41	56.35	67.60	59.81$_{d}$	31.31	32.45	35.49	34.58	36.13	35.90	34.31^{a}
300	54.99	54.98	34.16	49.98	43.74	55.54	48.9^{e}	25.09	29.16	32.67	32.57	29.62	26.21	29.22^{c}
350	29.15	34.76	19.02	38.89	33.49	43.90	33.2^{f}	34.37	30.16	32.63	35.84	30.69	34.34	33.00^{b}
400	12.42	12.38	0.00	25.48	20.50	25.24	16.01^{g}	22.85	34.11	14.93	29.47	38.20	22.73	27.05^{d}
Mean genotype	77.29^{a}	66.71^{b}	57.00^{c}	74.01^{a}	65.89^{b}	74.11^{a}		25.87^{c}	32.77^{a}	32.90^{a}	31.20^{b}	30.81^{b}	30.29^{b}	

Means with the same letter are not significantly different (P ≤ 0.05); Lsd for leaf area: Dose rate =7.18; Genotype = 5.86; Lsd for Chlorophyll content: Dose rate =0.98; Genotype = 0.88

Source: Field data, Afram (2018)

Discussion

The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals, which can damage different important compounds of plant cell (Kovacs and Keresztes, 2002). Insertion or deletion of DNA may occur during the repairing process of damaged DNA which lead to mutation (Amenorpe, 2010).

In mutation breeding process, radiosensitivity is first carried out to determine plants sensitivity to gamma radiation. The prime objective in mutation breeding had been to upgrade the well adopted plant varieties by altering one or two major agronomical traits which limit their productivity and quality. Genotype, doses applied and their interactions were significantly difference for most of the maize traits studied. Data analyses of radiosensitivity test confirmed maize genotypes reacted differently to gamma radiation due to inherent genetic differnces. The findings colobrates report that genotypes of rice displayed varied response to gamma radiations (Tabasum et al., 2011). The different LD_{50} and LD_{50} for each genotype, confirmed the need to determine the sensitivity and the optimal dose of any maize genotype before mass irradiation of actual samples to avoid loss of experimental materials.

The mean LD_{50} (Optimal) dose and the RD_{50} for the six maize genotypes was observed at 258.5 Gy and 345.8 respectively. It therefore implies that acute radiation administered at the optimal dose might be sufficient for causing useful mutation in any of the six maize genotypes. The reduction in germination and survival may be due to penetrating of ionizing radiation in biological materials, acting directly on critical targets in the cell (Kovacs and Keresztes, 2002).

Analyses of plant height of the maize genotypes indicate a general decreasing trend in seedling height in respect to increasing radiation. Plant height has become the most widely used index in determining the biological effect of various physical and chemical mutagens in M_1 plants (Sheeba et al., 2005). There were different responses of genotypes to the irradiation in plant height and root lengths. Both parameters decreased in response to increasing radiation doses. However, the rate of decrease in seedling height in response to varying radiation doses has no definite relationship on decreasing root length. Mashev et al. (1995) and Al-Salhi (2004) also reported that the seedling height in rice decreased with the increasing irradiation doses, but the decrease was not proportional to the increase in dosage. They also reported a reduction in root length with each corresponding increase in radiation dose that is in line with present findings.

Plant height is a fundamental trait from breeding point of view. In maize, higher yields are usually obtained from shorter crops because the reduction of stature increases lodging resistance (Forell, 2015). Among the height range of maize, the medium height is mostly preferred since a severe reduction in height tends to decrease production and to hinder development during early stages of growth and also at harvesting (Feng et al., 2009). In this research, Dormabin was the tallest genotype (31 cm), while Dzinueve recorded the least height of 26 cm at 50 Gy. The reduction of plant height in M_1 generation could be due to growth inhibition induced through high-dose irradiation and the cell cycle arrest in the G_2/M phase during somatic cell division and/or to a variety of damages in the entire genome (Preuss and Britt 2003). The data on plant height was used to compute the RD_{50} which is the dose that results in 50% post-irradiation reduction of plant height, shoot development and other growth

parameters. RD_{50} is compared to the LD_{50} in determining the optimum dose in radiosensitivity in order not to cause unnecessary damage to planting materials.

Gamma irradiation has also been reported to significantly curtail the synthesis of chlorophyll content (Dale et al., 1997). In the present study, increasing gamma radiation reduced significantly chlorophyll content as compared to the control. However, two cultivars Dormabin and Obatanpa showed normal photosynthetic rate at increasing gamma radiation. Other reports indicated significant reduction in chlorophyll synthesis with increased levels of gamma irradiation in potato cultivars (Dale et al., 1997). Moreover, it has also been reported in citrus that non-irradiated plantlets demonstrated the highest amount of chlorophyll content as compared to irradiated (10-50 Gy) plantlets (Ling et al., 2008).

Mutagens generally reduce the reproductive ability of plant and increase the number of sterile florets much more than the environmental effects. The decrease in the growth parameters of maize after irradiation is considered to be due to chromosomal aberrations (Kiong et al., 2008). There is evidence that if the mutation occurs in the gametophyte cells (meiosis) it then becomes permanent and the trait is transferred into later generations (van Harten, 1998). Based on genotype × dose interaction, germination percentage and chlorophyll fluorescence, three cultivars namely, Pann 53, Dapango and Obatanpa have been selected and would be added to a new cultivar Honampa (yellow Maize) for further mutation breeding against maize streak disease (MSD). The use of high dosage (400 Gy) caused deleterious effect in physiological processes thus considered an extreme dose in the present study. The ultimate aim of a mutagenic treatment should be to induce mutations leading to genetic improvement of a specific trait. The mutagenic treatments with low

physiological and strong genetic effects may be considered for initiating a successful mutation breeding programme.

Chapter Summary

The radio sensitivity test was set up to determine the sensitivity of six (6) maize genotypes to radiation, the LD_{50} and RD_{50} doses before mutagenesis. Maize genotypes were observed to react differently to gamma radiation due to inherent genetic differnces. The most sensitive genotype was Dzinueve, followed by Obatanpa, Keta 60, Pan 53, Dapango and Dormabin being the least. The mean LD_{50} and RD_{50} for germination and plant height reduction were 258.5 Gy and 345.83 Gy, respectively. The mean LD_{50} value of 258.5 Gy for germination was therefore used as optimal dose for acute irradiation of the actual samples to prevent substantial destruction of planting materials. The germination, seedling height, root lengths, leaf area and photosynthesis of M_1 plants were inversely proposional to doses applied. Induced plants from higher doses (≥500 Gy) were too weak to survive. A decrease of 4 - 41% of germination percentage, 4 - 30% of seedling height and 9 - 38% of the root length occurred between 50 Gy - 250 Gy exposures. The photosynthetic pigment content was also observed to decreased by 6–21% compared with the control.

CHAPTER FOUR

GENETIC INDUCTION FOR VARIABILITY IN FOUR MAIZE GENOTYPES AND SELECTION OF USEFUL MUTANTS

Introduction

Among the emerging diseases of maize that demands urgent attention is the maize streak disease (MSD) caused by maize streak virus (MSV). There is no effective chemical control for viral diseases; a situation that makes MSD a threat in Africa. Maize streak disease caused huge economic loss to farmers (Martin and Shepherd, 2009; Mawere et al., 2006). Although the maize streak resistance gene has been identified and incorporated into several maize strains through crosses, records show that resistance breaks down with time because of the emergence of new strains of the MSV (Isnard et al. 1998; van der Walt et al. 2008). To simplify the process of introducing MSV resistance into commercially viable maize genotypes, breeders would prefer mutation breeding, tauted to be technically simple, quicker and cost effective than conventional and molecular methods (Gadani, 1990; Shepherd et al. 2009). Almost any source of radiation can be used for acute exposures. Acute irradiation of large sample is prefered when the optimal dose is established through radiosensitivity (Ahloowalia and Maluszynski, 2001).

In Chapter 3, the radio sensitivity test was determined for six (6) maize genotypes and the most sensitive genotype was Dzinueve, followed by Obatanpa, Keta 60, Pan 53, Dapango and Dormabin being the least. That means irradiating Dzinueve genotype with LD_{50} Dormabin would result in very low germination of Dzinueve genotype and irradiation of Dormabin with LD_{50} of Dzinueve would result to minimal effect of radiation. The mean LD_{50} and RD_{50} for germination and plant height reduction for all genotypes were therefore determined as 258.5 Gy and 345.83

Gy, respectively. The mean LD_{50} value of 258.5 Gy for germination was therefore used as optimal dose for acute irradiation of the actual samples to prevent substantial destruction of planting materials.

Maize genotypes found in Ghana lacks the resistant gene to MSD and the varieties accredited with tolerance to the disease have also become susceptible with time (Asare-Bediako et al., 2019). There is therefore a need to induce the maize genotypes and develop resistance genotypes against the pandemic MSD.

Objective of the Study

The main objective of the study was to induce and select useful maize mutants.

The specific objectives of the study were to:

i. Characterize the induced plants for maize streak disease resistance

ii. Identify other mutants

The null hypothesis tested:

i. Some induced plants are not resistant to maize streak disease (MSD).

ii. Mutants cannot be produced from induced maize genotypes.

Materials and methods

Source of materials

Four maize varieties (Obatanpa, Pann 53, Dapango and Honampa) were selected from six varieties based on their sensitivity to radiation (Chapter 3; Table 3.1).

Acute irradiation of the four selected maize genotypes

For each of the genotypes, 10 kg seeds were acutely irradiated at 288.5 Gy, at the Radiation Technology Centre (RTC) of Ghana Atomic Energy Commission (GAEC), Accra, Ghana, using cobalt 60 (^{60}Co) delivering at a dose rate of 300 Gy/hr.

The mutation induction, field trial and mutant selection were carried out following a modified mutation breeding scheme by van Harten (1998) as shown in Figure 4.1.

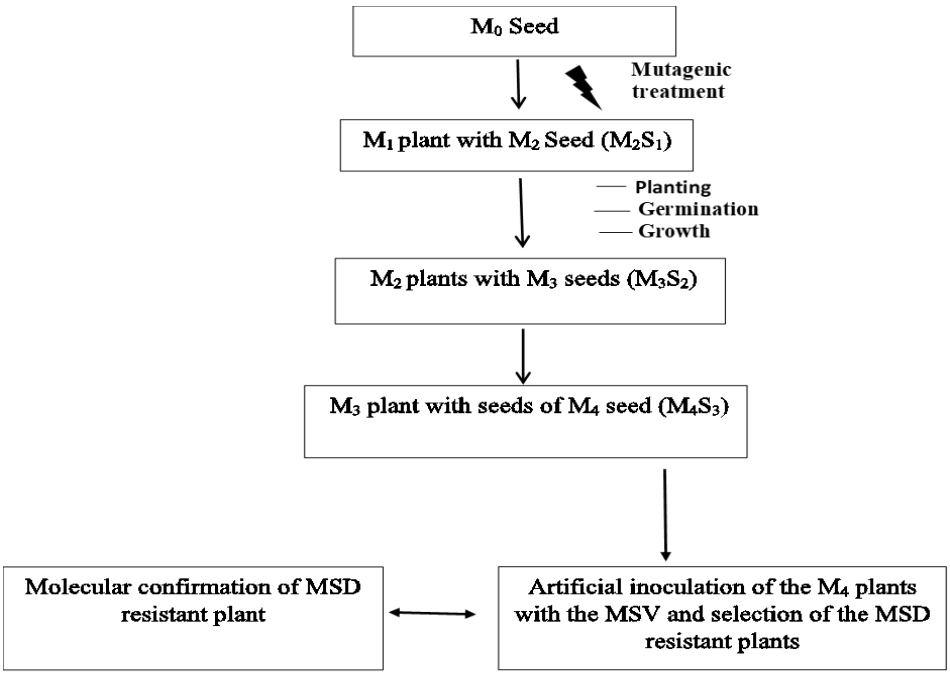

Figure 4.1: A modified mutation breeding scheme in seed propagated crops
Source: Field data, Afram (2018)

M_1S_0 Generation

The M_1 maize seeds were raised at the Soil and Irrigation Research Center (SIREC), University of Ghana, Kpong in the Eastern Region of Ghana. This site was chosen because of irrigation fascility for offseason trial for prevention of outcrossing of the induced maize plants. The site is latitude 6° 09' N, longitude 0° 04' E and 22m above sea level from November, 2014, to February, 2015. The site is situated some 80 km north east of Accra, and the soils in the area belong to the order Vertisols and fall under the Akuse series (Brammer, 1962). The rainfall pattern at the site is bimodal, with the major season occurring between May and July, and the minor season between September and November.

All the irradiated maize seeds along with their parental control were planted in the field immediately after the irradiation in a randomized complete block design (RCBD) in three replicates to raise the M_1 generation. A 6000 m^2 field was ploughed and harrowed and divided into eight (8) plots for each of the four (4) control seed and four (4) irradiated seed. Two seeds per hill were sown at a spacing of 40 × 80 cm. All the mutants generated were selfed according to (Falconer, 1989) to achieve homozygosity. Experimental management including fertilizer application and weed control measures were followed.

Characterization of M_1S_0 generation

Hundred and fifty (150) plants from each treatment were randomly selected based on maize sreak disease severity score. The following data were collected following standard procedures of CIMMYT (1985) unless otherwise stated:

Days to 50 % anthesis (DA): Number of days from planting to the date when 50 % of the plants in a plot have tassels shedding pollen.

Days to 50 % silking (DS): Number of days from planting to the date when 50 % of the plants in a plot have emerged silks.

Anthesis-silking interval (ASI): Difference between days to 50 % anthesis and days to 50 % silking.

Total leaf count (TLC): Mean of the number of leaves per plot after silking.

Plant height (PLHT): Average height of plants (cm) from the base of the plant to where tassel branching begins.

Ear height (EHT): Average height (cm) from the base of the plant to the node bearing the upper ear.

Ear leaf length (ELL): Length of leaf (cm) which subtends the uppermost ear after flowering.

Ear leaf width (ELW): Width of leaf (cm) which subtends the uppermost ear was measured mid-way along its length after flowering.

Ear leaf area (ELA): This was calculated using the formula: 0.75 x leaf length (cm) x maximum leaf width (cm) according to (Mokhtarpour et al., 2010).

Plant aspect (PASP): This is a general score for the appearance of the plants in the plot. Factors such as relative plant and ear heights, uniformity, reaction to MSD and lodging were considered. PASP was rated on a scale of 1 to 5 where: 1 = excellent overall phenotypic appeal, 2 = very good overall phenotypic appeal, 3 = good overall phenotypic appeal, 4 = fair overall phenotypic appeal and 5 = poor overall phenotypic appeal (Badu-Apraku et al., 2012).

Ear aspect (EASP): This is a score for the general appearance of all ears in the plot. Factors considered were ear size, grain filling, disease and insect damage and uniformity of size, color and grain texture. It was rated on a scale of 1 to 5 where: 1 = best, 2 = good, 3 = average, 4 = fairest, 5 = poorest ear aspect (Badu-Apraku et al., 2012).

Number of ear(s) per plant (NE/PLT): Total number of ears at harvest that bear kernels including the second ear as well as the top ear.

Field weight (FWT): Weight of harvested cobs (kg) per plot after harvest.

Ear length (EL): Length (cm) of the cob with grains per plot.

Ear diameter (ED): Diameter (mm) of the cob with grains per plot.

100-grain weight (HGW): Weight of hundred grains per plot.

Moisture percentage (MOIST %): Moisture tester (Aqua-Boy, Germany) was used to determine the moisture content of the grains per plot at biological maturity

Grain yield (GY) (t ha^{-1}): The field weight at 80 % shelling percentage was adjusted to 12.5 % moisture content. Yield per hectare (ha) was estimated by multiplying the yield per plant by plant density per ha and then converted to t ha^{-1}.

Disease incidence

Disease incidence per field was estimated as the percentage of plants along the transects showing MSD symptoms (Oppong et al., 2015).

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

MSD severity

Field observation and recording of maize streak virus (MSV) disease severity was assessed at seven-day interval, beginning at 14 days after planting, based on visual assessment of the whole plot following the standard procedure of Soto et al. (1982). A 1-5 hedonic scale was used, where One (1), represented no visible disease symptoms, Two (2), very few streaks on some leaves, Three (3), moderate streak symptoms on most leaves, Four (4) abundant symptoms on all leaves (>60%) and Five (5), leaf area affected and severe symptoms on all leaves (>80%) of leaves affected with no yield. A range of scores were used to define resistance types as follows: 1.0 (Immune), 1.1-1.4 (highly resistant), 1.5-2.4 (resistant), 2.5-2.9 (moderately resistant), and 3.0-5.0 (susceptible).

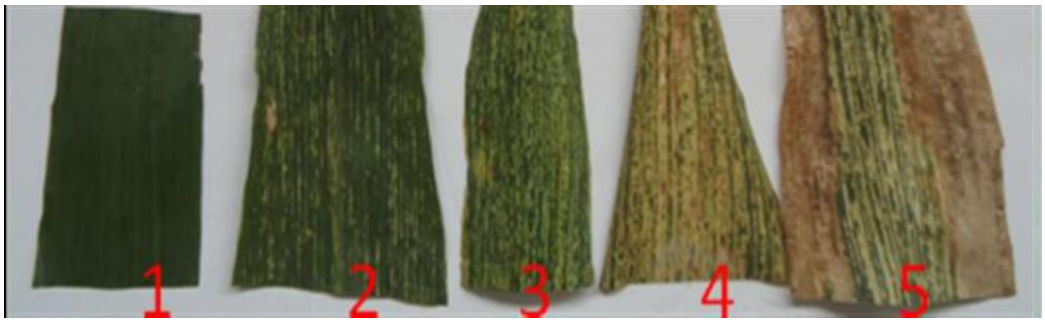

Figure 4.2: Maize streak virus disease scoring scale (courtesy Kyetere et al., 1999). 1= no infection, 2= mild; infection, 3= moderate infection, 4= severe infection, 5=very severe infection.

M_2 Generation

The M_2 induced plants were developed at the major planting season at the Nkwanta Municipal Agricultural Station in the northern part of Volta Region from June 2015 to September 2016. This site was chosen because it was identified as a hot spot for the MSD, during a reccjonnaissant survey prior to the experiment. It lies between latitudes 7 30° and 8 45° North and longitude 0 10° and 0 45° East. The average annual rainfall of the area ranges from 922 to 1,874 mm and the mean temperature is about 26.5°C (GSS, 2014). The dominant soil type was Acriosol (WRB, 2015).

Material and experimental design

Bulked seeds from M_1 generation were harvested individually from all the treatments and were grown in M_2 generation in Randomized Complete Block Design (RCBD) with three replications. 120 kg ha^1 rate of fertilizer was applied and cultural practices were followed to raise the M_2 plants.

Observation on quantitative traits

To study the induction of micromutation in quantitative traits 150 normal looking plants (excluding the macromutants plants) were randomly selected from each plot and recorded based on the classification given in Table 4.1.

Table 4.4: Classification of viable traits used in mutation rate and estimation

Classification	Viable physiological and morphological mutant trait	Descriptions
I	Plant architecture	Height, number of leaves, stem girth and presence or absent of props root
II	Flowering	Earlier or later than the parental control
III	Anthesis to silking interval (ASI)	Shorter or longer than the parental control
IV	Maize streak disease	Tolerance, resistance or susceptible when scored in reference to the parental control
V	Yield	Number of cobs per plant and grain weight

Source: Field data, Afram (2018)

Chlorosis

Plants were observed daily to record chlorosis within 7 to 10 days of sowing. Different types of chlorosis produced by the treatments, were determined from the total induced plants. The chlorosis was classified into the following types as per the classification of Gustafsson (1940).

Albina

A lethal mutation which is characterized by completely white leaves of the seedling. These seedlings survived for 21- 28 days after germination.

Xantha

A lethal mutation in which the leaf colour varies from orange yellow to yellowish white.

Viridis

A viable mutation characterized by yellowish green patches on leaves in the beginning and changes to green subsequently.

The Chlorophyll mutation frequency % and Total macro mutation frequency (%) were measured at 28 DAP following (FAO/IAEA. 2018).

$$\text{Chlorophyll mutation frequency \%} = \frac{Total\ number\ of\ chlorophyll\ mutants}{Total\ number\ of\ seedlings\ observed} \times 100$$

Total macro mutation frequency (%) = Chlorophyll mutation frequency (%) + morphological mutation frequency (%).

Selfing and Coefficient of Inbreeding.

Selfing was carried out to achieve homozygosity following the method of Good and Hallauer (1977). Special wax-coated tassel and silk bag was used to cover the tassels and silks of the plant before they started shedding (tassels) or receiving (silks) pollen. The eveloping young corn ear were located and covered before the silk appeared. The tassel was also covered with tassel bags, clipped before the pollen matured. Self-pollination was done between the hours of 8 am and 10 am, by shaking the pollen into the tassel bag and dusting it on the silk of the same plant. The silk was then covered with the bag and clipped.

Inbreeding depression as a result of selfing of the induced plants was computed following Falconer (1989) as follows:

$$Ft = 0.25\ (1 + 2Ft\text{-}1 + Ft\text{-}2) \qquad (1)$$

where Ft is the coefficient of the present generation, Ft-1 is the coefficient of the previous generation and Ft-2 is the coefficient of the generation before that.

First generation M_1S_0 -

Second generation $M_2S_1 = 0.25 (1 + 0 + 0) = 0.25$ (2)

Third generation $M_3S_2 = 0.25 (1 + 0.5 + 0) = 0.375$ (3)

`Fourth generation $M_4S_3 = 0.25 (1 + 0.75 + 0.25) = 0.5$ (4)

M_3 Generation

Materials and experimental design

One hundred and fifty (150) induced plants were selected from each genotype based on low MSD severity score, of 1 or 2, and advanced to M_3 stage. The induced plants were characterized on bases of phenotypic traits, at the minor season from September to December 2015, on the same field of the M_2. Plants. Rate of fertilizer application and other cultural practices were followed as described in the M_2 generation.

M_4 Generation

Material and experimental design

The experiment to test the M_4 putative mutant was carried out at the Crop Research Institute's research station at Kwadaso in the Ahanti Region. It is located in a deciduous forest zone with minimum rainfall of 1500 mm and lies between latitude 60 41' N, 10 36' W and having a coarse sandy-loam, Paleustult (Obeng, 2000). The reason for the choice of the Kwadaso station was to get access to screen house for the artificial inoculation of the M_4 putative mutants with MSV.

At M_3 generation, twenty induced plants with severity score of 1 (clean and healthy-looking plants without maize streak disease symptoms) were selected for M_4 trials. The M_4 generation was established in randomized complete block design with three replications. MSD and data on number of cobs per plant, cob weight, seeds per cob, hundred seeds weight and total yield per plant were recorded. Other observations like plant height, ear height, days to 50% tasseling, cob length, plant girth and prop roots) were collected. Top 10 induced plants were selected on the bases of MSD resistant and grain yield. Fertilizer were applied and other intercultural practices were followed during the crop growing period.

Population mean and variance of quantitative traits

The M_4 populations were subjected to statistical analysis to compare the effect of mutagenic treatment in induced plants vrs controls (Table 4.2). The ANOVA was determined at 5% α level and l.s.d was used to separate the means of putative mutants and parental controls for each of the four genotypes.

Data analyses

Variance components at M4 stage

The mean data of the induced genotypes recorded for each character were tabulated and subjected to analysis of variance (Panse and Sukhatme, 1964).

Table 4.5: Format for variance components

Source	Df	MS	Expected MS
Replication	(r-1)		
Genotypes	(g-1)	Mg	Ve + rVg
Error	(r-1) (g-1)	Me	Ve
Total	Rg-1		

Source: Panse and Sukhatme, (1964)

Where,

r = number of replications

g = number of genotypes

Mg = mean squares for genotypes

Me = mean squares for error

Ve = error variance

Vg = genotypic variance

$$SE(m) = \sqrt{\frac{Mg}{rt}}$$

$$SE(d) = \sqrt{\frac{2Mg}{rl}}$$

M_3 Generation

Days to 50 % flowering, plant height, disease severity and average grain yield of 50 randomly selected plants per genotype were recorded in each replication. The characters recorded in the M_3 progeny were subjected to student T-test as described by Posten (1978) to test the differences of traits of the induced plants and the parental controls.

M_4 generation

Descriptive statistics

Descriptive statistics was used to provide a brief summary of some of the important traits amoung the putative mutants at the end of the fourth mutant generation. Basic statistics viz., mean, range, minimum and maximum values, variance, standard deviations, standard error and coefficient of variation among the mutants were estimated for each trait studied using MS-EXCEL software 2013.

Estimation of genetic parameters

The genetic parameters like genotypic variance, phenotypic variance, genotypic coefficient of variation, phenotypic co efficient of variation, among the induced plant were calculated as given below.

Variance

Both genotypic and phenotypic variances were calculated by using the mean of squares of error and genotypes (Johnson et al., 1955).

a) Genotypic variance ($\sigma^2 g$) = $\frac{Mg - Ve}{r}$

b) Environmental variance ($\sigma^2 e$) = Ve

c) Phenotypic variance ($\sigma^2 p$) = $\sigma^2 e + \sigma^2 g$

r = number of replications.

Phenotypic coefficient of variation and genotypic coefficient of variation

Coefficient of variation was worked out using the formula given by Burton (1952).

a) Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\delta_p^2}}{Mean} \times 100$

b) Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\delta_g^2}}{Mean} \times 100$

PCV and GCV were classified as suggested by Sivasubramanian and Menon (1973)

Less than 10%	Low
10-20%	Medium
Greater than 20%	High

Broad sense heritability

It is the ratio of the genetic variance to the total variance according to Lush (1940) and expressed in percentage.

Heritability (h^2) in broad sense = $\frac{\delta_g^2}{\delta_p^2}$ x 100

Heritability Range (Robinson *et al.*, 1949)

0 – 30 Low

31 – 60 Medium

>61 High

Correlation of some traits

To assess the magnitude of association between morphological traits and disease severity, correlation coefficients were computed from the mean of traits, following Weber and Moorthy (1952). Correlation coefficients were compared against table r values given by Fisher and Yates (1963) at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance (Panse and Sukhatme, 1964).

Genetic diversity studies based on morphological characters

All the observed morphological characters in the induced maize plants were utilized for diversity study as follows;

Principal component and Cluster analysis

Principal component and cluster analysis (Ward, 1963) were carried out to identify the most important factors contributing to the variations and the diversity among selected mutants. The principal component analysis (PCA) was performed to group 24 induced plants selected from across the four genotypes into related groups using six morphological traits. The traits were ear height, leaf area, days to 50% silking, days to 50% tasselling, plant height and grain yield. The principal component analysis gives an indication of which traits contribute mainly to genetic variation and why particular mutants cluster together.

The hierarchical clustering was performed on the six different traits of the putative mutants. The cluster method used was between group-linkage, following squared Euclidean distance interval measure in SPSS version 20. The genetic associations between the putative mutants were evaluated using dendrogram.

Results

M_1S_0 Generation

The immediate effect of the acute irradiation on the M_1 plants of all the 4 maize genotypes resulted in reduction in germination percentage, plant height, days to 50% flowering, ear height, number of cobs per plant, hundred seeds weight and yield per plant. The magnitude of the effects varied among the maize genotypes. Analysis of variance of data on the M_1 characters showed significant ($P \leq 0.05$) differences among the treatments for all M_1 traits. However, there was no significant change in MSV disease expression and severity among the genotypes and the putative mutants and their respective parental control at the M_1 stage.

Germination

The comparison of the germination percentage of controls and induced plants vary from M_1S_0 – M_4S_3 generations (Figure 4.3). Controlled germination was significantly ($P \leq 0.05$) higher than the induced plants at various stages. Student T-Test at M_1S_0 (A) generation for Obatanpa Pan 53, Dapango and Honanpa were 1.44E-6, 1.03E-7, 2.97E-7 and 7.91E-7, respectively. For M_2S_1 (B) generation for Obatanpa Pan 53, Dapango and Honanpa were 5.08E-4, 8.78E-5, 4.02E-3, 9.13E-4, respectively; M_3S_2 (C) generation for Obatanpa Pan 53, Dapango and Honanpa were 8.78E-5, 1.57E-5, 1.57E -5, 3.04E-4, respectively and also for M_4S_3 (D) were 2.0 E-5, 1.01E-5, 4.55E-5, 1.57E-5 respectively.

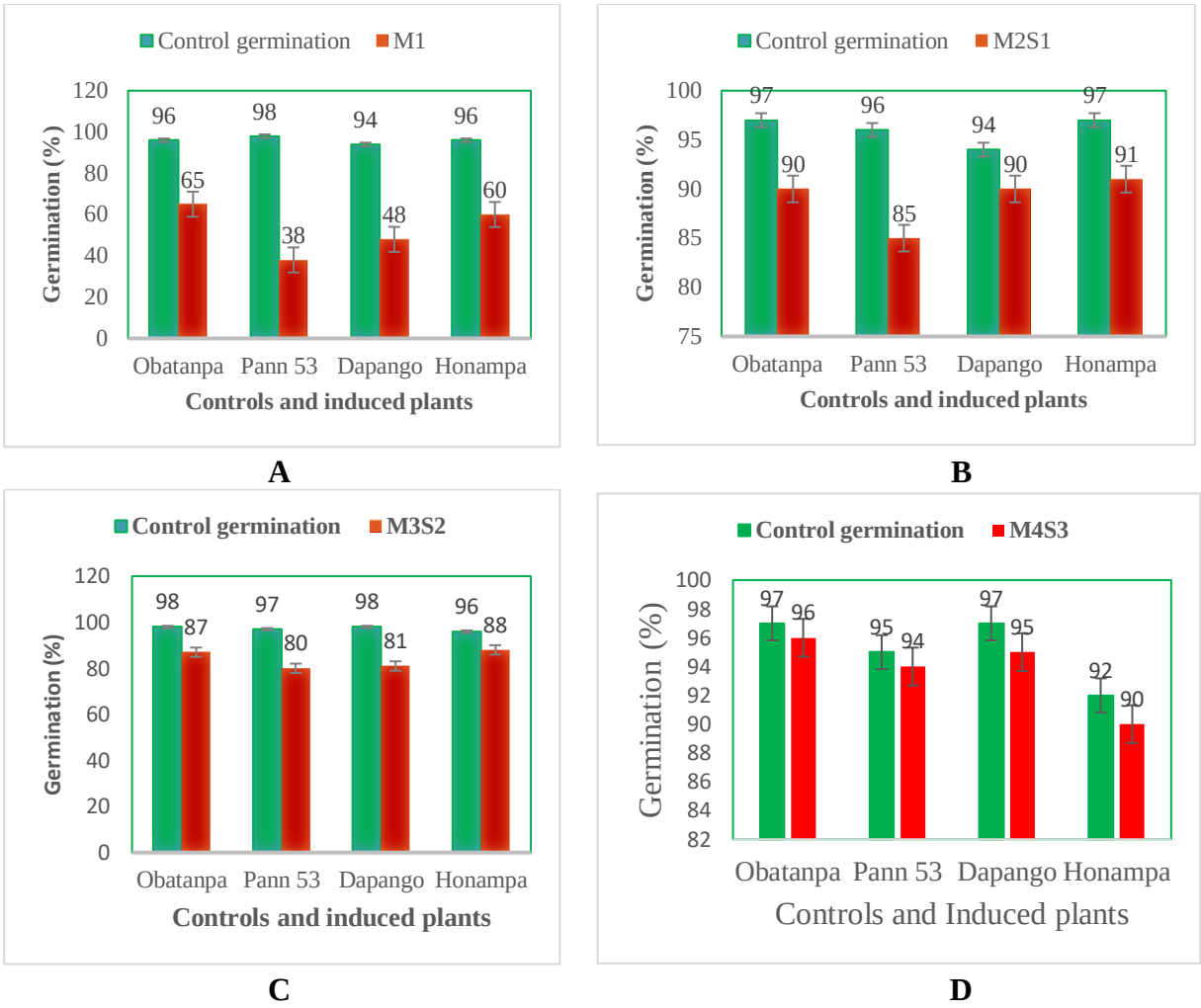

Figure 4.3: Germination percentage of controls and induced plants: at M_1 (A); M_2 (B); M_3 (C) and M_4 (D) generations.

MSD incidence and severity score

There were significant ($P \leq 0.05$) different in MSD incidence (22.1%) among the putative mutants at the M_1 stage. The highest incidence was recorded in Pann 53, (33.5%), followed by Obatanpa 21.29%, Honampa 18.2% and Dapango 15.47%. There was however high mean severity score among the putative mutants than their parental controls. The mean severity score for the putative mutants was 3.5 whiles that of the control was 3.0 (Table 4.3).

Table 4.3: Mean incidence and severity score of MSD among induced plants and their parental control at M_1 stage.

Genotype	MSD Incidence (%)		MSD Severity score (1-5)	
	Control	Induced plant	Control	Induced plants
Obatanpa	23.4	21.9	2.1	2.1
Pann 53	37.3	33.5	3.2	3.2
Dapango	18.33	15.5	2.3	2.3
Honampa	19.4	18.2	2.0	2.0
Mean	24.60	22.27	2.33	2.33
l.s.d. (P< 0.05)	4.08	3.54	0.23	0.23

Source: Field data, Afram (2018)

Plant height

Decrease in plant height was highly significant ($P \leq 0.01$) among the M_1 of the four induced plants as compared to their respective controls. The mean decrease in plant height was 25 cm among the M_1 genotypes. There was however an increase in plant height among the induced geotypes from M_2 to M_3 in reference to the controls. The decrease in Pann 53 at M_1 was 38 cm, followed by Dapango 33 cm, Obatanpa 17 cm with Honampa having the least decrease of 12 cm. Obatanpa induced genotype recorded the highest increase from 116 cm to 230 cm at the M_2S_1 stage. The rest of the induced genotypes also recorded increase in height from the M_2 to the M_3 stage. Cumulative average differences were observed in all the four cultivars from 100 cm between the M_1S_0 and M_2S_1 and then 60 cm between M_3S_2 and M_4S_3. (Table 4.4).

Table 4.4: Mean height (cm) of M_1S_0 - M_3S_4 induced plants and their respective controls

Genotype	Plant height (cm)							
	Control	M_1S_0	Control	M_2S_1	Control	M_3S_2	Control	M_4S_3
Obatanpa	133	116	214	230	188	179	133	128
Pann 53	126	88	231	226	186	175	111	95
Dapango	123	90	203	205	163	171	98	127
Honampa	112	100	193	197	156	157	107	94
Mean	123.5	98.5	210.25	214.5	173.25	170.5	112.25	111
Stdev	8.74	12.79	16.28	16.01	16.15	9.57	14.86	19.06
Cv (%)	7.07	12.99	7.74	7.46	9.32	5.62	13.24	17.17

Source: Field data, Afram (2018)

Days to flowering

Days to 50% flowering of M_1 population for all the induced plants ranged from 51 to 60. The respective parental controls did not show any variation in the measured parameter. The increase in days to flowering in the M_1 population was significant ($P \leq 0.05$) for all the induced genotypes except Pann 53. There were variations in the number the number of days flowering among the four putative mutants from the M_1S_0 to M_4S_3 stage. Days to 50% flowering for Obatanpa was 58 days in the M_1S_0 stage, 60 days at M_2S_1 stage, 58 days in the M_3S_2 stage and 59 days in the M_4S_3 stage whiles the Obatanpa control took an average of 57 days to flower (Table 4.6).

Table 4.6: Days to 50% tasselling of M_1S_0 – M_4S_3 putative mutants and their respective controls.

Genotype	Control	M_1S_0	Control	M_2S_1	Control	M_3S_2	Control	M_4S_3
Obatanpa	56	58	56	60	57	58	57	59
Pann 53	51	51	51	53	51	52	51	52
Dapango	50	53	51	54	53	52	51	53
Honampa	59	60	59	61	59	60	59	60
Mean	54	55.5	54.25	57	55	55.5	54.5	56
Stdev	4.24	4.20	3.95	4.08	3.65	4.12	4.12	4.08
Cv (%)	3.2	3.3	2.9	3.0	1.6	1.7	2.2	2.3

Source: Field data, Afram (2018)

Grain yield and yield indices

The yield performance of the putative mutants was highly significant ($P \leq 0.01$). Grain yield per plant varied among the putative mutants from 2.3 t ha^{-1} to 0.7 t ha^{-1}. Dapango genotype recorded the highest grain yield of 2.3 t ha^{-1}, followed by Obatanpa, 1.9 t ha^{-1}, Honampa, 1.2 t ha^{-1} and Pann 53 having the least grain yield of 0.7 t ha^{-1}. Mean square values for other yield indices except for number of cobs per plant showed highly significant ($P \leq 0.01$). values. The 100 seed weight for the four genotypes were 10. 20, 30 and 50, for Dapango, Obatanpa, Honampa and Pann 53 respectively (Table 4.7).

Table 4.6: Yield and yield indices of four M_1 induce plant and their parental controls

Genotype	No. of ear per plant		100 grain weight (g)		Grain yield (t ha^{-1})	
	Control	induced	Control	induced	Control	induced
Obatanpa	2.00	1.00^{NS}	81.00	54.10*	3.50	2.87*
Pann 53	2.00	1.00 ^{NS}	118.00	22.50 ^{NS}	3.93	0.67 ^{NS}
Dapango	1.00	1.00 ^{NS}	99.60	29.10 ^{NS}	3.87	2.27 ^{NS}
Honampa	2.00	1.00 ^{NS}	70.80	49.50*	3.37	1.23 ^{NS}
Mean	2.00	1.00	92.40	38.40	3.67	1.51
l.s.d. (P< 0.05)	0.52	0.24	6.99	14.99	0.07	0.15

*significant at ($P \leq 0.05$): NS = Not Significant
Source: Field data, Afram (2018)

Chlorosis

The M_2 putative mutants exhibited various shades of chlorosis among the various genotypes on the field. The various shades of chlorosis ranged from xanthan, viridis, albina and virido-albina (Figure 4.4a, b, c and d). The induced genotypes indicated low to moderately high mutation frequency among the four genotypes. None of the genotypes exhibited more than four types of the chlorophyll mutation. Dapango maize genotype recoded the highest mutation frequency based on individual plant assessment with an estimated frequency of 0.2, followed by Obatanpa 0.03, Pann 53 0.15, and Honampa recording the least frequency of 0.25 (Table 4.7).

A: Chlorophyll mutant, Xantha

B: Viridis

C: virido-albina

D: Albina

Figure 4.4: Macromutation (chlorophyll); (A) Xantha (B); Viridis (C) virido-albina and (D) Albina.

Table 4.7: Chlorophyll mutations and mutation frequency of M_2 cultivars

M_2 Genotype	Total plant Population	Number of chlorophyll mutants	Mutation frequency (%)
Obatanpa	2000	6	0.3
Pann 50	2000	3	0.15
Dapango	2000	4	0.2
Honampa	2000	5	0.25
Mean	2000	4.5	0.23
St.Dev.	0.0	1.29	0.06
Cv (%)	0.0	28.6	26.08

Source: Field data, Afram (2018)

Flowering, plant height, disease score and yield indices of M_2 progenies and their parental controls

Days to 50% flowering

Mean days to flowering of the M_2 populations was significant ($P \leq 0.05$) amoung the varous traits recorded. Days to flowering in the induced plants ranged from 52.67 to 60.67 while it was 51.0 and 58 .67 for respective controls (Table 4.9). The grand mean days to flowering in the M_2 population was 56.67 days and the control was 54.08. Pann 53 had the least number of days to flowering (52.67 days) followed by Dapango (53.67 days), Obatanpa (59.67 days) and Honampa (60.67 days) (Table 4.9).

Plant height

Variation in plant height (cm) of the M_2 population was significant ($P \leq 0.05$). Mean plant height of the M_2 population varied from 230.43 to 204.63 cm and 231.15 to 202.60 cm in the parental controls. All the M_2 putative mutants had an increased in plant height in reference to their parental controls except Pann 53 putative mutant that

had a decreased in plant height of about 6.6 cm as compared to the parental control (Table 4.9).

Grain yield

Records of yield contributing traits; number of cobs and grain yield varied among the putative mutants and their respective controls. Analysis of variance of M_2 population means and variances for the characters showed significant ($P \leq 0.05$) differences among the treatments for all the yield indices. Grain yield among the putative mutants ranged from 8.167, 7.87, 7.3 and 6.43 t ha^{-1} for Dapango, Pann 53, Obatanpa and Honampa respectively (Table 4.8).

Table 4.8: Flowering, plant height, disease score and yield indices of M_2 progenies and their parental controls

Genotype	Days to 50% tasseling		Plant height (cm)		Number of ears per plant		Grain yield (t ha^{-1})		MSD score (1-5)	
	Control	mutant	control	mutant	control	mutant	control	mutant	Control	mutant
Obatanpa	55.67	59.67*	214.13	230.43 *	1.61	1.38^{NS}	5.53	4.30*	1.38	1.23^{NS}
Pann 53	51.00	53^{NS}	231.15	225.49 *	1.36	1.43^{NS}	4.73	3.87^{NS}	1.12	1.19^{NS}
Dapango	51.00	53^{NS}	202.60	205^{NS}	1.44	1.48^{NS}	4.40	4.17*	1.31	1.18^{NS}
Honampa	58.67	61^{NS}	193.06	197^{NS}	1.68	1.73^{NS}	3.80	2.43^{NS}	1.30	1.18^{NS}
Mean	54.08	56.67	210.24	214.44	1.52	1.50	4.61	3.70	1.28	1.19
Lsd (P≤0.05)	1.96	2.78	11.51	16.28	0.19	0.28	0.35	0.500	0.17	0.25

*significant at ($P \leq 0.05$): Not Significant

Source: Field data, Afram (2018)

MSD incidence and severity score

There were low MSD incidence and severity score among the M_2 population. Putative mutants had an average disease score ranging from 2.0 to 3.3 and the parental control also had 2.0 to 3.4 score. The incidence of MSD in the induced plants ranged from 19.2% to 36.3% (Table 4.9).

Table 4.9: Mean incidence and severity score of MSD among the M_2 putative mutants and their parental control

Genotype	MSD Incidence (%)		MSD Severity score (1-5)	
	Control	Putative mutant	Control	Putative mutant
Obatanpa	22.7	24.2	2.3	2.1
Pann 53	39.5	36.3	3.4	3.3
Dapango	20.8	19.7	2.3	2.3
Honampa	18.4	19.2	2.0	2.0
Mean	25.35	24.85	2.50	2.43
St. Dev.	9.60	7.96	0.62	0.60
CV (%)	0.38	0.32	0.25	0.25

Source: Field data, Afram (2018)

M_3 generation

Analysis of variance for plant height, yield and yield indices of the M_3 progeny indicated significant ($P \leq 0.05$) differences among the progenies of each treatment in the genotypes. Grain yield of the M_3 progenies, varied from 5.70 to 2.93 t ha^{-1} as compared to 4.62 t ha^{-1} in the parental control. Though majority of putative mutants produced higher mean yield than the parent, the increase was significant only for Obatanpa and Dapango M_3 progenies. In Dapango genotype, grain yield of the M_3 progenies was 5.97 t ha^{-1} as against 5.40 t ha^{-1} in the control (parent variety). Analysis of variance for 50% days to flowering, and 100 grains weight indicated significant ($P \leq 0.05$) differences among the M_3 progenies except for number of ears

per plant which was not significant among the mutants and their parental controls (Table 4.10).

Table 4.10: Flowering, plant height, disease score and yield indices of M_3 progenies and their parental controls

Genotype	Days to 50% tasseling		Plant height (cm)		Grain yield (t ha^{-1})	
	control	mutant	control	mutant	Control	Mutant
Obatanpa	57.00	58.00 ^{NS}	188.33	178.70*	5.07	5.97*
Pann 53	51.00	51.67 ^{NS}	186.25	174.79*	4.30	4.63^{NS}
Dapango	52.67	52.67 ^{NS}	162.93	170.96*	5.40	5.97*
Honampa	56.33	57.00 ^{NS}	156.03	157.41 ^{NS}	3.73	4.70*
Mean	54.25	54.83	173.39	170.46	4.62	5.28
Lsd (P≤0.05)	1.08	1.52	11.2	15.84	0.44	0.62

*significant at ($P \leq 0.05$): NS – Not significant
Source: Field data, Afram (2018)

MSD incidence and severity score

There was high incidence of MSD in the experimental site as incidence shot from 26% among the M_2 segregating mutants to 83.1% in the M_3 mutant population. Analyses of variance showed increased incidence of the MSD incidence in the M_3 progenies. There was a high disease incidence in the induced maize plants. Pann 53 had 88.2%, Honampa, 87.5% Dapango 85.4% and Obatanpa 71.3% (Figure 4.5a). Disease severity score also increase for Pann 53 induce plant followed by Honampa, Dapango and Obatanpa with the least severity score (Figure 4.5b).

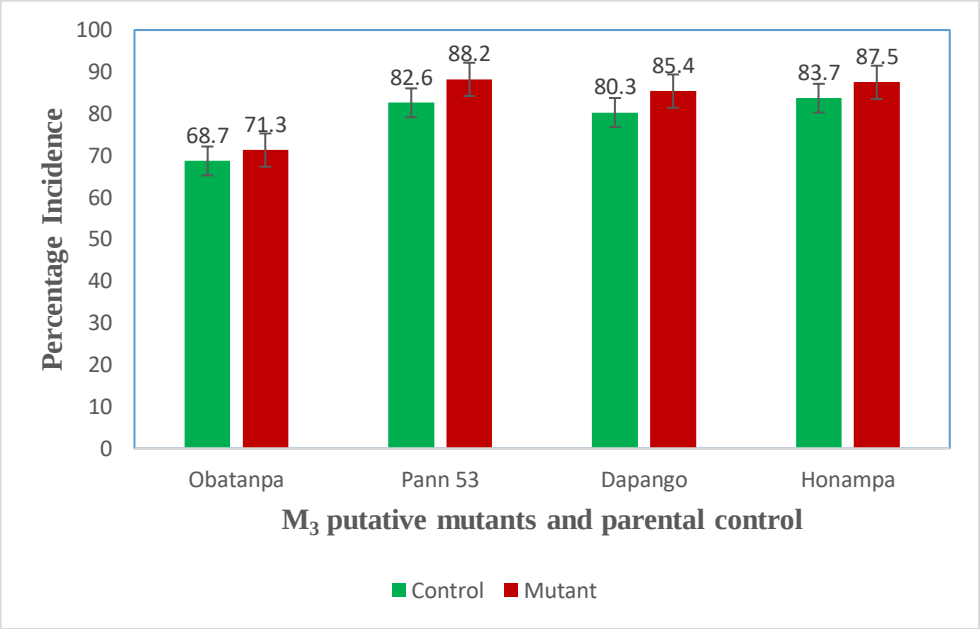

Figure 4.5a: MSD incidence in M_3 putative mutants and parental control

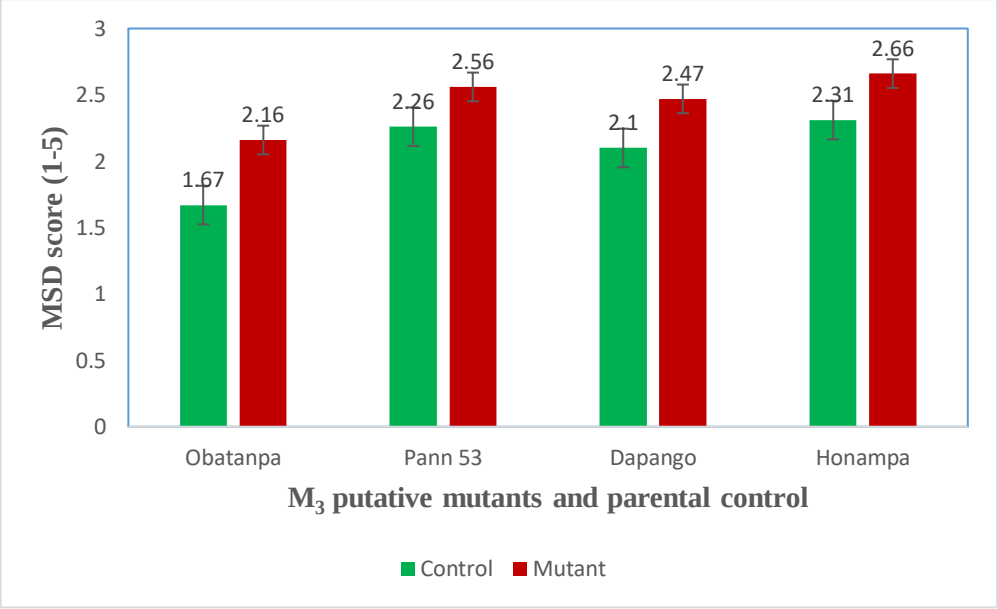

Figure 4.5b MSD severity score in M_3 putative mutants and parental control

M_4 Generation Descriptive statistics for morphological traits

Observations recorded on six morphological traits were subjected to descriptive statistical analysis. The statistical parameters considered were; mean, variance, standard deviation, standard error, minimum and maximum values and coefficient of variation were calculated for the induced genotypes and their respective controls. The descriptive statistics of the various morphological traits are presented in. All the traits observed were significantly ($P \leq 0.05$) different among the four induced plants. The coefficient of variation in grain yield for Obatanpa induced plant was 4.5 t $ha^1$and the control was 4.07 t ha^1.

The minimum days to 50% tasselling in Obatanpa induced genotype was 57 days whiles the control had 56 days. The anthesis to silking interval (ASI) for Honampa induced, was 1 to 3 days while the control ranged from 2 to 3 days. Pann 53 also had 1 to 3 days of ASI for induced plants and 2 to 3 days for the control. The mean plant height in Dapango induced genotype was 132 cm and that of the control was 101 cm. (Table 4.11).

Table 4.11: Descriptive statistics for six maize morphological traits in four induced genotypes and control.

Genotype	Particulars/traits		Days to 50% tassel	Days to 50% silking	ASI	Plant height (cm)	Ear height (cm)	Grain yield (t ha^{-1})
Obatanpa	Mean value	control	57±0.18	59±0.17	2±0.20	134±7.4	53±5.15	3.6±0.6
		induced	58±0.20	60±0.20	2.2±0.16	140±7.6	54±3.52	4.5±0.50
	Stdv	control	0.68	0.65	0.76	27.58	19.28	2.29
		induced	0.73	0.75	0.61	28.66	13.19	1.95
	Variance	control	0.46	0.42	0.57	760.64	371.72	5.27
		induced	0.53	0.57	0.37	821.8	174.2	3.82
	CV (%)	control	1.19	1.10	48.10	20.61	36.23	4.07
		induced	1.26	1.26	26.74	20.41	24.41	4.5
	Range	control	56 - 58	58 – 60	1 – 3	84 - 173	23 - 84	28 -98
		induced	57 - 59	59 – 62	1 – 3	112- 210	35 - 74	1.1 – 7.9
Pann 53	Mean value	control	51±0.18	53±0.19	1.9±0.16	110±9.6	49 ±5.4	4.2±1.2
		induced	51±0.28	52±0.22	2±0.10	107±5.03	40±2.86	4.8±0.92
	Stdv	control	0.69	0.72	0.61	36.01	20.27	4.6
		induced	1.07	0.82	0.39	18.83	10.72	3.4
	Variance	control	0.48	0.52	0.37	1296	411	4.8
		induced	1.14	0.68	0.15	354.61	114.95	12.04
	CV (%)	control	1.37	1.37	31.92	32.69	41.0	7.4
		induced	2.10	1.56	19.61	17.59	26.66	7.1
	Range	control	50 - 52	52 – 54	1 – 3	35 - 185	25 - 100	1.2 – 4.8
		induced	50 – 54	52 – 55	1 – 3	80 - 149	27 - 65	1.7 – 5.1
Dapango	Mean value	control	52±0.25	54±0.17	2±0.20	101±10.1	45±3.16	4.8±0.62
		induced	51±0.26	53±0.25	2±0.07	132±5.06	56±3.13	5.0±0.64
	Stdv	control	0.90	0.62	0.71	35.45	10.96	2.1
		induced	1.00	0.93	0.26	19.4	12.3	2.4
	Variance	control	0.81	0.38	0.51	12.57	120.26	47.49
		induced	1.01	0.87	0.07	359.8	137.6	57.8
	CV (%)	control	1.74	1.15	33.12	35.07	24.32	4.5
		induced	1.96	1.75	12.90	14.32	21.00	6.1
	Range	control	50 - 53	52 – 54	1 – 4	26 - 145	20 - 60	1.5 – 8.4
		induced	50 - 53	52 – 55	2 – 3	110- 184	40 - 80	1.8 – 9.0
Honampa	Mean value	control	56±0.16	58±0.18	2±0.11	93±7.86	36±3.96	3.4±0.75
		induced	56±0.14	57±0.14	2±0.12	101±6.75	43±2.0	3.6±0.46
	Stdv	control	0.57	0.65	0.38	27.23	13.79	2.68
		induced	0.51	0.51	0.42	23.39	6.95	1.6
	Variance	control	0.33	0.42	0.15	741	190.26	7.22
		induced	0.26	0.26	0.18	547	48.33	2.62
	CV (%)	control	1.02	1.11	17.96	29.09	37.36	6.09
		induced	0.92	0.88	21.32	23.08	16.10	11.10
	Range	control	55 - 57	58 – 60	2 – 3	60 - 145	24 - 64	1.9 – 6.4
		induced	55 - 57	57 – 59	1 – 3	35 - 130	30 - 54	1.1 – 6.6

Source: Field data, Afram (2018)

Disease incidence and severity

Analysis of incidence/severity relationships in the putative mutants suggests that high disease incidence results in increased severity in the induced plants and the controls alike. Obatanpa induced plant recorded the lowest severity of 1.5, followed by Pann 53 and Honampa with severity score of 2 for each induced maize plant and the most severe score being Dapango induced with a severity score of 2.3 (Table 4.12).

Table 4.12: Maize streak disease incidence and severity in the M_4 putative Mutants.

Genotype	% incidence		Severity scale (1- 5)	
	Control	Mutant	Control	Mutant
Obatanpa	55.2	31.7	2.8	1.4
Pann 53	68.6	34.5	3.4	2.4
Dapango	65.7	37.5	3.2	2
Honampa	60.8	35.4	3.0	2.2
Mean	62.58	34.78	3.10	2.00
St. Dev.	5.88	2.40	0.26	0.43
CV (%)	0.09	0.07	0.08	0.22

Source: Field data, Afram (2018)

Estimates variability, heritability in broad sense and genetic advance.

The putative mutant of the four genotypes showed wide range of variation for all the six quantitative traits studied. The genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance for these nine characters are presented in Table 4.13.

Coefficient of variability

Estimates of genetic parameters which included genotypic and phenotypic coefficients of variation, heritability in broad sense (h^2) and genetic advance as

percentage of mean were presented in Table 4.13. A perusal of the data revealed that genotypic coefficient of variation (GCV) was lesser than phenotypic coefficient of variation (PCV) for all traits studied. Plant height was found to possess higher PCV and GCV of 18.74 and 15.33 than the ear height of PCV (15.21) and GCV of (4.41). Leaf area had a PCV of 18.88 and GCV of 10.58. Days to 50% tasseling and silking were close and also the least in both PCV and GCV in the estimated characters. Grain yield had the highest estimate of PCV (19.34) but fell marginally in GCV of (8.11).

Heritability in broad sense

The heritability estimates for different characters of the putative mutants ranged from 8.44% for ear height to 95.53% for days to 50% tasseling, while it ranged from 66.89% for plant height and 31.39% for leaf area. Heritability in grain yield was 17.60% and that of days to 50% silking was 94.38% (Table 4.13)

Table 4.13: Genetic parameters of quantitative traits of four putative mutants

Characters	Range	Mean	PCV (%)	GCV (%)	h^2 (%)
Days to 50% Tasselling	50 – 61	55.33	10.5	9.94	95.53
Days to50% Silking	53 – 65	58.50	9.65	9.37	94.38
Leaf area (cm^2)	254.4- 624	438.54	18.88	10.58	31.39
Plant height (cm)	74.0 – 153.4	111.69	18.74	15.33	66.89
Ear height (cm)	27.8 – 66.0	46.60	15.21	4.41	8.44
Grain yield (t ha^{-1})	1.8 – 6.84	3.68	19.34	8.11	17.60

Source: Field data, Afram (2018)

Inbreeding depression

There was a decrease in plant vigor and productivity as a result of continuous self-fertilization of the maize genotypes from M_2 to the M_4 stage. A t-test analysis (Table 4.14), reveal that the rate of inbreeding depression was significant ($P \leq 0.05$) for three traits. There was a significant change in mean of the measured traits of the induced plants. Days to 50% Tasseling, Days to 50% Silking and grain yield were significant at $P_{\alpha = 0.05}$ = 0.04; 0.05 and 0.05, respectively.

Table 4.14: A t- test of inbreeding depression of traits of M_1S_0 - M_4S_3 generation

Morphological traits	M_1S_0	M_2S_1	M_3S_2	M_4S_3	Inbreeding depression value	T – test (α=0.05)
Days to 50% Tasseling	55.37	58.12	57.11	60.33	8.34 *	0.04
Days to 50% Silking	58.5	60.47	59.22	62.42	6.51*	0.05
Leaf area (cm^2)	687.58	587.33	496.27	668.53	2.83^{NS}	0.74
Plant height (cm)	212.33	224.14	220.36	208.69	1.91^{NS}	0.55
Ear height	98.42	96.28	98.13	95.60	3.06^{NS}	0.56
Grain yield (t ha^{-1})	6.44	5.137	4.18	5.97	7.31*	0.05

Source: Field data, Afram (2018)

Principal component analysis

The Eigen values' contribution to total variation are presented in Table 4.15. The first two PCA, PC1 and PC2 accounted for 54.40 and 87.61% of the total variability of the measured traits. PC1 had higher weighing for plant height. PC2 had higher weighing for days to 50% silking and tasselling.

Table 4.15: Principal component analysis of morpho-physiological traits of four putative mutants

Variables	Eigenvectors	
	PC1	PC2
Ear Height	-0.5290	-0.0278
Leaf Area	-0.5042	0.0021
Nda to Silking	0.0587	-0.7013
Nda to Tassel	0.0540	-0.7020
Plant Height	-0.5225	-0.1209
Grain yield	-0.4320	-0.0052
Eigen values	3.2640	1.9930
Individual %	54.4000	33.2100
Cumulative %	54.4000	87.6100

Source: Field data, Afram (2018)

Graphic representation of the principal components is presented in (Fig. 4.6) shows putative mutants that are closely related and those that are not. Genotypes found on the PC1 close to each other are related. Genotypes far from the PC1 are different and are likely to be mutants. Genotypes found in the first quadrant had high weighing for grain yield, those found in the second quadrant PC2 has high weighing for days to flowering, PC3 has high weighing for grain yield and PC4 has genotypes that has high weighing for plant height and leaf area.

The 24 genotypes are grouped into four quadrants. Quadrant one contained Honan 4, Pann 4, Honan 2, Honan 6, Pann 6, Pann 2 and Dap 4. Quadrant two contained Dap 3, Dap 5, Obatan 3, Pann 3, Pann 5 and Honan 3. Quadrant three contained Obatan 2, Obatan 5, Honan 5, Obatan 1, Dap 1, Pann 1 and Honan 1. Quadrant four contained Obatan 4, Obatan 6, Dap 2 and Dap 6. Genotypes found in the first quadrant had higher values for grain yield and ear height. Genotypes found in the second quadrant had higher values for days to 50% tasselling and flowering. Genotypes found in the third quadrant had higher values for plant height and leaf area.

Table 4.16 showed correlation of the variables. Grain yield and ear height had high positive correlation (0.6735) with each other. Days to 50% tasselling and silking also had high positive correlation (0.9837) with each other but negatively correlated with ear height and leaf area.

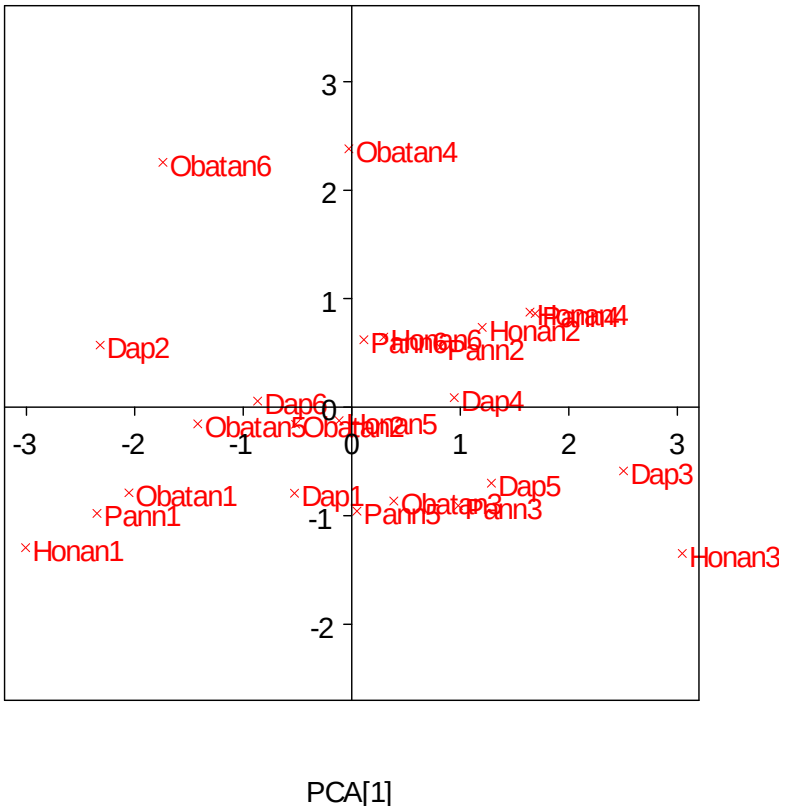

Figure 4.6: PCA showing genetic relationship among 24 induced genotypes

Table 4.16: Correlation among six traits of 24 induced genotypes

Characters	MSD	Leaf Area	Nda to Silking	Nda to Tassel	Plant Height
Leaf_Area	-0.456*				
Nda_to_Silking	0.067	-0.099*			
Nda_to_Tassel	0.049	-0.084*	0.937**		
Plant_Height	-0.565*	0.854*	0.063	0.071	
Grain_yield	-0.335*	0.549*	-0.054	-0.072	0.659*

* Significant at $p < 0.05$, ** Highly significant at $p < 0.01$
Source: Field data, Afram (2018)

Cluster analysis

The result of the dendrogram is shown in Figure 4.8. Similarities were observed in the mutants sharing the same cluster as in groups I, II and III. All group I genotypes (Hon control, Hon 33, Ob control, Pan, Pan control, Dap control, Hon 4, Pan17) were susceptible (SS) to MSD except Dap control which was tolerant. All group II (Pan17, Dap 10, Hon 16, Pan 28) and III (Dap 36, Hon 11, Ob 9 Dap 7, Dap 19, Ob 37, Pan 14, Ob 22, Ob 26) genotypes were tolerant (TL) and resistant or not susceptible (NS) to MSD, respectively (Figure 4.7)

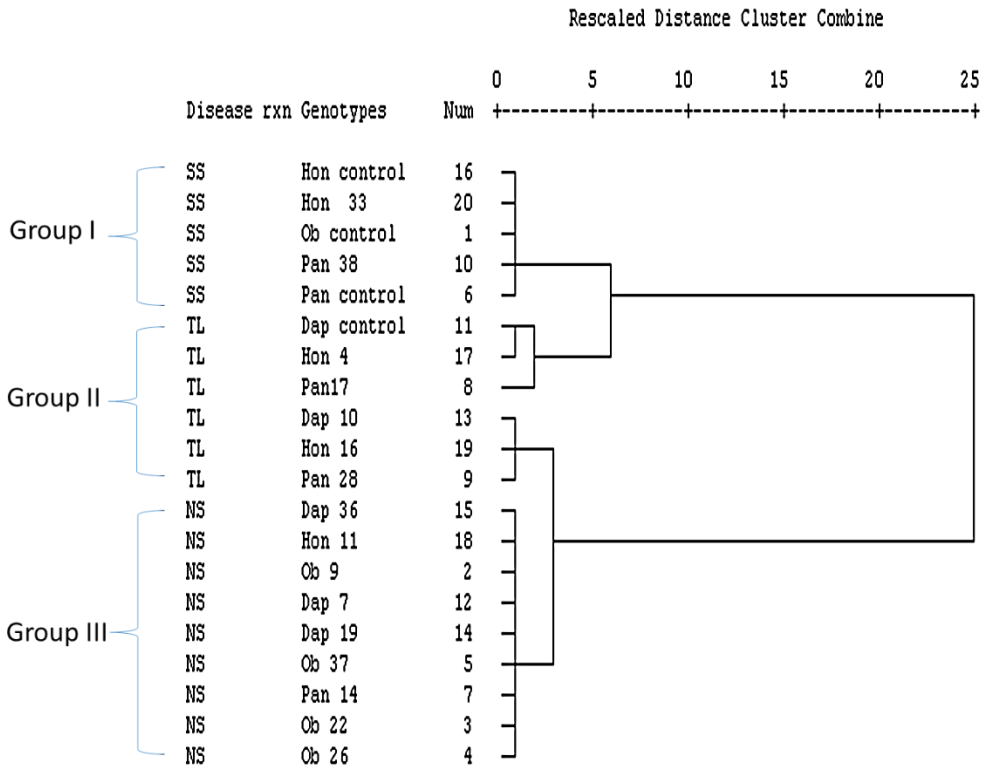

Figure 4.7: Dendrogram showing MSD severity score for 16 putative mutants and 4 non-irradiated parental controls.

Selection of useful mutants

Based on the various levels of characterization, clean putative mutants not expressing disease symptoms and producing significantly higher grain yield than the respective parental controls were selected across the four induced genotypes (Table 4.17). The selected mutants were scored based on MSD severity, early flowering and grain yield. Obatanpa variant was ranked first followed by Dapango, Pann 53 and Honampa. Pann 53 ranked first in 53 days to 50% flowering, followed by Dapango (55), Honampa (56) and Obatanpa (58). Obatanpa variant had the highest grain yield of 6.8 t ha^{-1}, followed by Dapango, Pann 53 and Honampa. Data on days to 50% flowering indicated that all the four putative mutants are intermediate maturing plants (Table 4.17).

Table 4.17: Selected MSD mutants based on disease score, flowering and grain yield.

Genotype	MSD severity score (1 - 5)	Days to 50% flowering	Grain yield (t ha^{-1})	Genotype rank
Obatanpa	1	58	6.8	1st
Dapango	1.5	55	5.8	2nd
Pann 53	2	53	5.2	3rd
Honampa	2.5	56	4.8	4th

Source: Field data, Afram (2018)

Discussions

Maize is an important food crop in Ghana, accounting for more than 50 percent of the country's total cereal production. The Ghana Grains Development Project and the Food Crops Development Project made major investments to improve maize yield. Despite these efforts, the average maize yield in Ghana remains one of the lowest in the world, much lower than the average for Africa south of the Sahara (Jayne, et al., 2016). This study sought among other things to mitigate

the threat posed by MSD by breeding the disease resistant lines through mutation breeding. Gamma rays is one of the efficient and effective mutagens that has been clearly established because of high frequency of viable mutations reported by several authors (Nandpuri, 1970; Paliwal 1983). Crop varieties released through induced mutation are gradually increasing in number. The "mutation varieties – Data Bank" of FAO/IAEA data base contains information on 1548 mutant varieties of which Africa has just few contributions (Maluszynski, et al., 1995).

Acute irradiation and establishment of induced plants and controls
Germination

Germination of the seeds revealed dose-dependent response between the induced genotypes and the parental controls which were not induced. The acute dose of radiation delivered, which is also referred to as the LD_{50}, is to kill half of the irradiated genotypes, a concept that give rise to useful mutants which is expected in this study to achieve the breeding objective of developing maize streak disease resistant lines. Germination percentage among the mutants were initially lower in the M_1 phase but improved along the breeding cycles. This was in contrast to the controls which has a stable and consistent germination from the M_1 to the M_4 phase. Induced Obatanpa genotype had 65%, 90%, 87% and 95% germination from the M_1 to the M_4 phase, whiles the control had 96%, 97%, 98% and 998% from the M_1 to the M_4 phase. The germination percentage result is in agreement with several authors (Hegazi, & Hamideldin, 2010; Marcu, et al, 2014) who also reported reduced germination percentage in okra (*Abelmoschus esculentus*) and maize seed after irradiation.

According to Fan and Sokorai, (2005). Gamma radiation exerts negative effects through the increase number of free radicals resulting from water radiolysis,

which damage important cellular components and perturb vital processes. Reactive oxygen species are believed to be a major contributing factor to stress injuries and cause rapid cellular damage because they are highly reactive. Increased radiation doses enhanced the membrane permeability resulting in higher loss of leachates and reduced germination percentage (Chaturvedi et al., 2012)

Characterization of the induced plants for maize streak disease resistance
Observed mutagenic effect on plant morphology

Artificial induction of mutation through physical means provide good scope for crop improvement through selection of mutants having desired morphological and physiological traits. In mutation breeding experiment, the mutagenic treatments (mutagens, their doses and method of application) should be effective in inducing genetic changes. The primary effect of mutagens is ascertained immediately by some physiological damage to the plants in M_1 generation in the form of reducing plant growth and productive traits in addition to some hereditary changes. In the present study, the gamma irradiation brought about a decrease in the mean values of all the characters observed in M_1 generation as compared to control in all the induced genotypes. Similar decrease in M_1 traits following mutagenic treatments have also been reported in different crops, such as pepper, rice and sponge gourd by (Ramalingam, 1980; Rao et al., 1983; Kumar et al., 2002). There was 20% reduction in the mean height of the M_1 generation as compared to the control. However, there were a marginal increase of 2% in mean plant height of the putative mutants at the M_2 stage over the controls. The mutants however became shorter than the parental controls at the last two phases of the mutation breeding.

Flowering also varied among the genotypes with respect to the radiation effect on the morphology of the genotypes. Flowering in maize, both tasselling and

silking are maturity index which is important determinant in the plant's life cycle. All the mutants have one or two days longer than the parental controls. Notwithstanding, some of the individual mutants took lesser days than the controls to flower but were not selected due to plant architecture and disease severity score. According to Cairns et al., (2013) breeding early maturing maize with improved biotic stress tolerance will mitigate the threat of climate change and will also improve food security in Africa.

Crop improvement through mutation induction brings about other macro morphological effect on various plant parts. Chlorophyll mutations are considered as the major group of macromutations. Mutations producing drastic effects, which could be recognized with certainty in a single plant, were macromutation. There were scores of chlorophyll mutants observed in the M_2 generations in this study. The chlorophyll mutants ranged from xanthan, albina, xanthaviridis. Similar differential spectrum of morphological mutations in different mutagenic treatments has also been reported by Jahangir and Chandrasekharan (1978) in okra and Sood and Masuda, (2005) in Tomatoes. From crop improvement point of view, chlorophyll mutations are considered to be of less breeding value but they act as indicators of effectiveness of mutagenic effect (Jayabalan and Rao, 1988).

MSD incidence and severity.

Breeding for disease resistance is a major aspect of plant breeding, which may take at least 20% of a plant breeder's time, effort and budget (Kozjak, 2012). Nevertheless, numerous resistance problems remain unsolved and present major constraints to the roduction of food, feed, fiber and industrial commodities. The plant breeder, in search for resistance to a particular pathogen, such as in any other

desirable character, needs genetic variation to begin with. In addition, the breeder needs an appropriate screening method to detect the desired character (Van der Plank,1963).

The MSD incidence among the induced genotypes were low (22.7%) at the M_1 stage, moderate (26%) at the M_2 stage and high at M_3 and the M_4 stage (80 and 63%) (Table 4.9 and Figure 4.5a). The disease severity was equally low at the M_1 and M_2 phase with the mutants and the controls showing the same mean severity of 2.3. The mutants began to show more MSV disease susceptibility at the M_3 and M_4 stage when the disease incidence shot up to 80%. It was at the M_3 and the M_4 levels that mutagenic effect began to show up in terms of disease resistant. Obatanpa genotype was outstanding in selection for resistance and hence very mutable according to the breeding objective. There were scores of Obatanpa putative mutants showing no disease symptoms on the field, this was followed by genotype Dapango, Honampa and Pann 53 being the least resistance among the mutants (Figure 4.5b). Discovery of mutants at M_3 and M_4 generation has been reported by several authors including Gustafsson et al., (1971) in barley, Shereen, et al., (2009) in rice and Singh and Datta, (2010) in wheat.

Mutant detection is generally based on phenotypic observation, which means that the mutated gene has to have a fairly strong expression with little disturbance by environmental interaction for a reasonable chance of being detected. Selection gets of course, more complicated by the fact that many genes are "silent" through long periods of the plant's life and are only activated at certain stages of plant development, in certain organs, at certain times of the daily metabolic cycle (Ahloowalia et al., 2004).

Grain yield and inbreeding depression

Mutations are mostly recessive and they cannot be selected until the second and or latter generation, whereas dominant mutations occur at very low frequencies and can be selected in the first generation. To increase the chances of early detection of outcrossing plants like maize, there is a need for selfing to attain homozygosity to fix mutated gene early. Self-pollination was carried out among the induced plants from the M_2 to M_4 generation. The selfing however resulted in decrease in vigor and productivity of days to 50% flowering and grain yield for the induced plants. Days to 50% tasseling and flowering increased from 55.37 and 58.5 in M_0S_1 to 60.33 and 62.42 in M_4S_3 for tassel and silking respectively. Grain yield reduced from 6.44 t ha^{-1} in M_1S_0 to 5.97 t ha^{-1} in M_4S_3. The other three traits, plant height, ear height and leave area were not affected by the inbreeding depression in the study. The decrease in vigor and productivity that accompanies continuous self-fertilization in maize is a major frustration of breeders. This finding has been established by pioneer breeders like Genter, (1971) and Good and Hallauer, (1977).

Genetic variability for morphological characters

Variability is the measure of variation or the degree of difference among the individuals in a population which ultimately predicts the selection criterion for the improvement of any breeding programme. This is even more important and most expected when the germplasm is induced through purposeful mutation. Genetic variability in any crop is a pre-requisite to initiate the breeding programme for the selection of superior entries over the existing cultivar. The wholesome success of genetic improvement of any character may depend on the nature and magnitude of variability present in the gene pool for that character. Significant difference between

maximum and minimum values of each trait indicates the presence of considerable genetic variation in all the quantitative traits studied. Among the six quantitative traits, days to 50% flowering and grain yield are the main parameters in any maize improvement programme. In the present study, maximum grain yield was 6.8 t ha^{-1} and the minimum days to 50% tasseling and silking was 50 and 53 days. The maximum plant height and ear height for the four putative mutants was 153 cm and 66 cm. These results are in accordance with the findings of Kportor, (2012) who also reported 50 – 55 minimum days to tasseling and silking in some improved varieties of maize in Ghana.

The extent of variability among the mutant with respect to different quantitative characters was estimated in terms of phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV). In this study, (GCV) was lesser than (PCV) for all traits studied. Grain yield had the highest estimate of PCV (19.34) but recorded a low GCV of (8.11). Plant height was found to possess higher PCV and GCV of 18.74 and 15.33 than the ear height of PCV (15.21) and GCV of (4.41). The high PCV values suggest that these characters were unnder the influence of environmental control. Similar reports were given by (Akbar et al. 2008; Reddy et al., 2013) for grain yield per plant and plant height. Leaf area had a PCV of 18.88 and GCV of 10.58. Days to 50% tasseling and silking were close and also the least in both PCV and GCV in the estimated characters. Heritability (broad sense) estimates among the putative mutants were high for days to flowering and plant height except grain yield which recorded moderate estimate of heritability. It implies that selection and improvement is expected for these characters in future breeding programme as the genetic variance is mostly due to the additive gene action. The results are in

consonance with the reports given by (Natraj et al., 2014) for grain yield per plant, ear height, plant height,

Association analysis

Association analysis measured the natural relationship between the induced plant traits and determined the components on which selection can be done. Five traits of 24 selected mutants were correlated. Plant height positively correlated with grain yield (0.65). Leaf area and ear height also had positive corerrelation with grain yield (0.53) and (0.67) respectively. Plant height and ear height had strongly positive correlation with leaf area. However, number of days to 50% tasselling and silking had negative correlation with grain yield (-0.07) and (-0.06). Negatively correlation was expressed by MSD (-0.04) and tasseling (-0.068). These results are in accordance with findings of (Jayakumar, 2007; Alaei, 2012) who reported that grain yield of maize was found to be significantly and positively correlated with plamt height, leaf area and ear height. According to Ribaut, et al. (1997), the corroborative reports of positive correlation between grain yield and other traits components suggests that any one of the traits could be used to select indirectly for grain yield. Direct selection for yield is not effective since yield is a complex and indirect selection could be made for the component characters contributing to yield through character association as it provides information about the characters that are correlated with each other in improving dependent variable especially yield. Positive correlation of leaf area with grain yield are important indices for selection in improvement towards high grain yield. High grain yield with broader leaves area will enhance surface area interception of solar rays for higher accumulation of energy needed for more photosynthates. The excess photosyntate is stored in the form of higher yield.

Cluster analysis has been made to determine the extent of diversity among 24 selected putative mutants. The 24 putative mutants have been divided into three different clusters based on the Maize streak disease resistant. Cluster I contained the susceptible genotypes. These included all the controls and three putative mutants. Cluster II contained four putative mutants that has mild symptoms of the disease which is termed tolerant and cluster III, contained clean and symptomless induced mutants. The Grouping of the mutants according to resistant to MSD suggested that, the mutation induction has activated resistance genes 'R gene' of some induced maize genotype whiles others are lost through the same process. This result is in consonant with the research findings of Kozjak and Meglič, (2012) that, most proteins coded by R genes either silenced (masked) and or activated during mutation induction process.

Selection of useful mutants

Based on the various levels of morphological screening and field evaluation, putative mutants showing improved plant architecture, increased grain yield and resistant to maize streak disease were tagged and selected. Obatanpa-induced-genotype topped with a grain yield of 6.8 t ha^{-1}, followed by Dapango, Honampa and Pann 53. Data on days to 50% flowering indicated that all the four putative mutants are intermediate maturing plants. Seeds from the selected mutants will be planted in a separate trial and artificially inoculated with MSV from viruliferous leafhoppers and will be further screened using molecular technique for mutant confirmationin the next chapter.

Chapter Summary

Genetic induction of four selected maize genotypes was undertaken to create variability through mutation breeding for selection of maize streak resistant mutants. The seeds of the four maize genotypes were subjected to acute irradiation (gamma rays) and planted to raise the M_1. Seeds from the selected M_1 plants of each treatment were bulked to raise the M_2 generation and through to the M_4 generation. Field observation was recorded on clean and symptomless plants selected in each generation. The immediate effect of mutagenic treatments was ascertained on the basis of germination percentage, days to flowering, plant height, and grain yield. In general, mutagenic treatments brought about decrease in the mean values of all the characters observed in M_1 generations as compared to the respective controls. Gamma rays induced higher frequency of chlorophyll mutation at the M_2 phase. Analysis of variance and t-test revealed highly significant differences among the induced and control genotypes for all the characters under investigation. The Phenotypic coefficient of variation and Genotypic coefficient of variation estimates were high (> 30%) for plant height and days to 50% flowering. Phenotypic and genotypic correlation based on yield estimates revealed that plant height, and leave area possess highly significant positive correlation. Cluster analyses placed the mutant into three main clusters based on maize streak disease reaction. Cluster three contained the mutants that are completely resistant to maize streak virus, whiles cluster one and two contained both the controls and tolerant plants. Mutant selection from M_1 to M4 was based on phenotypic expression of traits which had limitations. The selected putative mutants will be further developed and artificially inoculated with viruliferous leafhoppers for molecular screening and confirmation.

CHAPTER FIVE

ARTIFICIAL INOCULATION OF MUTANTS WITH MAIZE STREAK VIRUS (MSV) AND MOLECULAR CONFIRMATION FOR DISEASE RESISTANCE

Introduction

Maize streak virus disease causes significant yield loss in maize when the virus infects susceptible plant at the early growth stage (Sharma and Misra, 2011). The economic impact of the disease makes it one of the major biotic constraints on maize production. The disease threatens the livelihood of small-scale farmers throughout Africa (Martin and Shepherd, 2009). Breeding for MSV disease resistant maize lines has been suggested to be the most cost-effective measure for reducing yield loss (Wisser et al., 2006). Despite the successes achieved in breeding against MSV disease, sporadic outbreaks of MSV have continued to occur in much of Africa, with significant yield losses. Maize streak virus infection of maize plants is caused by leafhoppers that feed on leaf mesophyll tissues of infected grass and transmit the acquired virus to healthy plants (Mesfin and Bosque-Pérez, 1998).

Transmission of MSV into maize by Cicadulina mbila is associated with injection of saliva into phloem tissues (Gray and Banerjee, 1999). Cicadulina mbila is known to move to different plant tissues while feeding, and this behaviour is dependent on nutritive and health status of host plant (Mesfin et al., 1995; Lett et al., 2001). Salivary sheaths produced during feeding usually extends to the vascular bundle, remain unbranched or terminate in the phloem (Mesfin et al., 1995). The minimum access acquisition period (AAP) of the virus by C. mbila is15 seconds but could be extended to about 5 min in a controlled environment. (Peterschmitt et al., 1996; Alegbejo et al., 2002). The reported minimum inoculation access period (IAP)

for C. mbila is 5 min, but usually takes 1-3 h from the initial access (Asanzi et al., 1995). The virus does not multiply in the vector, therefore the quantity of the virus acquired by the insect is crucial in relation to transmission (Markham et al., 1984). Therefore, successful viral transmission depends on the availability of the virus in plant, and the dose acquired. The longer the viruliferous insects are allowed to feed on healthy plants, the more likely the virus is transmitted (Okoth et al., 1987).

Artificial inoculation of plant disease is essential for studies of various aspects of plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction and disease control (Xu and Ko, 1998). Disease assessment through artificial inoculation method provides a foundational understanding of ecological enrichment on disease control (Jie et al. 2009). Among the methods developed, three main procedures of artificial inoculation are generally employed; spraying the spore suspension on test genotypes using an atomizer; injection of spore suspension into the plants surface or into the intercellular air spaces of a leaf with the help of a hypodermic needle and immersion of seedlings of test genotypes in a spore suspension before transplanting them into fields (Baayen, & Schrama, 1990). The conventional method like spraying has the disadvantage of causing considerable variation in spore distribution. Syringe infiltration is also one of the promising ways, but not suitable for viral transmission. (Bennett, 1969). In many field experiments, artificial inoculation by using the natural insect vector is considered most effective (Alexander et al., 1993).

Marker-assisted selection (MAS) is a technique in precision plant breeding which involves fragments of DNA revealing mutations/variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool (Bardakci, 2001; Gonzalez-

Chavire et al., 2006). There are different marker systems used in the analysis of genetic diversity in plants in marker-assisted plant breeding programmes (Akkaya et al., 1992; Bolibok et al., 2005). These markers include Restriction fragment length polymorphism (RFLPs) and Polymerase chain reaction (PCR)-based molecular markers, such as Random amplification of polymorphic DNA (RAPDs), Simple sequence repeat (SSRs), Amplified fragment length polymorphisms (AFLPs) and Single nucleotide polymorphisms (SNPs). These molecular techniques have a collective advantage over morphological markers as they are unaffected by environmental or physiological factors (Akter et al., 2008). Simple sequence repeat (SSR) markers, also known as microsatellites (He et al., 2003), have been extensively used to characterise germplasm collections in major cereal crops including wheat (Ijaz and Khan, 2009) and maize (Cholastova et al., 2011). Different repeat numbers in SSRs can be treated as separate "alleles" and the site can be treated as highly polymorphic with multiple alleles for the detection of variation in populations (Akkaya et al., 1992). Microsatellites are highly abundant, simple to analyse, co-dominant, economical and are easily assayed using PCR with primers specific to conserved regions flanking the repeat array (Yu et al., 2000). Compared with other marker types, SSRs are advantageous due to their abundance in plant genomes and large number of alleles per locus making them highly polymorphic even among closely related cultivars due to naturally occurring mutations, and thus they can distinguish between closely related species (Brown et al., 1996; Wöhrmann, and Weising, 2011;), providing greater power of discrimination. Hence, they are useful for assigning heterotic groups for maize lines (Xia-Su et al., 2004).

For developing resistant germplasm, artificial inoculation is essential to ensure disease development when natural infection is not possible, to optimise

genotypic host differentiation, and to reduce the influence of morphological characters that can contribute to disease avoidance (Mesterhazy, 1995). Virus-induced diseases cause severe damages to cultivated plants resulting in crop losses. Some diseased plants are able to re-gain health, further grow and develop normally. Thus, disease recovery involves the achievement of a tolerant state in which the replicating virus and the plant can co-exist without disease. Using *Arabidopsis thaliana* as a model system, Kørner et al. (2018), identified genes and mechanisms involved in recovery and further demonstrate that recovered leaves still contain replicating and infectious virus. The most efficacious means to determine the resistance gene of crop to a particular disease is through artificial inoculation (Mesterhazy et al., 2003).

The general objective of this study was to challenge MSD resistant mutants with MSV and to confirm the resistance using simple sequence repeat (SSR) primer.

The specific objectives of the study were to:

I. Infest elite mutant seedlings with viruliferous leafhopper colony.
II. Screen the infected mutant lines with MSV specific primer for the present or absence of MSD.

The null hypothesis was:

- Leafhoppers will not infest putative mutants with the MSV.
- Simple sequence repeat (SSR) will not detect mutants that are resistance to MSD,

Materials and methods

Study site

The experiment was conducted at the entomology section of the Crops Research Institute of CSIR, Kwadaso Station, in the Ashanti Region of Ghana. It is located in a deciduous forest zone with minimum rainfall of 1500 mm and lies between latitude 60 41' N, 10 36' W and having a coarse sandy-loam, Paleustult (Obeng, 2000).

Experimental materials.

Elite mutant seeds (M_4S_3) and controls in chapter four (4) were advanced to the ensuing experiment.

Establishment of screening nurseries and artificial inoculation of maize seedlings with maize streak virus

Establishment of screening nurseries

The screening nursery was established at the entomology Division of Crops Research Institute Kwadaso, Kumasi in the Ashanti Region of Ghana based on the recommendation of Bosque-Pérez & Alam (1992). It is made with a metal framed cage (1.25 x 1.25 x 1.50 m) covered with fine insect proof mesh of pore size 0.05 mm. A zipper is fixed on one side of the mesh cage to allow easy access to the plants and insects. The cage was placed over a metallic stand about 0.75 m high and the leg of the stand placed in a water trough to trap predatory insects from getting acess to the cage. Leafhopper (*Cicadulina mbila*, Naude) colonies were collected from the wild using iron frame covered with dark cotton cloth. The leafhoppers were put in the cage and reared and fed on young seedlings of wild pearl millet (*Pennisetum tryphoides*) plant for 8 weeks.

Artificial inoculation of maize seedlings with maize streak virus

Artificial inoculation for MSV disease expression was done following the method of Bosque-Perez and Alam (1992). Two to four weeks old maize plant showing MSD symptoms from farmer's field were transplanted into pots and used as source of inoculum. The leafhoppers were given 48 hours of Acquisition Access Period (AAP) on the MSV-infected maize plants. Seven (7) days to the inoculation, 168 seeds comprising 42 seeds each of the four putative mutant lines with their parental controls were planted in disposable cups filled with loamy soil. The young maize plant at 2-3-leaf stage transferred to cage. The viruliferous leafhoppers were then given a two-day inoculation access period (IAP) to infect the maize seedlings with MSV.

Transplanting

Ten (10) inoculated putative mutant seedlings and 10 control seedlings were immediately planted for each veriety, on the plot. Plots of four (4) derived varieties were distributed in three blocks (Replications) in a Randomized Complete Block Design (RCBD), making a total plant population of 240. The transplanting was done after the field was waterered copiously to provide moisture and cooling to the plant roots. Standard agronomic practices for maize experimental fields were followed.

Plant data and phenotypic evaluation

Agronomic data and phenotypic evaluation were carried out as described in experiment three chapter four.

Data analyses

All the morphological data recorded for each trait was subjected to Analyses of variance (ANOVA) using GenStat (12th edition) statistical package. The least

significant difference (lsd) was used to separate means when significant differences were observed. Student's T-test was used to compare significant differnces between controls and inoculated plants.

SSR Marker Assisted Screening of putative mutants for MSV.

Sampling of leaves, DNA extraction and DNA quality assessment

Leaves from 8 weeks old inoculated and transplanted putative mutant maize seedlings were sampled, cleaned with 70% ethanol, transferred immediately into plastic bags and transported to the laboratory on ice for storage at -4 ^{o}C until further processing. The total DNA was extracted from the leaf tissue using a modified CTAB method (Appendix 1). About 2 g of the chilled leaf sample was placed in 2 ml eppendorf microfuge tube, freeze-dried with liquid nitrogen and ground into a fine powder. A volume of 800 µl of CTAB buffer containing 2% CTAB, 2% Polyvinylpyrrolidone (PVP) solut, 1.4 M NaCl, 20 mM EDTA pH 8.0, 0.1 M Tris-HCl pH 8.0 and 1% β-mercaptoethanol was added with gentle shaking and placed in water bath at 65 ^{o}C for 30 min. The tubes were allowed to cool at room temperature and then equal volume of chloroform to iso-amyl alcohol in the ratio 24:1 was added and spun with the centrifuge (Eppendorf, Germany) for 12 min at 12000 rpm. The supernatant was pipetted into 1.5 ml eppendorf microfuge tube and the chloroform iso-amyl alcohol wash was repeated. The supernatant was again pipetted into a new 1.5 ml eppendorf microfuge tube, ice-cold isopropannol was added and kept in the freezer overnight at -20 ^{o}C to enhance DNA precipitation. The precipitated DNA was centrifuged at 14,000 rpm for 5 min to obtain DNA pellets and the isopropannol was carefully decanted. Pellets were washed with 10 mM ammonium acetate on a shaker for 15 min and spun at 6000 rpm for 4 min. Ammonium acetate was decanted and 80% ethanol was added to the pellets and spun

at 6000 rpm for 4 min. Ethanol was decanted and pellets were vacuum dried in DNA mini centrifuge (Jouan Nordic Gydevang, Denmark). The precipitated DNA was re-suspended in 50 µl of 1.0 mM Tris-HCl pH 8.0 and 0.1 mM EDTA pH 8.0 1X TE buffer.

The quality of each DNA isolate was established by electrophoresis on 0.8 % agarose gel stained by adding 5 µl/ml ethidium bromide solution. Each DNA sample (10 µl) was added to 2 µl loading dye (6X Bromophenol blue) in different eppendorf tubes. The mixtures were spun for 30 s at 4680 rpm and then loaded separately in the wells on the gel submerged in 1X TBE loading buffer. After loading, they were then run at 120 volts for 45 min and observed under the UV transilluminator (Scie-plas, UK). Samples were finally stored at 4 °C until required for use.

The MSV specific Primer

The total DNA of samples were amplified using MSV specific primer (MSV11) as reported by Oluwafemi et al., (2008). The PCR cocktail was prepared by adding all the constituents of the master-mix for each of the primer except the DNA into 0.2 ml PCR tubes on ice. In a 15 µl PCR reaction volume, 13.5 µl of master-mix was mixed with 1.5 µl of DNA. The sequences of the primers used were 5’ TTCATCCAATCATCATC 3’F / 5’ GGAAAATCTACTTGGGC3’R (Rybbicki and Hughes, 1990). The reaction mixtures were centrifuged briefly before placing in a thermocycler (Flex cycler2 Base Unit, Germany). The PCR cycles for the reaction mixture was programmed at a temperature of 94 °C for 2 min for initial denaturation; 35 cycles of denaturation at 94°C for 30 s, annealing at 50 °C for 40 s, extension at 72 °C for 1 min followed by 1 cycle of denaturation 94 °C for 30 s, 50 °C for 40 s, 72 °C of final elongation for 7 min.

Electrophoresis and visualization of amplified products

The KAPA Universal Ladder Kit was used for the electrophoresis and visualization of amplified products. The kit is designed for determining the approximate size and quantity of double-stranded DNA on agarose gel. The kit contained eighteen DNA fragments (in base pairs): 100, 150, 200, 300, 400, 500, 600, 800, 1000, 1200, 1600, 2000, 3000, 4000, 5000, 6000, 8000, and 10000. It also contains four reference bands (500, 1000, 1600, and 4000) for orientation. The KAPA kits are formulated with DNA loading dye for direct loading on agarose gel.

The PCR products were electrophoresed on 2% agarose gel system. The gel was prepared by weighing 4 g of agarose into a beaker containing 200 ml of 1X TBE buffer. The mixture was swirled to mix, melted in a microwave oven and allowed to cool to about 45 °C. The molten gel was stained by adding 5 µl/ml ethidium bromide solution before pouring into an electrophoresis tank with combs creating wells. The gel was allowed to solidify before being used. The solid gel was then placed in a gel box containing 1X TBE buffer making sure that it was completely submerged prior to removing the combs. Two µl of bromophenol blue loading dye was added to the PCR products and 10 µl was loaded into each 1.5 mm wide gel well. The first well was loaded with 5 µl of the 250 bp DNA ladder (M) followed by the PCR products from the DNA extracted from all the fourteen genotypes(1-14) under the artificial infestations, then known positive control (P) for the presence of MSV and lastly placed is a negative control (N), (purified water instead of DNA). The electrodes of the gel box were joined (red to red and black to black) and switched on to 90 volts. The gel was removed, visualized under UV transilluminator (Scie-plas, UK) and photographed after running for about 120 min.

Results

Germination and inoculation

All the 240 seeds of the four (4) genotypes planted germinated at the end of the 5th day after planting. Dapanngo genotypes germinated first with high vigour, followed by Pann 53, Honampa and Obatanpa (Figure 5.1). One nursery cage housing Leafhopper (*Cicadulina mbila*, Naude), contained all the 240 seedlings with high rate of success of inoculation when leaf samples were tested (Figure 5.2).

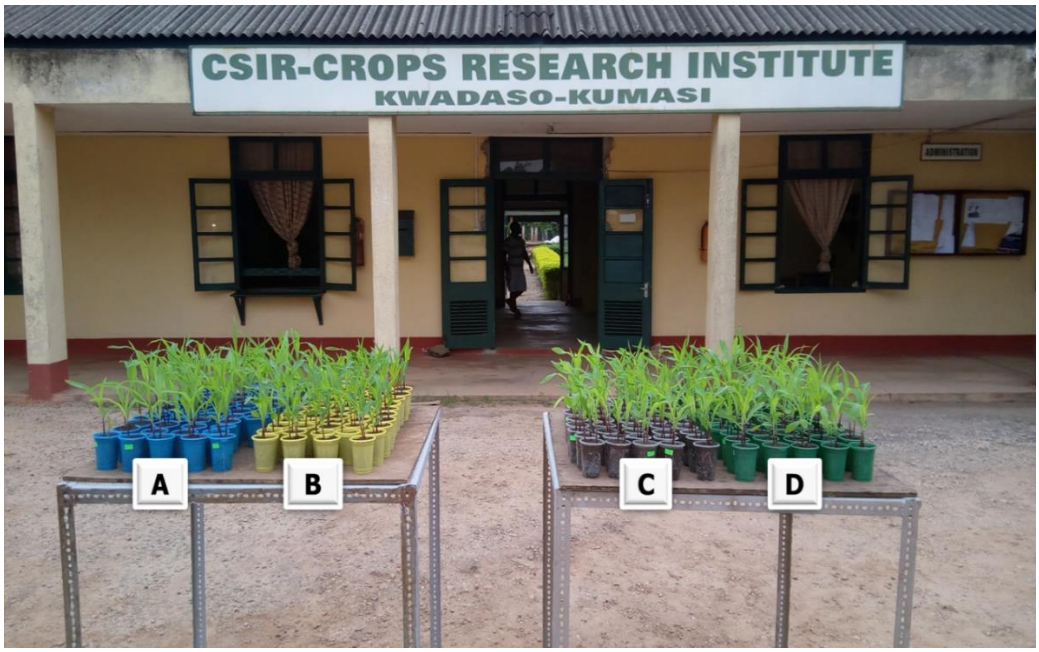

Figure 5.1: Inoculated putative mutants and controls. A = Dapango, B = Honampa, C = Obatanpa and D = Pann 53. Inoculated with MSV at two leaf stage.

Figure 5.2: Insectary at Crops Research Institute (CRI) Kwadaso-Kumasi, where the colonies of leaf hopper were reared for inoculation.

Field establishment of the challenged maize plants

High number of the inoculated putative mutants and control plants at 2-3 leaf stages surved after transplanting. Seventy two percent of controls and 80% of putative mutants surved. Obatanpa derived mutant recorded the highest survival rate of 83%, followed by Dapango, Honampa and Pann 53 being least (Table 5.1).

Table 5.5: Transplanting and survival of the MSV inoculated maize plants on the field

Genotype	Inoculated and transplanted		% Number survived	
	Control	Mutant	Control	Mutant
Obatanpa	30	30	70	83
Pann 53	30	30	46	60
Dapango	30	30	63	73
Honampa	30	30	60	67
Total	120	120	100	100

Source: Field data, Afram (2018)

MSD severity of controls (3.16) were higher than the putative mutants (2.61) (Table 5.2). A t–test comparism of the severity score for the putative mutants and their respective parental controls were significant ($P \leq 0.02$). The t-test value for Obatanpa putative mutant and the control was 0.02. Pann 53 was 0.04, whiles Dapango also had 0.02. They were no significant difference in disease expression between Honampa mutants and their parental control (Table 5.2).

Table 5.6: A t-test of mean severity MSV score of induced and parental control of four maize genotypes.

Genotype	Control	Mutant	T-test probabilities (α =0.05)
Obatanpa	2.78	1.43	0.02*****
Pann 53	3.25	2.96	0.40*****
DaPanngo	3.13	2.18	0.02*****
Honampa	3.49	3.86	0.08 ^{NS}
Mean	3.16	2.61	-
STDV	0.26	0.90	-

* - Significant; NS - Not significant; 1 - no symptoms; 2 – mild symptoms; 3 – moderate symptoms; 4 – severe; 5 – very severe symptoms.

MSV inoculation and change in plant height

There was a significant (P≤0.05) difference in the plant height of the mutants and controls after the MSV inoculation. Maize streak disease resistant mutants had a mean height of 140 cm, whiles susceptible controls had a mean height of 135cm. Obatanpa mutant was taller than the rest with a height of 160.38 cm, followed by 140.62 cm and 120 cm for Dapanngo and Honampa, respectively (Figure 5.3).

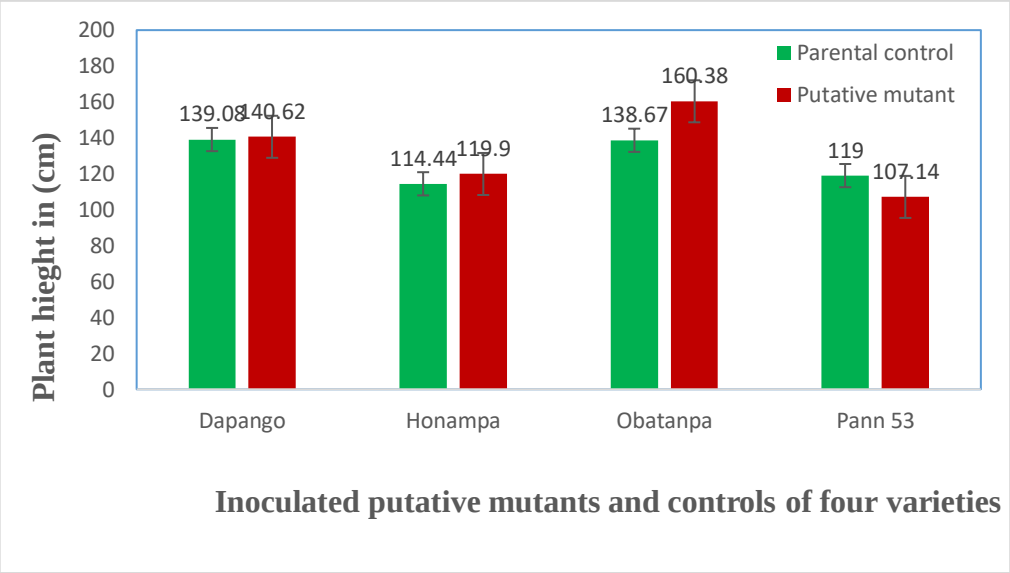

Figure 5.1: Plant height of inoculated putative mutants and parental controls

Days to 50% Anthesis, Silking and Anthesis to Silking interval.

There were variations in the flowering and anthesis among the mutants and the parental controls after the MSV challenge. A t-test between the mutants and their respective controls shows significant (P ≤ 0.01) differences in days to 50% tasseling, days to 50% anthesis for Obatanpa and Dapango mutants (Table 5.3). The 50% days to flowering and anthesis were not significant for Pan 53 and Honampa mutants and their controls. Mutant genotypes Obatanpa, Dapango Pann 53, and Honampa had 54, 46, 46 and 51 days to 50% tasseling, respectively. Similarly, Putative mutants Obatanpa, Dapango, Pann 53 and Honampa had 57, 50, 49 and 55 days to 50%

anthesis respectively. There were no significant differences in the anthesis to silking interval (ASI) among the genotypes and their respective controls.

Table 5.7: Days to 50% anthesis, Silking and anthesis to silking interval after MSV inoculation.

Genotype	Days to 50% tasseling		T-test	Days to 50% anthesis		T-test	Anthesis to silking interval		T-test
	control	Mutant		control	Mutant		control	Mutant	
Obatanpa	50.33	54.33	0.02 *	53.33	57.00	0.04 *	3.00	3.00	0.21 NS
Pann 53	46.00	45.67	0.33 NS	48.67	49.33	0.32 NS	2.67	2.67	0.11 NS
Dapango	49.00	46.33	0.04 *	52.33	50.00	0.01 *	2.67	2.67	0.11 NS
Honampa	51.33	51.33	0.50 NS	53.00	55.33	0.06 NS	2.33	2.33	0.09 NS
Mean	48.67	48.41	-	51.08	51.91	-	2.67	2.67	-
STDV	2.22	2.45	-	3.00	2.41	-	0.24	0.24	-

* - Significant; NS - Not significant; T-test was done at $\alpha = 0.05$.

Source: Field data, Afram (2018)

Grain yield (t ha^{-1}) of four putative mutants and controls after MSV inoculation

There was significant ($P \leq 0.05$) difference in grain yield of mutants and controls after the inoculation. Putative mutants had a mean yield of 4.23 t ha^{-1}, which was significant at ($P_{(\alpha = 0.05)} = 0.01$) while the parental control which were all susceptible to the MSD had a mean grain yield of 3.5 t ha^{-1}. Obatanpa mutant was the higher in grain yield 5.3 t ha^{-1} followed by 4.8 t ha^{-1} for Dapango mutants 4.5 t ha^{-1}, 2.3 t ha^{-1} Honampa and Pann 53 respectively (Figure 5.4).

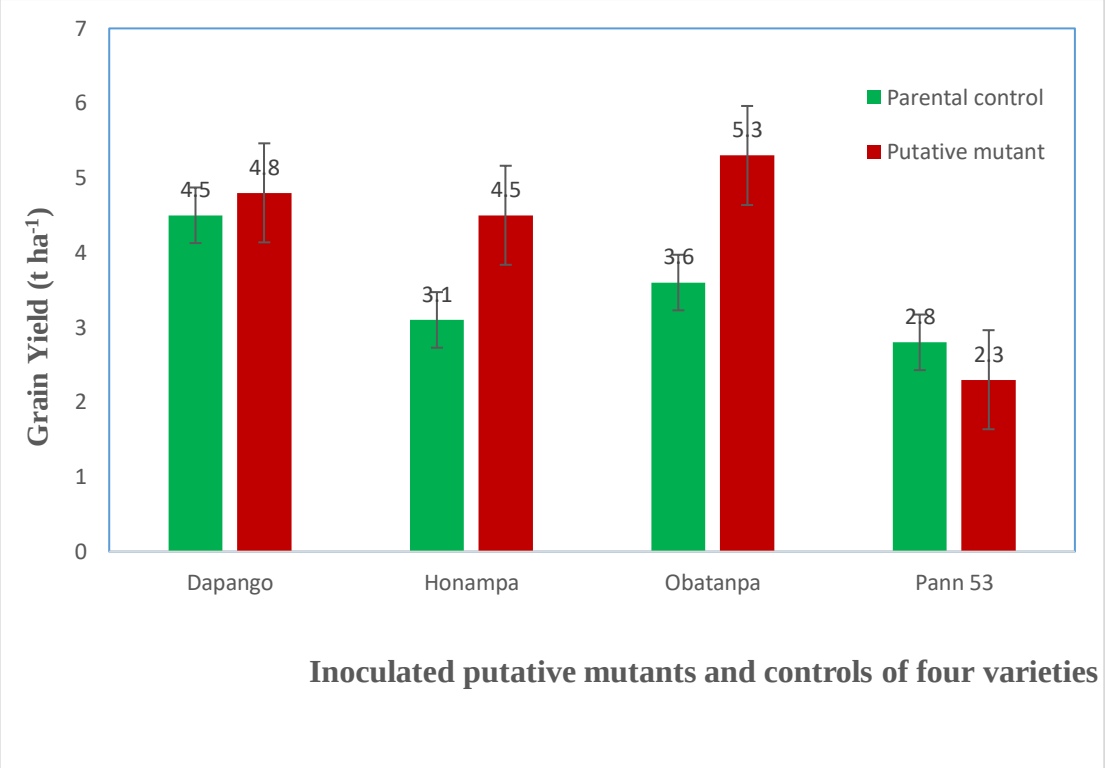

Figure 5.2: Grain yield of inoculated putative mutants and parental controls

Generation of codes and MSD status of 14 putative mutants

The generation of codes for the putative mutants and their morphological classification of their MSD status is shown in Table 5.4. The disease status was based on severity scores on the field for 14 putative mutants. The selected putative mutants were assigned codes following standard practices of CIMMYT (CIMMYT, 1985). The first three codes (TZS) referred to Tropical Maize Species, the second three codes (KPO) referred to location; the two figure codes (15) referred to the year of naming the genotype and the last digits code (0001) is the place holder for serial number of genotypes. The full code for putative genotypes one (1) and two (2) were TZS/KPO/140001 and TZS/KPO/150002, respectively. All the 14 putative genotypes were phenotypically resistant to MSD. There were six (6) putative mutants obtained from Obatanpa maize genotype followed by three (3), three (3) and two (2) mutants for Pann 53, Dapango and Honampa genotypes, respectively.

Table 5.8: Generation of codes and MSD status of 14 putative mutants.

Selected genotype	Generated codes	MSD status
	TZS/KPO/150001	Resistant
	TZS/KPO/150002	Resistant
OBATANPA	TZS/KPO/150003	Resistant
	TZS/KPO/150004	Resistant
	TZS/KPO/150005	Resistant
	TZS/KPO/150006	Resistant
	TZS/KPO/150007	Resistant
PANN 53	TZS/KPO/150008	Resistant
	TZS/KPO/150009	Resistant
	TZS/KPO/150010	Resistant
DAPANNGO	TZS/KPO/150011	Resistant
	TZS/KPO/150012	Resistant
	TZS/KPO/150013	Resistant
HONAMPA	TZS/KPO/150014	Resistant

Source: Field data, Afram (2018)

Molecular confirmation of maize streak disese status of 14 putative mutants

PCR amplification of the MSV from leaves of putative mutant maize lines (lanes 1-14) and control (lane 2) were shown in Figure 5.5. The result showed absence of bands at lanes one (1), three (3), four (4) and five (5) confirming MSD resistance status. The four MSD resistant mutants are Obatanpa mutants (TZS/KPO/150001 TZS/KPO/150003 TZS/KPO/150004 and TZS/KPO/150005). Bands were observed in lanes two (2), six (6), seven (7), eight (8), nine (9), ten (10), eleven, twelve, theirteen and fourteen indicating the presence of the virus and their susceptibility status. Positive and negative controls had presence and absence of bands at lanes ‘P’ and ‘N’, respectively.

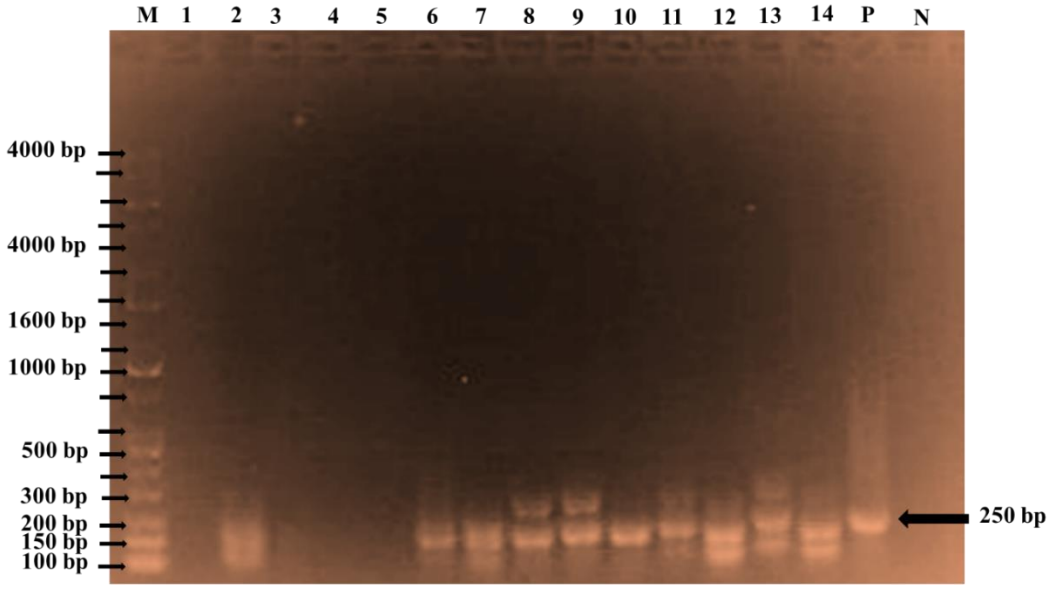

Figure 5.3: Agarose gel electrophoresis image of 250 bp image of MSV 11 F/R specific primer for screening maize streak virus. Susceptible genotypes had a band in lanes 2 and 6-14; and resistant genotypes lack a band in lanes 1 and 3-5. M denotes 1 kb DNA ladder from KAPA with 18 DNA bands of various sizes indicated. Lanes 1 – 14 (**1-TZS/KPO/150001**; 2-TZS/KPO/150002; **3 - TZS/KPO/150003; 4 - TZS/KPO/150004; 5 - TZS/KPO/150005;** 6 - TZS/KPO/150006; 7 - TZS/KPO/150007; 8 - TZS/KPO/150008; 9 - TZS/KPO/150009; 10 - TZS/KPO/150010; 11 - TZS/KPO/150011; 12 - TZS/KPO/150012; 13 - TZS/KPO/150013 and 14 - TZS/KPO/150014) represents 14 induced genotypes; P - Positive control and N - Negative control (distilled water).

Discussion

MSV inoculation and disease expression of putative mutants

The inoculation of the putative mutants with MSV at the 2-3 leaf stage was carried out and total of 83 inoculated and control plants became stunted and eventually died of disease presure (Table 5.1). Maize streak disease symptoms were visible on the susceptible putative mutants and all the parental control just after one week of inoculation and transplanting, confirming that inoculation was effective. Pale streak less than 10 mm was observed concentrated at the basal portion of the leaf of some of the putative mutants on the field while the controls were exhibiting exacerbated symptoms. The putative mutants had a mean severity score of 2.6 on 1-5 scale of severity whiles the controls had a mean severity score of 3.16. indicating

lower disease expression in some putative mutants than controls. Putative Obatanpa mutant was the most resistant genotype with a severity score of 1.43, followed by Dapango (2.18), Pann 53 (2.96) and Honampa (3.86). Field observation showed a number of Obatanpa putative mutants were completely clean of maize streak symptoms. The most plant death occurred in Pann 53 genotype. The putative mutant had 40% death and the parental control had 54% death. This corroborated with Bock (1982) who reported a slim chance of survival for maize seedlings infected with the virus as early as the second leaf stage of growth. The death of some the controls and inoculated plants could be attributed to the viruliferous leafhoppers. According to (Rose, 1978; Van Rensburg and Giliomee, 1990), population density of viruliferous leafhoppers and the concentrations of viruses within the salivary glands of the leafhoppers influence disease severity and plant survival.

Plant height which is a function of growth and development was equally significant in the study. A t-test comparison between the mutants and the parental control shows a significant ($P_{(\alpha = 0.05)} = 0.01$) effect on plant height. There was 22% reduction in mean plant height of the putative mutants from the M_4 generation as against 26% reduction in mean plant height of the parental control. The putative mutant had a mean height of 140 cm whiles the parental controls had a mean height of 135 cm. Obatanpa mutant was the tallest after the MSV inoculation. Field records showed that the mutants become taller than the controls after the MSV challenge. The difference recorded in the heights of the putative mutants and the parental control may be as a result of the maize streak disease suppression on plant's biomass accumulation and of inhibition of photosynthesis and carbon assimilation. According to (Wollenweber et al., 2005; Palme et al., 2014), plant height is an important index in plant breeding which directly affects the yield of crops. In maize

breeding, Semi-dwarf plants are desired, because such plants are more resistant to lodging and are fertilizer responsive.

MSV inoculation and days to 50% tasseling

Putative mutants had an average of two more days to 50% tasseling in comparism to their respective controls. Significant ($P_{(\alpha = 0.05)} = 0.01$) differences were observed among Obatanpa and Dapango derived mutants (Table 5.3). Days to 50% tasseling ranged from 45 to 54 days. The putative mutants could be classified as intermediate maturing plants suitable for some agroecological zones of the country.

Days to 50% anthesis

Days to 50% anthesis is an important character for determining maturity period in maize crop. Pollen grains remain viable for a shorter period of time as compared to silks. Pollination must not occur within 1-8 days of anthesis to ensure perfect synchronization and high kernel filling and productivity. Statistical analysis revealed a significant ($P \leq 0.01$) difference among the two top performing putative mutants, Obatanpa and Dapango. Days to 50% anthesis ranged between 49 to 57 days. Dapango putative mutant took minimum days (49) to 50% anthesis, while Obatanpa recorded the maximum days of (57) to 50% anthesis. In an earlier experiment to evaluate newly released maize varieties in Ghana for yield and stability under three nitrogen application rates in two agro-ecological zones, Azinu (2014) also reported similar values among some selected maize hybrids. There was no remarkable difference in the anthesis to silking interval among the maize genotypes as all the putative mutants had 3 days interval except Honampa which had two days interval.

MSV inoculation and Grain Yield of putative mutants

The goal of crop breeding is to develop varieties with a high yield potential and desirable agronomic characters. In maize breeding, the most important qualities sought by breeders have been high yield potential; resistance to major diseases and insects; and improved grain and eating quality. Analysis of variance revealed that there were significant differences among the putative mutants for yield. The grain yield for the mutants ranged from 2.3 t ha^{-1} to 5.3 t ha^{-1} and grand mean of 3.9 t ha^{-1}. Obatanpa mutant had the highest yield. Although all the putative mutants have been cyclically selfed with inbreeding depression, the yield is still encouraging especially for induced Obatanpa (5.3 t ha^{-1}). The grain yield in this study is similar to that of Kpotor (2012) who evaluated newly released maize varieties in Ghana, and recoorded a grain yield of 4.73 t ha^{-1} for 'Mamaba' a Quality Protein Maize (QPM). It was observed that the grain yield of native Dapango was marginally higher than Obatanpa followed by Honampa and Pann 53 but after induction Obatanpa had higher grain yield than Dapango, followed by Honampa and Pann 53.

Molecular confirmation of putative mutants

PCR is one of the most effective molecular means in identification, detection and diagnosis of plant viruses. It involves the use of molecular markers or primers (Sharma and Misra, 2011). The specific MSV detection primer used (MV 11) was reported to have a size of 250bp (Oluwafemi et al., 2008). Decisively, the primer detected MSV in ten (10) out of fourteen (14) putative mutants under the artificial infestations with viruliferous leafhoppers. The result corroborated the investigations by Mesterhazy (1995), who reported that in developing resistant germplasm, artificial inoculation is essential to ensure disease development when natural

infection is not conclusive and effective so as to optimise genotypic host differentiation, and to reduce the influence of morphological characters that can contribute to disease avoidance.

The molecular analyses confirmed putative mutants (TZS/KPO/150001, TZS/KPO/150003, TZS/KPO/150004 and TZS/KPO/150005) as mutants. These genotypes were all mutated from Obatanpa. Mutations in general are recessive traits which occur at low frequencies, one in a million per gene. If two independent mutations are necessary in recessive alleles to obtain resistant phenotype, the frequency lowers to 10^{-18} per nucleotide (Gressel and Levy, 2006). Mutagenesis is however used to accelerate spontaneous mutations in driven evolution (Kozjak and Meglič, 2012). Using chemical mutagen (EMS) in arabidopsis about ten mutations per gene were recorded among 192 genes in 3,000 M_2 plants examined (Greene et al., 2003) with an average of 720 mutations in single M_2 plant (Till et al., 2003). For the improvement of disease resistance, the induction of spontaneous mutations by irradiation usually cause point mutations (deletions or insertion of DNA), (Li et al., 2002; Li and Zhang, 2002; Rogers et al., 2009). Mutagenesis is a fundamentally important DNA technology which seeks to change the base sequence of DNA and test its effect on gene or DNA function. The results obtained in this study is a justification of mutation breeding and has thus satisfied the breeding objective outlined in the experiment. According to Ahloowalia and Maluszynski (2001), induced mutations have been employed to improve major crops such as wheat, rice, barley, cotton, peanuts, and beans, which are seed propagated. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, more than 1800 cultivars obtained either as direct mutants or derived

from their crosses have been released worldwide in 50 countries. Mutation breeding remained a viable option to compliment conventional breeding.

In Ghana, somatic embryogenesis has been developed through in vitro mutagenesis and pollen mutagenesis of desirable genotypes and is being applied for induction of virus-resistant cocoa trees (Novak and Brunner., 1992). Amenorpe (2010) also used mutation breeding for modification of amylose starch in cassava. Breeding for host plant resistance along with performance traits requires the use of an effective breeding method and selection strategy. Such a selection strategy and related indices should contribute to reducing the time required for breeding, and saving resources for further development and testing. The four maize germplasm mutants identified and confirmed fit into the efficient selection indices and selection strategy for maize inbred lines that combines selecting for traits of primary interest such as anthesis-silking interval and resistance to maize streak virus (MSV) with other characteristics that directly and indirectly influence grain yield. In mutation breeding, a mutant possessing all the desired trait, may be released directly as a new improved variety. It may also be back crossed to a mother strain to improve one or two undesirable trait or crossed to a well adapted and high yielding local variety. In this study since all the mutants are mutated from Obatanpa line, there may be a need for multi-locational trials to establish genetic stability.

Chapter Summary

Marker-assisted selection (MAS) which involves the use of primers to follow regions of the genome that encode specific characteristics of a plant genetically linked to a disease resistance locus is the most effective means to predict the presence of the resistant or the susceptible allele. The reliability of the prediction will depend upon the closeness of the genetic linkage. Primers that co-segregate with

the target trait are absolutely reliable and can be regarded as diagnostic. To be effective, the primer must detect a polymorphism between the plants being analyzed. Detailed analyses of morphological and phenotypic data of putative mutants after the MSV inoculation was not enough to pass the putative mutants as complete mutants. The phenotypic analyses classified 14 of the genotypes as mutant but only four has been confirmed through simple sequence repeat (SSR). There is therefore the need for molecular confirmation in any purposeful mutation breeding programme. The mutants confirmed in this study also have good agronomic traits such days to 50% anthesis and grain yield. Detection of sequence variation at the DNA level offers several important advantages and hope for plant breeders. There are essentially an unlimited number of such DNA markers since sequence variation, in the form of single-base changes, insertions and deletions, or large sequence differences, are abundant. The result of the study establishes the fact that molecular techniques have become part of plant mutagenesis research.

CHAPTER SIX

GENERAL CONLUSIONS AND RECCOMMENDATIONS

Conclusion

Breeding for disease resistance requires resistant gene combination in susceptible host plants for hybridization and where there is no reliable source of resistant gene, induced mutation becomes the viable option. The objective of this study was to use nuclear technique to induce mutation in four selected maize genotypes to breed against the maize streak virus (MSV) disease. The study began with the measure of sensitivity of maize genotypes to radiation and the Lethal dose (LD_{50}) for mutation induction. The optimum dose which reduces growth rate and seed production by 50% (RD_{50}) was the next parameter established after which all the selected putative mutants were characterised and classified based on severity of maize streak disease. Putative mutants were artificially inoculated with MSV in a screen house and 'true mutants' confirmed using simple sequence repeat (SSR).

The LD_{50} doses for the six genotypes were ranked from a maximum dose of 299 Gy for Dormabin followed by 281 Gy for Dapango, 269 Gy for Pannar 53, 250 Gy for Keta 60, 231 Gy for Obatanpa and 221Gy for Dzinueve. Application of the acute dose of 288.5 Gy was observed during the characterization of the induced plants for maize streak disease resistance. Maize streak disease expression was higher in the control plants than the induced genotypes at the end of the M_4 stage. The severity of MSD was observed to be lower in the induced genotypes than respective controls. Some induced genotypes of Obatanpa had severity score as low as 1.4 and the control scored 2.8. Similarly, some of the induced genotypes of Dapango had a severity score of 2 and the controls scoring 3.2 followed by Pann 53 which had 2.4 while the control was 3.4. Honampa on the other hand scored 2.2 and

the control was 3.0. Following characterization, 14 individual plants across the four genotypes were observed to show resistance to maize streak disease and also produced higher grain yield than the respective controls. The 14 maize plants were considered as putative mutants.

Molecular screening confirmed four (4) out of the fourteen putative mutants as resistant to MSD while the remaining ten (10) were susceptible. The four mutants which were all obtained from Obatanpa were coded as TZS/KPO/140001, TZS/KPO/140003, TZS/KPO/140004 and TZS/KPO/140005. The confirmed mutants also have excellent agronomic traits such days to 50% anthesis and grain yield. It is therefore recommended that:

The MSD resistant mutants developed in this study should be further evaluated for the stability of the resistant gene.

The resistant gene should be introgressed into commercial varieties or formulated into hybrids.

QTL mapping and analyses should be done to identify genomic regions associated with the MSD resistant mutants.

.

REFERENCES

Ahloowalia, B.S. & Maluszynski, M. (2001). Induced mutations: a new paradigm in plant breeding. *Euphytica*, 118, 167–173.

Ahloowalia, B.S., Maluszynski, M. & Nichterlein, K. (2004). Global impact of mutation-derived varieties. *Euphytica*, 135, 187–204.

Akbar, M., Shakoor, S., Hussain, A. & Sarwar, M. (2008). Evaluation of maize 3-way crosses through genetic variability, broad sense heritability, characters association and path analysis. *Journal of Agricultural Research*, 46(1), 39-45.

Akkaya, M. S., Bhagwat, A. A., & Cregan, P. B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics, 132*(4), 1131-1139.

Akter, J., Islam, M.S., Sajib, A.A., Ashraf, N., Haque, S. & Khan. H. (2008). Microsatellite markers for determining genetic identities and genetic diversity among jute cultivars. *Australian Journal of Crop Science*, 1, 97-107.

Alaei, Y. (2012). Correlation analysis of corn genotypes morphological traits. *International Research Journal of Applied and Basic Sciences*, 3, 2355-2357.

Alegbejo, M.D., Olojede, S.O., Kashina, B.D. & Abo, M.E. (2002). Maize streak mastrevirus in Africa: distribution, transmission, epidemiology, economic significance and management strategies. *Journal of Sustainable Agriculture, 19*(4), 35-45.

Alene, A. D., Menkir, A., Ajala, S. O., Badu-Apraku, B., Olanrewaju, A. S., Manyong, V. M., & Ndiaye, A. (2009). The economic and poverty impacts of maize research in West and Central Africa. *Agricultural Economics*, 40(5), 535-550.

Alexander, H. M., Antonovics, J., & Kelly, A. W. (1993). Genotypic variation in plant disease resistance-physiological resistance in relation to field disease transmission. *Journal of Ecology*, 325-333.

Al-Salhi, M., Ghannam, M.M., Al-Ayed, M.S., El-Kameesy, S.U. & Roshdy, S. (2004). Effect of gamma irradiation on the biophysical and morphological properties of corn. *Nahrung*, 48, 95–98

Amenorpe, G. (2010). *Mutation breeding for in planta modification of amylose starch in cassava (Manihot esculenta, Crantz)*, Unpublished Doctoral dissertation, University of the Free State.

Amenorpe, G., Amoatey, H.M. and Asare, D. (2004). Breeding for drought tolerance/avoidace and mealyness in cassava using gamma radiation. GAEC/BNARI 2004 Annual Report, 22-23.

Asanzi, M., Bosque-Perez, N. A., Nault, L. R., Gordon, D. T., & Thottappilly, G. (1995). Biology of Cicadulina species (Homoptera: Cicadellidae) and transmission of maize streak virus. *African entomology*, 3(2), 173-179.

Asare-Bediako E., Kvarnheden, A., van der Puije, G.C., Taah, K.J., Frimpong, K.A., Amenorpe, G., Appiah-Kubi, A., Lamptey, J.N.L., Oppong, A., Mochiah, M.B., Adama, I. & Tetteh, F.M. (2017). Spatio-temporal variations in the incidence and severity of maize streak disease in the Volta Region of Ghana. *Journal of Plant Pathology and Microbiology*, 8(3), 401-407.

Asare-Bediako, E., Taah, K. J., van der Puije, G., Amenorpe, G., Appiah-Kubi, A., and Akuamoa-Boateng, S. (2019). Evaluation of maize (zea mays l.) genotypes for high grain yield and resistance to maize streak virus infections under diverse agro-ecological zones. *Research Journal of Plant Pathology*, 2, 2-11.

Auerbach, (1962). Mutation: An Introduction to Research on Mutagenesis.Part 1 Methods. Oliver and Boyd., London, 176 p.

Azinu, A., R. (2014). *Evaluation of hybrid maize varieties in three agro-ecological zones in Ghana*. Unpublished master's thesis, Department of Crop Science, University of Ghana,

Baayen, R. P., & Schrama, R. M. (1990). Comparison of five stem inoculation methods with respect to phytoalexin accumulation and Fusarium wilt development in carnation. *Netherlands Journal of Plant Pathology*, 96(6), 315-320.

Badu-Apraku, B., Fakorede, M. A. B., Menkir, A. & D. Sanogo, D. (eds.). (2012). Conduct and management of maize field trials. International Institute of Tropical Agriculture, Ibadan, Nigeria, 59.

Bardakci, F. (2001). Random Amplified Polymorphic DNA (RAPD marker). *Turkish Journal of Biology*, 25, 185-196.

Barrow, M.R. (1992). Development of maize hybrids resistant to maize streak virus. *Crop Protect*, 11, 267-271.

Bennett, C. W. (1969). Seed transmission of plant viruses. In advances in virus research, 14, 221-261.

Bjarnason, M. S. (1986). Progress in breeding for resistance to the maize streak virus disease. In: B. Gelow (ed.), First Eastern, Central, Southern Africa Regional Maize Workshop to Feed Ourselves, March 10-17, 1985, Lusaka, Zambia. CIMMYT, Mexico.

Bock KR (1982) Geminivirus diseases in tropical crops. *Plant Dis*, 66, 266–270

Bock, K.R. (1974). Maize streak virus. CMI/AAB Description of plant viruses, No. 133.

Bolibok, H., M. Rakocyz-Trojanowska, A. Hromada, & R. Pietrzykowski. (2005). Efficiency of different PCR-based marker systems in assessing genetic diversity among winter rye (Secale cereale L.) inbred lines. *Euphytica*, 146, 109-116.

Bosque-Perez, N.A. & Alam. M.S. (1992). Mass rearing of Cicadulina leafhoppers to screen for maize streak virus resistance: a manual. International Institute of Tropical Agriculture, Ibadan, Nigeria, 22.

Bosque-Perez, N.A., Olojede, S.O. & Buddenhagen, I.W. (1998). Effect of maize streak virus disease on the growth and yield of maize as influenced by varietal resistance levels and plant stage at time of challenge. *Euphytica*, 101, 307-317.

Brammer, H. (1962). Soils: in agriculture and land use in Ghana. J.B. Wills ed., Oxford University Press London, Accra, New York, 88-126.

Briddon, R. W., Pinner, M. S., Stanley, J., & Markham, P. G. (1990). Geminivirus coat protein gene replacement alters insect specificity. *Virology*, 177(1), 85-94.

Broertjes, C., & Van Harten, A. M. (2013). Applied mutation breeding for vegetatively propagated crops, (12). Elsevier.

Brown, J.K., Fauquet, C.M., Briddon, R.W., Zerbini, M., Moriones, E., Navas-Castillo, J. (2012) Classification and nomenclature of viruses; ninth report of the International Committee on Taxonomy of Viruses; Eds.; Elsevier Academic Press: London, UK, 351–373.

Brown, S. M., Szewc-McFadden, A. K., & Kresovich, S. (1996). Development and application of simple sequence repeat (SSR) loci for plant genome analysis. *Methods of genome analysis in plants*, 1, 147-158.

Bua, B. & Chelimo, B. (2010). The reaction of maize genotypes to maize streak virus disease in central Uganda. *Second RUFORUM Biennial Meeting held from 20-24 September, 2010.* Entebbe, Uganda.

Burton, G.W. (1952). Quantitative inheritance in grasses. Proc. 6th Intl. Grassland Congress. Pennsylvania State College, USA, 273-83.

Cairns, J. E., Hellin, J., Sonder, K., Araus, J. L., MacRobert, J. F., Thierfelder, C., & Prasanna, B. M. (2013). Adapting maize production to climate change in sub-Saharan Africa. *Food Security*, 5(3), 345-360.

Caldwell, D.G., McCallum, N., Shaw, P., Muehlbauer, G.J., Marshall, D.F. & Waugh, R. (2004). A structured mutant population for forward and reverse genetics in barley (Hordeum vulgare L.). *Plant Journal*, 40(1), 143–50.

Çelik, Ö., & Atak, Ç. (2017). Applications of ionizing radiation in mutation breeding. *New Insights on Gamma Rays*, 111-132.

Chahal, G.S. & Gosal, S.S. (2002). Principles and procedures of plant breeding. Oxford: Alpha Science International Ltd, 399–412.

Chapman, G.P. and Peat, W.E. (1992). An introduction to the grasses (including bamboos and cereals). CAB International.

Chaturvedi, A., Sreedhar, M., Singhal, R.K., Venubabu, P., Kumar, D. & Aparna, M. (2012). Effect of γ irradiation on germination of rice (Oryza sativa L.). *International Journal of Bioresource and stress Management*, 3, 18-21.

Cholastova, T., Soldanova, M. & Pokorny, R. (2011). Random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) marker efficacy for maize hybrid identification. *African Journal of Biotechnology*, 10, 4794-4801.

CIMMYT. (1985). Managing trials and reporting data for CIMMYT International maize testing program. Mexico D. F. CIMMYT.

Cruz, C. D. & Regazzi, A. J. (1997). Modelos biométricos aplicados ao melhoramento genético. Viçosa, MG: Editora da UFV.390 p.

Dale, M.F.B., Grifuths, D.W., Bain, H. & Goodman B.A. (1997). The effect of gamma irradiation on glycoalkaloid and chlorophyll synthesis in seven potato cultivars. *J. Sci. Food Agric.*, 75, 141-147.

Damsteegt, V. D. (1983). Maize streak virus: I. Host range and vulnerability of maize germ plasm. *Plant Disease*, 67(7), 734-736.

Darren, P. M. & Shepherd, D. N. (2009). The epidemiology, economic impact and control of maize streak disease. *Food Sec.*, 1, 305–315.

DeVries, J. & Toenniessen, G.H. (2001). Securing the harvest: biotechnology, breeding, and seed systems for African crops. CABI.

Doebley, J. (2004). The genetics of maize evolution. *Ann. Rev. Gen*., 38, 37–59.

Dong, C.M., Vincent, K. & Sharp, P. (2009). Simultaneous mutation detection of three homoeologous genes in wheat by High Resolution Melting analysis and Mutation Surveyor (R). BMC *Plant Biology*, 9, 143.

Dreher, K., Khairallah, M., Ribaut, J-M., Pandey, S. & Srinivasan, G. (2000). Is marker assisted selection cost-effective compared to conventional plant breeding methods? The case of quality protein maize. Fourth annual conference of the International Consortium on Agricultural Biotechnology Research (ICABR). Ravello, Italy.

Dresselhaus, T. H. O. M. A. S., Carman, J. G., & Savidan, Y. (2001). Genetic engineering of apomixis in sexual crops: A critical assessment of the apomixis technology. Flowering of Apomixis: From Mechanisms to Genetic Engineering, Y. Savidan, J.G., Carman, and T. Dresselhaus, eds (Mexico: CIMMYT, IRD, European Commission DG VI), 229-243.

Effron, Y., Kim, S.K., Fajemisin, J.M. Marek, J.H., Tang, C.Y., Dabrowski Z.T., Rossel, H.W. & Thottappilly, G. (1989). Breeding for resistance to maize streak virus: a multi-disciplinary team approach. Plant Breeding, 103, 1-36.

Esnault, A.M., Legue, F. & Chenal, C. (2010). Ionizing radiation: advances in plant response. Environ. *Exp. Bot.* 68, 231–237.

Fajemisin, J. M., & Shoyinka, S. A. (1976). Maize streak and other maize virus diseases in West Africa. In Proceedings, International maize virus disease colloquium and workshop. (pp. 52-60). Ohio Agricultural Research and Development Center.

Fajemisin, J. (2003). Overview of maize viruses in Sub-Saharan Africa. Plant virology in Sub-Saharan Africa, In: J. H. Hughes and J. Odu (eds.), International Institute of Tropical Agriculture Conference, Ilesa, Nigeria, 158-171.

Fajemisin, J.M., Debrowski, Z.T., Effron, Y. & Kim, S.K. (1986b). Weather factors associated with recurring maize streak epidemics. Proc. of the seminar on agrometeorolgy and crop protection in the lowland, humid and sub-humid tropics. Cotonou, Benin, 267-276.

Falconer, D. S. (1989). Introduction to quantitative genetics 3rd ed. Harlow: Longman Scientific & Technical.

Falconer, D. S. and Mackay T. C. (1996). Introduction to quantitative genetics. 4th Edition. Longman Group Ltd., London, 160, 274-281.

Fan, X. & Sokorai, K. J. B. (2005). Assessment of radiation sensitivity of fresh-cut vegetables using electrolyte leakage measurement. *Postharvest Biology and Technology*, 36, 191–197.

FAO/IAEA (2009). A symposium of International Atomic Energy Agency, Vienna, Austria, 316–8.

FAO/IAEA. (2014). Mutant varieties database (http://mvgs.iaea.org/Search)

FAO/IAEA. (2018). Manual on mutation breeding - Third edition. Spencer-Lopes, M.M., Forster, B.P. and Jankuloski, L. (eds.), Food and Agriculture Organization of the United Nations. Rome, Italy. 301 pp.

FAO/UNESCO (1998). FAO/UNESCO soil map of the world, revised legend, world resources report 60. FAO, Rome, Italy. pp. 146.

FAOSTAT. (2016). Food and Agricultural Organization of the United Nations (FAO), FAO statistics database.

Feng, G., Liu, Z. F & Li, Y. Y. (2009). Genetics of lodging in tolerance to maize stem puncture, *Acta Agronomica Sinica*, 35, 2133-2138.

Fisher, R.A. and F. Yates. (1963). Statistical tables. 6th ed. New York.

Forell, G. V., Robertson, D., Lee, S. Y., & Cook, D. D. (2015). Preventing lodging in bioenergy crops: a biomechanical analysis of maize stalks suggests a new approach. *Journal of experimental botany*, 66(14), 4367-4371.

Gadani, F., Mansky, L.M., Medici, R., Miller, W.A. & Hill, J.H. (1990) Genetic engineering of plants for virus resistance. *Arch Virol* 115:1–21.

Gager, C.S. (1908). Effects of the rays of radium on plants. Memories of the New York Botanical Garden, 4, 278.

Gaul, H. (1964). Mutation in plant breeding. *Radia. Bot.*, 4, 155-252.

Genter, C. F. (1971). Yields of S1 lines from original and advanced synthetic varieties of maize 1. *Crop science*, 11(6), 821-824.

Gibson, L. & Benson, G. (2002). Origin, history, and uses of corn (Zea mays). Iowa State University, Department of Agronomy. Accessed August 19, 2016. http://agronwww. agron.iastate.edu/Courses.

Giroux, M. J., Shaw, J., Barry, G., Cobb, B. G., Greene, T., Okita, T. & Hannah, L. C. (1996). A single mutation that increases maize seed weight. Proceedings of the National Academy of Sciences, 93(12), 5824-5829.

Gonzalez-Chavire, M.M., Torres-Pacheco I. & Villordo-Pineda. E. (2006). DNA markers. *Advances in Agricultural and Food Biotechnology* 2, 99-134.

Good, R. L., & Hallauer, A. R. (1977). Inbreeding depression in maize by selfing and full-sibbing 1. Crop Science, 17(6), 935-940.

Goodman, M. M., & Galinat, W. C. (1988). The history and evolution of maize. Critical reviews in plant sciences, 7(3), 197-220.

Goodspeed, T.H. (1929). The effect of x rays and radium on species of the genus Nicotiana, *J. Heredity*., 20, 243.

Gorter, G.J.M.A. (1953). Studies on the spread and control of the streak disease of maize. Union of South Africa Department of Agricultural Science Bulletin. *Botany and plant pathology series*, 18, 341

Gray, S.M. & Banerjee, N. (1999). Mechanisms of arthropod transmission of plant and animal viruses. *Microbilogy and molececular Biology Rev*, 63, 128-148.

Greene, E.A., Codomo, C.A., Taylor, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H., Enns L.C., Burtner, C., Johnson, J.E., Odden, A.R., Comai, L. & Henikoff, S. (2003). Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genetics*, 164, 731–740.

Gressel, J. & Levy, A. (2006). Agriculture: the selector of improbable mutations. Proceedings of the National Academy of Sciences, 103, 12215-12216.

GSS (Ghana Statistical Service) (2014). 2010 Population and housing census: districts analytical report. Accra, Ghana: Ghana Statistical Service.

Gustafsson, A. (1940). The mutation system of the chlorophyll apparatus. Lunds. Univ. Arssks., 36, 1-4.

Gustafsson, A., Hagberg, A., Persson, G., & Wiklund, K. (1971). Induced mutations and barley improvement. *Theoretical and Applied Genetics*, 41(6), 239-248.

Hallauer, A. and Miranda, J. (1988). Quantitative genetics in maize breeding. Iowa State University. Press.

Hallauer, A. R., Carena M. J. and Miranda, J. B. (2010). Quantitative genetics in maize breeding. Springer Science + Business Media, New York, 57, 463.

Hameed, A., Mahmud, T.S., Atta, B.M., Haq, M.A., Sayed, H. (2008). Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid eroxidation in desi and kabuli chickpea. *Pak. J. Bot.* 40, 1033–1041.

Hammond, R. W. (1994). Agrobacterium-mediated inoculation of PSTVd cDNAs onto tomato reveals the biological effect of apparently lethal mutations. *Virology*, 201(1), 36-45.

Hansson, C. M., Buckley, P. G., Grigelioniene, G., Piotrowski, A., Hellström, A. R., Mantripragada, K., & Dumanski, J. P. (2007). Comprehensive genetic and epigenetic analysis of sporadic meningioma for macro-mutations on 22q and micro-mutations within the NF2 locus. BMC genomics, 8(1), 16.

Haq, M.A, Sadiq, M. & Hassan M. A. (2001). Multiple disease resistant chickpea mutant variety. Mutation Breeding Newsletter CM 88, 45, 5–6.

Harkins, G. W., Martin, D. P., Duffy, S., Monjane, A. L., Shepherd, D. N., Windram, O. P., ... & Flett, B. (2009). Dating the origins of the maize-adapted strain of maize streak virus, MSV-A. *The Journal of general virology*, 90, 3066.

Harrison, B.D. (1985). Advances in geminivirus research. *Ann. Rev. Phytopathol*. 23, 55-82.

He, G., Meng, R. Newman, M. Gao, G. Pittman, R.N. & Prakash. C. (2003). Microsatellites as DNA markers in cultivated peanut (Arachis hypogaea L.). BMC *Plant Biology* 3, 1- 6.

Hegazi, A. Z. & Hamideldin N. (2010). The effect of gamma irradiation on enhancement of growth and seed yield of okra (Abelmoschus esculentus L. Monech) and associated molecular changes. *Journal of Horticulture and Forestry*, 2, 38-51.

Hill, D. S. & Waller, J. M. (1988). Pests and diseases of tropical crops. 2. Handbook of pests and diseases.

Hitchcock, A.S. & Chase, A. (1971). Manual of the grasses of the United States. In two Volumes. Revised by. USDA Misc. Pub. 200.

Hugo de Vries, (1901) Die mutations theorie. I von veit and co. Leipzig.,1-648.

IAEA. (2014). Radiation protection and safety of radiation sources: International basic Safety standards. Basic standard series. CD-ROM. Available at: https://www-pub.iaea.org/MTCD/publications/PDF/Pub1578_web-57265295.pdf

IAEA. (2011). Mutation induction for breeding. International Atomic Energy Agency, Vienna. Available from: URL: http://mvgs.iaea.org/Laboratory Protocals.aspx (accessed 14 May 2016).

IBPGR, (1991). Descriptors for maize. International maize and wheat improvement center, Mexico City/International Board for Plant Genetic Resources, Rome ISBN 92-9043-189-1.

IITA, (1981). Cereal improvement programme. *Ann. Rep.* 1980, 85-90.

Ijaz, S., & Khan I.A. (2009). Molecular characterisation of wheat germplasm using microsatellite markers. *Genetics and Molecular Research*, 8, 809-815.

ISAAA, (2001). Advances in maize streak virus disease research in eastern and southern Africa. Workshop report, 15-17 September 1999, KARI and ISAAA AfrLCentre, Nairobi, Kenya. Wambugu, F. and Wafula. J. (eds.). ISAAA Briefs No.16. ISAAA: Ithaca, NY. 43.

Isnard, M., Granter, M., Frutos, R., Reynaud, B. & Peterschmitt, M. (1998). Quasi-species nature of the maize streak virus isolates obtained from a population used to assess maize cultivar response to infection. *J Gen Virol*, 79, 3091–3099.

Jahangir, K. S. & Chandrasekharan, P. (1978). Comparative mutagenic effects of gamma rays and diethyl sulphonate in bhendi. (*A. esculentus* L. Moench). *Madras Agric. J.*, 65, 211-217.

Jain, S.M. & Maluszynski, M., (2004). Induced mutations and biotechnology on improving crops. In: Mujib, A., Cho, M., Predieri, S. and Banerjee, S. (eds.) In vitro applications in crop improvement: recent progress. IBH, Oxford, India, 169-202.

Jain, S.M. (2006). Mutation-assisted breeding for improving ornamental plants. In: Proceedings of the 22nd International Eucarpia Symposium Section Ornamentals: *Breeding for Beauty*, 714, 85–98.

Jayabalan, N., & Rao, G. R. (1988). Induced chlorophyll mutation studies in tomato. *Genetica Agraria*, 42(3), 263-272.

Jayakumar, J., Sundaram, T., Arun, P. D., & Ragu, R. R. A. (2007). Correlation studies in maize (Zea mays L.) evaluated for grain yield and other yield attributes. *Int. J. Agric. Sci*., (2), 57-60.

Jayne, T. S., Mason, N. M., Burke, W. J., & Ariga, J. (2016). Agricultural input subsidy programs in Africa: an assessment of recent evidence, 1879-1895.

Jie, L., Zifeng, W., Lixiang, C., Hongming, T., Patrik, I., Zide, J., & Shining, Z. (2009). Artificial inoculation of banana tissue culture plantlets with indigenous endophytes originally derived from native banana plants. Biological Control, 51(3), 427-434.

Johnson, H.W., Robinson H.F. & Comstock, R.E. (1955). Estimation of genetic and environmental variability in soybean. *Agron. J*., 47, 314-318.

Jonker, B. & Flett, B.C. (1997). A preliminary study of seed transmission of maize streak virus in maize. In: ARC-Grain Crops Institute (ed.). Maize Streak Disease Symp. ARC, Nelspruit, South Africa, 33.

Kamaruddin, N. Y., Abdullah, S., & Harun, A. R. (2016). The effect of gamma rays on the radiosensitivity and cytological analysis of Zingiber officinale Roscoe varieties Bentong and Tanjung Sepat. *International Journal of Advances in Agricultural & Environmental Engineering*, 3(1), 143-145.

Kearsey, M. J. & Pooni, H. S. J. (1996). The genetical analysis of quantitative traits. Chapman and Hall, London.

Kim, J.S., Lee, E.K., Back, M.H., Kim, D.H. and Lee, H.S. (2000). Influence of low dose g radiation on the physiology of germinative seed of vegetable crops. *Korean J Environ Agricult*, 19, 58–61.

Kim, S.K., Efron, Y., Fajemisin, J.M. & Buddenhagen, I.W. (1989). Mode of gene action for resistance in maize to maize streak virus. *Crop Sci*, 29, 890–894.

Kiong, A.L.P., Lai, A.G., Hussein, S. & Harun, A.R. (2008). Physiological responses of Orthosiphon stamineus plantlets to gamma irradiation. *Am-Eurasian. J. Sustain. Agric*, 2, 135–149.

Kørner, C. J., Pitzalis, N., Peña, E. J., Erhardt, M., Vazquez, F., & Heinlein, M. (2018). Crosstalk between PTGS and TGS pathways in natural antiviral immunity and disease recovery. *Nature plants*, *4*(3), 157.

Kovacs, E. and Keresztes, A. (2002). Effect of gamma and UV-B/C radiation on plant cell. *Micron*, 33, 199–210.

Kozjak, P., & Meglič, V. (2012). Mutagenesis in plant breeding for disease and pest resistance. In Mutagenesis. InTech.

Kpotor, P. (2012). *Evaluation of newly released maize varieties in Ghana for yield and stability under three nitrogen application rates in two agro-ecological zones*. Unpublished master's thesis, Department of Crop Science, Kwame Nkrumah University of Science and Technology.

Kumar, S., Kumar, S., Singh, B., Virendra, J. A. L. & Kumar, S. (2002). Influence of gmma radiation of seed on growth, sex expression and yield in sponge gourd (*Luffa cylindrica* L.) *Plant archives*. 2(2), 25-28.

Kyetere, D.T., Ming, R., McMullen, M.D., Pratt, R.C., Brewbaker, J. & Musket, T. (1999). Genetic analysis of tolerance to maize streak virus in maize. *Genome* 42, 20–26.

Lagat, M., Danson, J., Kimani, M. & Kuria, A. (2008). Quantitative trait loci for resistance to maize streak virus disease in maize genotypes used in hybrid development. *African Journal of Biotechnology*, 7, 2573–2577.

Lazarowitz, S. G., Pinder, A. J., Damsteegt, V. D., & Rogers, S. G. (1989). Maize streak virus genes essential for systemic spread and symptom development. *The EMBO Journal*, 8(4), 1023-1032.

Legesse, B. W., Pixley, K. V., & Botha, A. M. (2009). Combining ability and heterotic grouping of highland transition maize inbred lines. *Maydica*, 54(1), 9-14.

Lett, J.M., Granier, M., Grondin, M., Turpin, P., Molinaro, F., Chiroleu, F., Peterschmitt, M. & Reynaud, B. (2001). Electrical penetration graphs from Cicadulina mbila on maize, the fine structure of its stylet pathways and consequences for virus transmission efficiency. *Entomol. Exp. Appl. V.* 101(2), 93-109

Li, X & Zhang, Y. (2002). Reverse genetics by fast neutron mutagenesis in higher plants. *Functional & Integrative Genomics*, 2, 254–258

Li, X., Lassner, M. & Zhang Y. (2002). Deletagene: a fast neutron deletion mutagenesis-based gene knockout system for plants. *Comparative and Functional Genomics*, 3, 158- 160.

Ling, A.P.K., Chia, I., Hussein, Y.S. & Harun, A.R. (2008). Physiological responses of *Citrus sinensis* to gamma irradiation. *World Applied Sci.*, 5, 12-19.

Lomonossoff, G.P. (1995). Pathogen-derived resistance to plant viruses. *Annu Rev Phytopathol*, 33, 323–343.

Lush, J.L. (1940). Intra-sire correlations and regression of off springs on dams as a method of estimating heritability of characters. Proceedings of the American Society of Animal Production, 33, 293-301.

Magenya, O.E., Mueke, J. & Omwega, C. (2008). Significance and transmission of maize streak virus disease in Africa and options for management: A review. *Afri J. Biotech*, 7, 4897-4910.

Maluszynski, M., Ahloowalia, Beant S. & Sigurbjornsson, B. (1995). Application of in vivo and in vitro mutation technique for crop improvement. *Euphytica*., 85, 303-315.

Marcu, D., Besenyei, E. & Cristea V. (2014). Radiosensitivity of maize to gamma radiation based on physiological responses. Studii şi comunicări. *Ştiinţele Naturii*. Tom. 30.

Marcu, D., Cristea, V. & Darban, L. (2013). Dose-dependent effects of gamma radiation on lettuce (Lactuca sativa var. capitata) seedlings. *Int Journal Radiat Biology*, 89, 219–223.

Marcu, D., Damian, G., Cosma, C. & Cristea, V. (2013). Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (Zea mays). *Journal of biological physics*, 39(4), 625-634.

Markham, P. G., Pinner, M. S., & Boulton, M. I. (1984). Transmission of maize streak virus by leafhoppers, a new look at host adaptation. Mitteilungen der Schweizerischen Entomologischen Gesellschaft Bulletin de la Societe entomologique suisse.

Martin, D. P. & Shepherd, D. N. (2009). The epidemiology, economic impact and control of maize streak disease. *Food Security. In Press.*

Mashev, N., Vassilev, G. & Ivanov, K. (1995). A study of N-allyl N-2 pyridyl thiourea and gamma radiation treatment on growth and quality of peas and wheat. Bulg. *J. Plant. Physiol*, 21, 56–63.

Masuda, M., Yuasa, T. & Murakani, K. (2005). Growth characters and genetic inheritance in tomato cv. First mutant with short internodes induced by seed irradiation with ion beam. Scientific Reports of the Faculty of Agriculture, Okayam University, Japan., 94, 25-29.

Mawere, S., Vincent, V., Meyer, J.D. & Pixley, K.V. (2006). Resistance of four inbred lines to inoculation with 20 isolates of maize streak virus from Zimbabwe. *Plant Disease*, 90, 1485-1489.

Mba, C. (2013). Induced mutations unleash the potentials of plant genetic resources for food and agriculture. *Agronomy*, 3, 200-231.

McCallum, C.M., Comai, L., Greene, E.A. & Henikoff, S. (2000). Targeting induced local lesions in genomes (TILLING) for plant functional genomics. *Plant Physiology*, 123, 439-442.

Mead, R. (2017). Statistical methods in agriculture and experimental biology. Chapman and Hall/CRC.

Medina, F.I.S., Amano, E. & Tano, S. (2004). FNCA Mutation Breeding Manual. Forum for Nuclear Cooperation in Asia. Available from: URL: http://www.fnca.mext.go.jp/english/mb/ mbm/e_mbm.html (accessed 15 Dec. 2015).

Mejlhede, N., Kyjovska, Z., Backes, G., Burhenne, K., Rasmussen, S.K. & Jahoor, A. (2006). EcoTILLING for the identification of allelic variation in the powdery mildew resistance genes Mlo and Mla of barley; *Plant Breeding*, 125(5), 461–7.

Mesfin T. & Bosque-Pe´rez, N.A. (1998). Feeding behaviour of Cicadulina storeyi China (Homoptera: Cicadellidae) on maize varieties susceptible or resistant to maize streak virus. *Afr. Entomol*, 6, 185-191.

Mesfin, T., Hollander J.D. & Markham, P.G. (1995). Feeding activities of Cicadulina mbila Hemiptera: Cicadellidae on different host-plants.Bull. *Entomol. Res*, *85*(3), 387-396.

Mesterhazy, A., Bartok, T. and Lamper, C. (2003): Influence of wheat cultivars, species of Fusarium and isolate aggressiveness on the efficacy of fungicides for control of fusarium head blight. *Plant Dis*, 87, 1107–1115.

Mesterhazy, A., Bartok, T., Mirocha, C. G. and Komoroczy, R. (1995). Nature of wheat resistance to fusarium head blight and the role of deoxynivalenol for breeding. *Plant Breed*, 118, 97–110.

MOFA, S. R. I. D. (2011). Agriculture in Ghana-facts and figures (2010). Ministry of Food and Agriculture (MoFA)-Statistics, Research and Information Directorate (SRID).

MOFA, S. R. I. D. (2017). Agriculture in Ghana-facts and figures (2010). Ministry of Food and Agriculture (MoFA)-Statistics, Research and Information Directorate (SRID).

Mokhtarpour, H., Teh, C.B.S., Saleh, G., Selmat, A.B., Asadi, M.E. & Kamar, B. (2010). Non-destructive estimation of maize leaf area, fresh weight, and dry weight using leaf length and leaf width. *Communications in Biometry and Crop Science*, 5(1), 19–26.

Mopelola, L. O., & Victor, E. (2016). Chemical composition, functional and sensory properties of maize-based snack (Elekute) enriched with African oil bean seed (Pentaclethra macrophylla benth). *African Journal of Food Science*, 10(12), 379-384.

Morris, M. L., Tripp, R. and Dankyi, A. (1999). Adoption and impacts of improved maize production technology: a case study of the Ghana grains development project. *Economic Programme Paper*, 99-101.

Moussa, H. R. (2006). Role of gamma irradiation in regulation of NO_3 level in rocket (Eruca vesicaria sub sp. sativa) plants. Russ. *Journal of Plant Physiol*., 53, 193.

Muller, H.J. (1929). Artifical transmutation of gene. Science., 66, 84-87.

Nandapuri K.S., Sandhu K.S. & Randhawa, K.S. (1970). Effect of irradiation on variability of okra. A. esculentus L. Moench. *Journal etica Agraria*., 37, 204-205.

Nataraj, V., Shah, J.P. & Vandana, D. (2014). Estimates of variability, heritability and genetic advance in certain inbreeds of maize (Zea mays L.*)*. *International Journal of Applied Biology and Pharmaceutical Biology*, 5(1), 205-208.

Nilan, R.A. (1981). Recent advances in barley mutagenesis. In: fourth barley genetics symposium. Edinburgh University Press, Edinburgh, 823–31.

Njoroge, K. and Gichuru, L. (2013). Diallel analysis of Turcicum leaf blight resistance in Kenyan maize lines. *African Journal Agricultural Research*, 8, 2877-2883.

Novak, F. J., & Brunner, H. (1992). Plant breeding: induced mutation technology for crop improvement. *IAEA Bull*, 4, 25-33.

Nuss, E.T. & Tanumihardjo, S.A. (2010). Maize: a paramount staple crop in the context of global nutrition. *Compr. Rev. Food Sci. Food Saf*, 9, 417–436.

Obeng, H. (2000). Soil classification in Ghana. Centre for Policy Analysis (CEPA), 1-35.

Obilana, A. T., & Hallauer, A. R. (1974). Estimation of variability of quantitative traits in BSSS by using unselected maize inbred lines 1. *Crop science*, 14(1), 99-103.

Okoth, V. A. O., Dabrowski, Z. T., Thottappilly, G., & Van Emden, H. F. (1987). Comparative analysis of some parameters affecting maize streak virus (MSV) transmission of various Cicadulina spp. populations. *International Journal of Tropical Insect Science*, 8(3), 295-300.

Oluwafemi, S., Thottappilly, G. and Alegbejo, M. (2008). Search for variability among mastreviruses showing symptoms of maize streak virus from different regions of Nigeria by polymerase chain reactions. *Journal of Plant Protection Research*, 48, 529-539.

Oppong, A., Offei, S. K., Ofori, K., Adu-Dapaah, H., Lamptey, J. N., Kurenbach, B. & Varsani, A. (2015). Mapping the distribution of maize streak virus genotypes across the forest and transition zones of Ghana. *Archives of virology*, 160(2), 483-492.

Page, W. W., Smith, M. C., Holt, J., & Kyetere, D. (1999). Intercrops, Cicadulina spp., and maize streak virus disease. *Annals of applied biology*, 135(1), 385-393.

Paliwal, N.K., Agarwals, S. and Singh, V.P. (1983). Effects of morphactin and gamma irradiation on the growth and yield in okra (A. *esculentus* (L) Moench). *Indian Journal of Botany*, 6, 5-9.

Palme, K. Li, X., Teale, W.D. (2014). Towards second green revolution: engineering nitrogen use efficiency. *Journal Genet Genomics*, 41, 315–316.

Panse, V. G. & Sukhatme, P.V. (1964). Statistical methods of agricultural workers. ICAR publication, New Delhi, 359-372.

Pernet, A., Hoisington, D.A., Dintinger, J., Jewell, D.C., Jiang, C., Khairallah, M.M., Letourmy, P., Marchand, J.L., Glaszmann, J.C. & González, L. D. (1999). Genetic mapping of maize streak virus resistance from the Mascarene source. II. Resistance in line CIRAD390 and stability across germplasm. *Theor Appl Genet*, 99, 540–553.

Peterschmitt, M., Granier, M., Frutos, R., & Reynaud, B. (1996). Infectivity and complete nucleotide sequence of the genome of a genetically distinct strain of maize streak virus from Reunion Island. *Archives of virology*, 141(9), 1637-1650.

Pingali, P.L. & Pandey, S. (2001). Meeting world maize needs. In: Pingali, P.L (ed.). 2001. CIMMYT 1999-2000. World maize food facts and trends. Meeting world maize needs: Technological opportunities and priorities for the public sector. Mexico, D.F. CIMMYT.

Pingali, P.L. (2001). Population and technological change in agriculture. In N.J. Smelser and P.B. Baltes (eds.), International Encyclopedia of the Social and Behavioral Sciences. London: Pergamon.

Pinstrup-Anderson, P., Pandya-Lorch, R. & Rosegrant, M.W. (1999). World food prospects: critical issues for the early twenty-first century. IFPRI 2020 Vision Food Policy Report. Washington, D.C.: IFPRI.

Piperno, D.R. & Flannery, K.V. (2001). The earliest archaeological maize (Zea mays L.) from highland Mexico: new accelerator mass spectrometry dates and their implications. Proc. Natl. Acad. Sci. U. S. A., 98, 2101–2103.

Pixley, K. V., Dhliwayo, T. & Tongoona, P. (2006). Improvement of a maize population by full-sib selection alone versus full-sib with selection during inbreeding. *Crop Science*. 46, 1130-1136.

Posten, H. O. (1978). The robustness of the two-sample t-test over the Pearson system. *Journal of Statistical Computation and Simulation*, 6, 195–311.

Pratt, R.C. & Gordon, S.G. (2006). Breeding for resistance to maize foliar pathogens. *Plant Breed Rev*, 27, 119–174.

Preuss, S.B. & Britt, A.B. (2003). A DNA-damage-induced cell cycle checkpoint in *Arabidopsis*. *Genetics*, 164, 323–334.

Purseglove, J. W. (1988). Tropical crops: Monocotyledons Vol 1 and 2. ELBS edition, London, Longman. 607pp.

Rai, M.K., Kaliaa, R.K., Singh, R., Gangola, M.P. & Dhawana, A.K. (2011). Developing stress tolerant plants through in vitro selection - An overview of the recent progress. *Environmental and Experimental Botany*, 71, 89-98.

Ramalingam, R.S. (1980). Frequency and spectrum of mutations induced by irradiation and six chemical mutagens in chillies. *Agricola*., 13, 59-66.

Ramya, B., Nallathambi, G. & Ram, S.G. (2013). Mutagenic effectiveness and efficiency of gamma rays and EMS in black gram (V. mungo L.). *Int. Journal Scientific Research*, 2: 6–9.

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences, 1312(1), 105-112.

Rao, G.M., Rao, V.M. & Madhusudana R. G. (1983). Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice. *Cytologia.*, 48(3), 427-436.

Rao, L.J. (1990). Induced mutations recovered in M2 and subsequent generation in three varieties of okra (Abelmoschus esculentus (L.) Moench). Proc. Int. Seminar on New Frontiers in Hort., November, 25-28.

Rapoport, J.A. (1946). Carbonyl compounds and the mechanism of chemical mutation. *Doklady Akademii Nauk CCCP*, 54, 65–7.

Reddy, V.R., Jabeen, F., Sudarshan, M.R. & Rao, A.S. (2013). Studies on genetic variability, heritability, correlation and path analysis in maize (Zea mays L.) over locations. *International Journal of Applied Biology and Pharmaceutical Technology*, 4(1), 195-199.

Ribaut, J. M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G. O., & Hoisington, D. A. (1997). Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics*, 94(6-7), 887-896.

Robinson, H. F., Comstock, R. E. & Harvery, V. H. (1949). Estimates of heritability and degree of dominance in corn. *Agron. Journal*, 41, 353-359.

Rodier, A. Assié, J. Marchand, J.L. & Hervé, Y. (1995). Breeding maize lines for complete and partial resistance to maize streak virus (MSV). *Euphytica*, 81, 57–70.

Rogers, C., Wen, J., Chen, R. & Oldroyd, G. (2009). Deletion-based reverse genetics in Medicago truncatula. *Plant Physiology*, 151, 1077–1086.

Rose, D.J.W. (1978). Epidemiology of maize streak disease. *annual review entomology*, 23, 259-282.

Rutger, J.N. (2006). Thirty years of induction, evaluation, and integration of useful mutants in rice genetics and breeding. Plant Mutation Reports 1(2), 4–13.

Rybicki, E. P. and Hughes, F. L. (1990). Detection and typing of maize streak virus and other distantly related geminiviruses of grasses by polymerase chain reaction amplification of a conserved viral sequence. *Journal of General Virology*, 71, 2519-2526.

Shah, S.M. & Sharif, M. (1994). Studies on the effect of gamma radiation on some morphological characters of mungbean (Vigna radiata (L.) Wilczek) cultivars. *Science Khyber*, 7, 37–44

Sharma, K. and Misra, R. S. (2011). Molecular approaches towards analyzing the viruses infecting maize (Zea mays L.). *Journal of General and Molecular Virology*, 3, 1-17.

Sheeba, A., Abumalarmalhi., J., Babu, S. & Ibrahim, S.M. (2005). Mutagenic effects of gamma rays and EMS in M1 generation in sesame. *Resources on Crops*, 6(2), 300-306.

Shepherd, D.N., Dugdale, B. Martin, D.P., Varsani, A. Lakay, F.M., Bezuidenhout, M.E. (2014). Inducible resistance to maize streak virus. PLoS ONE 9(8): e105932. https://doi.org/10.1371/journal.pone.0105932.

Shepherd, D.N., Mangwende, T., Martin, D.P., Bezuidenhout, M., Kloppers, F.J., Carolissen, C.H., Monjane, A.L., Rybicki, E.P. & Thomson, J.A. (2007a). Maize streak virus-resistant transgenic maize: a first for Africa. *Plant Biotechnol* Journal, 5, 759–767.

Shepherd, D.N., Mangwende, T., Martin, D.P., Bezuidenhout, M., Thomson, J.A & Rybicki, E.P. (2007b). Inhibition of maize streak virus (MSV) replication by transient and transgenic expression of MSV replication-associated protein mutants. *Journal Gen Virol*, 88, 325–336.

Shereen, A., Ansari, R., Mumtaz, S., Bughio, H. R., Mujtaba, S. M. & Shirazi, M. U. (2009). Impact of gamma irradiation induced changes on growth and physiological responses of rice under saline conditions. *Pakistan Journal of Botany*. 41, 2487-2495.

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, 3(3), 307.

Shu, Q.Y. (2009). Induced plant mutations in the genomics era. Food and Agriculture Organization. United Nations, New York, USA.

Shu, Q.Y. and Lagoda, P.J.L. (2007). Mutation techniques for gene discovery and crop improvement. *Molecular Plant Breed*. *5*(2), 193-195.

Singh, B. & Datta, P.S. (2010). Gamma irradiation to improve plant vigour, grain development, and yield attributes of wheat. *Radiation Physics and Chemistry*, 79, 139–143.

Sivasubramanian, S., & Menon, M. (1973). Heterosis and inbreeding depression in rice. *Madras Agric. Journal*, 60(7), 1139-1140.

Slade, A.J., Fuerstenberg, S.I., Loeffler, D., Steine, M.N., Facciotti, D. (2005). A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. *Nature* Biotechnology, 23(1), 75–81.

Smalley, M., Daub, J. and Hallauer, A. (2004). Estimation of heritability in maize by parent-offspring regression. *Maydica*, 49, 221-229.

Smith, B.D. (2001). Documenting plant domestication: the consilience of biological and archaeological approaches. Proc. Natl. Acad. Sci. U. S. A., 98, 1324–1326.

Soto, P.E., Buddenhagen, LW., Asnani, V.L. (1982). Development of streak virus-resistant maize populations through improved challenge and selection methods. *Annals of Applied Biology*. 100, 539-546.

Sprague, G. F. and Tatum, L. A. (1942). General vs. specific combining ability in single crosses of corn. *Agronomy Journal*, 34, 923-932.

Stadler, L.J. (1928). Genetic effects of X rays in maize. Proc. Natl. Acad. Sci. USA., 14, 69-75.

Steel, R.G., Torrie, R.H. (1984). Principles and procedure of statistics. 2nd edition MCGraw-Hill, Inc. New York. 87pp.

Stephenson, P., Baker, D., Girin, T., Perez, A., Amoah, S. & King, G.J. (2010). A rich TILLING resource for studying gene function in Brassica rapa. *BMC Plant Biology*, 10, 62-97.

Storey, H.H. (1928). Transmission studies of maize streak disease. *Annals of Applied. BioIogy*, 59, 429-436.

Storey, H.H. (1936). Virus diseases of East African plants IV. A survey of the viruses attacking the Gramineae. *East African. Agric. Journal.* 1, 333-337.

Strable, J. & Scanlon, J.M. (1999). Maize (Zea mays): a model organism for basic and applied research in plant biology. In: Emerging Model Organisms: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2, 33–41.

Stuber, C. W. (1994). Heterosis in plant breeding. *Plant Breed Rev*, 12, 227-251.

Suprasanna, P., Sonawane, B.V. & Patade, V.Y. (2012). In vitro mutagenesis and selection: strategies and prospects for crop improvement. In: Kozgar, M.I. and Khan, S. (eds) Induced mutagenesis in crop plants. Bioremediation, biodiversity and bioavailability. Volume 6 (Special Issue 1), Global Science Books, Tokyo, Japan, 6-14.

Syafii, M., Cartika, I., & Ruswandi, D. (2015). Multivariate analysis of genetic diversity among some maize genotypes under maize-albizia cropping system in Indonesia. *Asian Journal of Crop Science*, 7(4), 244.

Tabasum, A., Cheema, A. A., Hameed, A., Rashid, M. & Ashraf, M. (2011). Radio sensitivity of rice genotypes to gamma radiations based on seedling traits and physiological indices. *Pakistan Journal of Botany*, 43(2), 1211-1222.

Teramura, A. H., & Sullivan, J. H. (1994). Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. Photosynthesis Research, 39(3), 463-473.

Tang, C.Y. & Bjarnason, M.S. (1993). Approaches for the development of maize germplasm resistant to maize streak virus. *Maydica*, 38, 301-307.

Thottappilly G, Bosque-Pérez NA, Rossel HW (1993) Viruses and virus diseases of maize in tropical Africa. *Plant Pathol*, 42, 494-509.

Till, B.J., Reynolds, S.H., Greene, E.A., Codomo, C.A., Enns, L.C., Johnson, J.E., Burtner, C., Odden, A.R., Young, K., Taylor, N.E., Henikoff, J.G., Comai, L. & Henikoff, S. (2003). Large-scale discovery of induced point mutations with high-throughput TILLING. *Genome Research*, 13, 524–530.

Till, B.J., Reynolds, S.H., Weil, C., Springer, N., Burtner, C. and Young, K. (2004). Discovery of induced point mutations in maize genes by TILLING. *BMC Plant Biology*, 4, 12- 98.

Timothy, D.H., Harvey, P.H. & Dowswell, C.R. (1988). Development and Spread of Improved Maize Cultivars and Hybrids in Developing Countries. USAID, Washington, DC.

Tomlekova, N. B. (2010). Induced mutagenesis for crop improvement in Bulgaria. Plant Mutation Reports, 2(2), 4-27.

Twumasi-Afriyie S, Sallah PY, Owusu-Akyaw M, Ahenkora K, Soza, R.F. (2012) Development and utilisation of quality protein maize in Ghana. Crop Research Institute, Kumasi, Ghana.

USDA, (United States Department of Agriculture), (2014). Economic Research Service. Corn Trade. Cited February 20, 2015. http://www.ers.usda.gov/ topics/crops/corn.aspx.

Vacaro, E., Barbosa-Neto, J. F., Pegoraro, D. G., Nuss, C. N. & Conceicao, L. D. H. (2002). Combining ability of twelve maize populations. Pesquisa-agropecuaria-Brasileira, 37, 67-72.

Van der Plank, J.E. (1963). Plant diseases epidemics and control. Academic Press, New York & London, 349.

Van der Walt, E., Martin, D.P., Varsani, A., Polston, J.E. & Rybicki, E.P. (2008). Experimental bservations of rapid maize streak virus evolution reveal a strand-specific nucleotide substitution bias. *Virol Journal*, 5, 104.

Van Harten, A.M. (1998). Mutation breeding: theory and practical applications. Cambridge University Press, Cambridge, UK.

Van Rensburg, G.D.J. & Giliomee, J.H. (1990). A comparison of females and males of Cicadulina anestae and C. mbila (Homoptera: Cicadellidae) as vectors of maize streak virus. *Hytophylactica*, 22, 241–243.

Vasal, S. K., Srinivasan, G., Crossa, J. & Beck, D. L. (1992). Heterosis and combining ability of cimmyt's subtropical and temperate early-maturity maize germplasm. *Crop Science*, 32, 884-890.

Vivek, B. S., Odongo, O., Njuguna, J., Imanywoha, J., Bigirwa, G. and Pixley, K. (2010). Diallel analysis of grain yield and resistance to seven diseases of 12 African maize (Zea mays L.) inbred lines. *Euphytica*, 172, 329-340.

Vollbrecht, E. & Sigmon, B. (2005). Amazing grass: developmental genetics of maize domestication. Bioch. *Soc.* Trans, 33, 1502–1506.

Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C. & Nelson, R. J. (1994). RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics*, 136(4), 1421-1434.

Wani, A. & Anis, M. (2008). Gamma ray and EMS-induced bold-seeded high-yielding mutants in chickpea (Cicer arietinum). *Turky Journal of Biolology*, 32, 1-5.

Ward, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of American Statistical Association*, 58(301), 236-244.

Waugh, R., Leader, D.J., McCallum, N. & Caldwell, D. (2006). Harvesting the potential of induced biological diversity. *Trend Plant Sci. 11*(2), 71-79.

Weber, C.R. & Moorthy, H.R. (1952). Heritable and non-heritable relationship and variability of oil content and agronomic characters in the F2 generation of soybean crosses. *Agronomy J*ournal, 44, 202-209.

Welz, H.G., Schechert, A., Pernet, A., Pixley, K.V. & Geiger, H.H. (1998). A gene for resistance to the maize streak virus in the African CIMMYT maize inbred line CML 202. *Mol Breed*, 4, 147– 154.

Wisser, R.J., P.J. Kurti-Balint & R.J. Nelson. (2006). The genetic architecture of disease resistance in maize: a synthesis of published studies. *Phytopathology*, 96, 120-129.

Wöhrmann, T., & Weising, K. (2011). In silico mining for simple sequence repeat loci in a pineapple expressed sequence tag database and cross-species amplification of EST-SSR markers across Bromeliaceae. Theoretical and applied genetics, 123(4), 635-647.

Wollenweber, B. Porter, J.R., Lubberstedt, T. (2005). Need for multidisciplinary research towards a second green revolution. *Curr Opin Plant Biolology*, 8, 337–341.

WRB (World Reference Base for Soil Resources), (2015). IUSS working group, international soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Xia-Su, X., Liu, J. & Liu. G. (2004). The use of SSRs for predicting the hybrid yield and yield heterosis in 115 key inbred lines of Chinese maize. *Hereditas*, 141, 207-215.

Xu, X. L., & Ko, W. H. (1998). A quantitative confined inoculation method for studies of pathogenicity of fungi on plants. Botanical Bulletin of Academia Sinica, 39.

Yonezawa, K. & Yamagata, H. (1977). On the optimum mutation rate and optimum dose for practical mutation breeding. *Euphytica*, 26, 423–6.

Yu, K., Park, S.J. Poysa, V. & Gepts. P. (2000). Markers into a molecular linkage map of common bean (Phaseolus vulgaris L.). *Journal of Heredity*, 91, 429-434.

APPENDICES

Appendix 1: Preparation of reagents

1. CTAB
   a. 2 % CTAB (Cetyltrimethylammonium bromide)
   b. 0.1 M TrisHCl {pH = 8}
   c. 20 mM EDTA
   d. 1.4 M NaCl
   e. 2 % (w/v) PVP (polyvinyl polypyrrolidine)
   f. 1.0 % β-mercaptoethanol (added just before use)
   g. mg/ml proteinase K (added just before use)

2. TE buffer (1000 ml)
   a. 1 M Tris pH 8.0 10 ml
   b. 0.5 M EDTA pH 8.0 2 ml
   c. 5 M NaCl 200 ml
   d. Distilled H2O complete volume to 1000 ml

3. Chloroform: isoamyl alcohol (24:1)
   a. Measure 960 ml/l Chloroform in beaker
   b. Add 40 ml/l Isoamyl alcohol into the beaker

4. 70 % ethanol (100 ml): Measure and mix 70 ml of absolute ethanol with 30 ml distilled water

5. 0.8 % Agarose: Weigh 0.8 g of agarose, add 100 ml of 1X TBE and heat in a microwave to dissolve

Appendix 2: Analyses of variance radiosensitivity seed germination

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype	5	11272.5679	2254.5136	372.30	<.0001
Dose rate	8	158404.7531	19800.5941	3269.82	<.0001
Genotype*Dose rate	40	10867.5432	271.6886	44.87	<.0001
Error	108	654.0000	6.0556		
Total	161	181198.8642			

Appendix 3: Analyses of variance radiosensitivity Plant height

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype	5	593.551803	118.710361	41.89	<.0001
Dose rate	8	4671.239049	583.904881	206.04	<.0001
Genotype*Dose rate	40	522.820914	13.070523	4.61	<.0001
Error	108	306.070867	2.833990		
Total	161	6093.682633			

Appendix 4: Analyses of variance radiosensitivity number of leaves

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype	5	22.8483216	4.5696643	25.99	<.0001
Dose rate	8	158.7398975	19.8424872	112.87	<.0001
Genotype * Dose rate	40	40.7733395	1.0193335	5.80	<.0001
Error	108	18.9856667	0.1757932		
Total	161	241.3472253			

Appendix 5: Analyses of variance Radiosensitivity Leaf area

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype	5	7650.6066	1530.1213	6.53	<.0001
Dose rate	8	149780.3347	18722.5418	79.87	<.0001
Genotype * Dose rate	40	7665.3199	191.6330	0.82	0.7625
Error	108	25315.4001	234.4019		
Total	161	190411.6612			

Appendix 6: Analyses of variance Radiosensitivity Root length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype	5	224.297953	44.859591	65.14	<.0001
Dose rate	8	1127.117464	140.889683	204.59	<.0001
Genotype*Dose rate	40	78.364314	1.959108	2.84	<.0001
Error	108	466.241067	4.317047		
Total	161	1504.152531			

Appendix 7: Analyses of variance Radiosensitivity photosynthesis

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Genotype	5	900.819359	180.163872	41.73	<.0001
Dose rate	8	675.829549	84.478694	19.57	<.0001
Genotype*Doserate	40	2043.642169	51.091054	11.83	<.0001
Error	108	466.241067	4.317047		
Total	161	4086.532144			

Appendix 8: Analyses of variance M_1 plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	128.98	64.49	2.21	
Dose rate	1	3861.33	3861.33	132.40	<.001
Genotype	3	1456.46	485.49	16.65	<.001
Dose rate* Genotype	3	728.16	242.72	8.32	0.002
Residual	14	408.31	29.16		
Total	23	6583.24			

Appendix 9: Analyses of variance M_1 maize streak disease severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0833	0.0417	0.15	
Dose rate	1	1.0417	1.0417	3.72	0.074
Genotyp	3	4.4583	1.4861	5.31	0.012
Dose rate* Genotype	3	0.4583	0.1528	0.55	0.659
Residual	14	3.9167	0.2798		
Total	23	9.9583			

Appendix 10: Analyses of variance M_1 100 grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	187.15	93.58	1.28	
Dose rate	1	17219.21	17219.21	235.01	<.001
Genotype	3	340.35	113.45	1.55	0.246
Dose rate* Genotype	3	5693.84	1897.95	25.90	<.001
Residual	14	1025.79	73.27		
Total	23	24466.34			

Appendix 11: Analyses of variance M_2 plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	167.21	83.60	0.97	
Dose rate	1	106.07	106.07	1.23	0.287
Genotype	3	4356.12	1452.04	16.80	<.001
Dose rate* Genotype	3	372.89	124.30	1.44	0.274
Residual	14	1210.34	86.45		
Total	23	6212.62			

Appendix 12: Analyses of variance M_2 maize streak severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.05747	0.02874	1.46	
Dose rate	1	0.04172	0.04172	2.12	0.167
Genotype	3	0.06833	0.02278	1.16	0.360
Dose rate* Genotype	3	0.04194	0.01398	0.71	0.561
Residual	14	0.27520	0.01966		
Total	23	0.48465			

Appendix 13: Analyses of variance M_2 100 grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	140.0	70.0	0.30	
Dose rate	1	4.5	4.5	0.02	0.891
Genotype	3	5000.2	1666.7	7.21	0.004
Dose rate* Genotype	3	1023.3	341.1	1.47	0.264
Residual	14	3238.4	231.3		
Total	23	9406.3			

Appendix 14: Analyses of variance M_3 plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	444.19	222.09	2.71	
Dose rate	1	51.20	51.20	0.63	0.442
Genotype	3	2785.63	928.54	11.35	<.001
Doserate* Genotype	3	384.54	128.18	1.57	0.242
Residual	14	1145.38	81.81		
Total	23	4810.94			

Appendix 15: Analyses of variance M_3 plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	444.19	222.09	2.71	
Dose rate	1	51.20	51.20	0.63	0.442
Genotype	3	2785.63	928.54	11.35	<.001
Dose rate * Genotype	3	384.54	128.18	1.57	0.242
Residual	14	1145.38	81.81		
Total	23	4810.94			

Appendix 15: Analyses of variance M_3 maize streak severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1816	0.0908	0.54	
Dose rate	1	0.0265	0.0265	0.16	0.696
Genotype	3	0.4209	0.1403	0.84	0.495
Dose rate * Genotype	3	0.8224	0.2741	1.64	0.226
Residual	14	2.3435	0.1674		
Total	23	3.7951			

Appendix 16: Analyses of variance M_3 100 grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	384.9	192.5	0.63	
Dose rate	1	1347.9	1347.9	4.42	0.054
Genotype	3	1504.6	501.5	1.64	0.224
Dose * Genotype	3	1223.9	408.0	1.34	0.302
Residual	14	4271.5	305.1		
Total	23	8732.8			

Appendix 17: Analyses of variance M_4 plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3540.1	1770.0	8.13	
Dose rate	1	6.8	6.8	0.03	0.863
Genotype	3	3292.4	1097.5	5.04	0.014
Dose rate * Genotype	3	1915.4	638.5	2.93	0.070
Residual	14	3048.5	217.7		
Total	23	11803.1			

Appendix 18: Analyses of variance M_4 maize streak severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2708	0.1354	0.92	
Dose rate	1	6.0000	6.0000	40.73	<.001
Genotype	3	0.7917	0.2639	1.79	0.195
Dose rate * Genotype	3	0.3333	0.1111	0.75	0.538
Residual	14	2.0625	0.1473		
Total	23	9.4583			

Appendix 19: Analyses of variance M_4 anthesis to silking interval

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3333	0.1667	0.64	
Dose rate	1	4.1667	4.1667	15.91	0.001
Genotype	3	0.5000	0.1667	0.64	0.604
Dose rate * Genotype	3	3.1667	1.0556	4.03	0.029
Residual	14	3.6667	0.2619		
Total	23	11.8333			

Appendix 20: Analyses of variance M_4 100 grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1618.4	809.2	7.33	
Doesrate	1	20.8	20.8	0.19	0.671
Genotype	3	322.2	107.4	0.97	0.433
Dose rate * Genotype	3	257.4	85.8	0.78	0.526
Residual	14	1545.8	110.4		
Total	23	3764.6			

Appendix 21 Germination percentage data of genotypes (y) (control and induced plant)

Doses rate (Gy)	Germination (%) of maize genotypes					
	Dapango	Dormabin	Dzinueve	Keta 60	Obatanpa	Pann 53
0	95	98	93	95	96	98
50	94	95	90	92	88	95
100	91	93	89	86	81	92
150	91	90	82	83	69	84
200	90	88	58	62	55	72
250	86	80	29	41	41	67
300	58	60	1	39	35	43
350	13	29	0	17	15	28
400	10	18	0	5	2	16
450	1	5	0	1	0	1
500	0	2	0	1	0	0
550	0	1	0	0	0	0
600	0	0	0	0	0	0
650	0	0	0	0	0	0
700	0	0	0	0	0	0
750	0	0	0	0	0	0
Mean	39	41	28	32	30	37
St Dev.	43.8	42.5	39.4	38.3	36.4	40.6
CV %	1.12	1.04	1.41	1.2	1.21	1.1