EFFECTS OF GAMMA RADIATION AND COLCHICINE ON AGRONOMIC PERFORMANCE OF SOY-BEAN [*GLYCINE MAX* (L.) MERRILL] VARIETIES

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NIGERIA.

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EFFECTS OF GAMMA RADIATION AND COLCHICINE ON AGRONOMIC PERFORMANCE OF SOY-BEAN [*GLYCINE MAX* (L.) MERRILL] VARIETIES

BY

Sadiq, Abdullahi SHEHU (BSc. Botany, Unimaid 2009)

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BOTANY

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
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NIGERIA.

SEPTEMBER, 2015.

DECLARATION

I declare that the work in this Dissertation entitled "EFFECTS OF GAMMA RADIATION AND COLCHICINE ON AGRONOMIC PERFORMANCE OF SOY-BEAN [*GLYCINE MAX* (L.) MERRILL] VARIETIES" has been carried out by me in the Department of Biological sciences. The information derived from the Literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another degree or diploma at this or any other institution.

Sadiq, Abdullahi SHEHU _____ _____

Signature Date

CERTIFICATION

This Dissertation titled: "EFFECTS OF GAMMA RADIATION AND COLCHICINE ON AGRONOMIC PERFORMANCE OF SOY-BEAN [*GLYCINE MAX* (L.) MERRILL] VARIETIES" meets the regulations governing the award of the degree of Master of Science in Botany of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this Dissertation to my Daughter Hafsat Sadiq Shehu (Hafiza) and my Family.

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ABSTRACT

A pot experiments was conducted to determine the effects of gamma radiation and colchicine on Agronomic performance of soybean varieties. The seeds of two varieties of Soybean (TGX 1448-2E and SAMSOY-2) were subjected to five treatments (0, 5, 10, 15, and 20 Krad) of gamma rays and five colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0 mM). Combinations of these two mutagens were also used to treat the two soybean varieties. The treatments were laid out in a complete randomized design. The plants were sampled for germination percentage two weeks after planting (2 WAP), plant survival (3 and 6 WAP), plant height (3 and 6 WAP), root nodule (6 WAP), leave number (6 WAP), root fresh weight (6 WAP), shoot fresh weight (6 WAP), fresh weight (6 WAP) and flower production for the assessment of growth parameters, the plants were also sampled for pod dry weight, 100 seed weight, grain yield and crude protein content at harvest. The results indicated a varying degree of effects in response to the mutagenic treatments. The higher doses of gamma rays (15 Krad and 20 Krad) were particularly lethal. Similarly, there was decrease in percentage germination with increase in colchicine concentrations observed in the treated seeds. The interaction of the two mutagens showed a significant decrease in percentage germination, plant survival, shoot fresh weight, fresh weight and pod dry weight. At higher doses of gamma ray or higher concentrations of colchicine (20 Krad and 2 mM) the results revealed an increase in grain yield, crude protein and 100 seed weight. The data showed that, variety TGX 1448-2E showed higher values in germination percentage, plant survival, plant height, root nodule, leaf number, shoot weight, flower production, total fresh weight, pod weight, 100 seed weight, total grain yield and crude protein content than the other variety. The result was highly significant

for percentage germination and survival rate. The findings from this study also revealed that some chlorophyll deficient mutants were manifested. Among these were chlorina, striata, virescent, lustescent produced by TGX 1448-2E and Lustescent and virescent types produced by SAMSOY-2. In conclusion, the impact of mutagens on both vegetation and yield parameters of soybean varieties varied with the level of mutagens. TGX 1448-2E was shown to be the most responsive to the treatment doses than SAMSOY-2 variety.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Soybean [*Glycine max* (L.) Merrill] belongs to the Order Fabales, Family Fabaceae, Sub-family *Faboideae*, Genus *Glycine* and Species *max*. The Genus *Glycine* was originally introduced by Carl Linnaeus (1737) in his first edition of "Genera Plantarum". The word '*glycine*' was derived from the Greek word – *glykys* (sweet) and likely refers to the sweetness of the pear-shaped (*apios* in Greek) edible tubers produced by the native North American twining or climbing herbaceous soybean called *Glycine apios*, now known as *Apios americana* (Singh *et al.,* 2006). The cultivated soybean first appeared in "Species Plantarum" by Linnaeus, under the name *Phaseolus max* L. The name *Glycine max* (L.) Merrill, as proposed by Merrill in 1917, has become the valid name for this useful plant (Singh *et al*., 2006).

1.2 Soybean Production

Soybean is a legume that grows in tropical, subtropical and temperate climates. It has 40 chromosomes (2n = 40) and is a self-fertile species with less than 1% out-crossing (IITA, 2009). Soybean was domesticated in the 11th century BC around northeast of China. It is believed that it might have been introduced to Africa in the 19th century by Chinese traders along the east coast of Africa. Reports indicate that soybean has been under cultivation in Tanzania in 1907 and Malawi in 1909 (IITA, 2009).

Soybean varies in growth and habit. The height of the plant varies from less than 0.2 to 2.0 m (0.66 to 6.56 ft). The pods, stems and leaves are covered with fine brown or gray hairs. The leaves are tri-foliate, having three to four leaflets per leaf, and the leaflets are 6–15 cm (2.4–5.9 in) long and 2–7 cm (0.79–2.76 in) broad (Lim, 2012). The leaves fall before the seeds are mature. The inconspicuous, self-fertile flowers are borne in the axial of the leaf and are white, pink or purple. The fruit is a hairy pod that grows in clusters of three to five; each pod is 3–8 cm long (1–3 in) and usually contains two to four (rarely more) seeds 5–11 mm in diameter (Lim, 2012).

Soybean occurs in various sizes, and in many hull or seed coat colours, including black, brown, blue, yellow, green and mottled. The hull of the mature bean is hard, water-resistant, and protects the cotyledon and hypocotyl from damage. If the seed coat is cracked, the seed will not germinate (Lim, 2012). The scar, visible on the seed coat, is called the hilum (colours include black, brown, buff, gray and yellow) and at one end of the hilum is the micropyle, or small opening in the seed coat which can allow the absorption of water for sprouting.

Soybean cultivation is successful in climates with hot summers, with optimum growing conditions in mean temperatures of 20˚ to 30 °C (68˚ to 86° F); temperatures of below 20 °C and over 40 °C (68° F, 104° F) stunt growth significantly. They can grow in a wide range of soils, with optimum growth in moist alluvial soils with a good organic content (Baohui *et al.,* 2006). Soybean, like most legumes, perform nitrogen fixation by establishing a symbiotic relationship with the bacteria *Bradyrhizobium japonicum* and *Rhizobium japonicum* (Herbert *et al*., 2009). For best results, an innoculum of the correct

strain of bacteria should be mixed with the soybean (or any legume) seeds before planting. Crop cultivars generally reach a height of around 1 m (3.3 ft), and take 80–120 days from sowing to harvesting (Baohui *et al*., 2006).

According to FAO (2005), total land area under soybean cultivation in the world was 95.2 million hectares and total production was 212.6 million tonnes. The three major soybean- producing countries were United States of America (29 million ha), Brazil (23 million ha), and Argentina (14 million ha). In terms of total production, United States of America produced 83 million tonnes, followed by Brazil (51 million tonnes) and Argentina (38 million tons). In Africa, soybean was grown on an average of 1.16 million hectares with an average production of 1.26 million tonnes in 2005. African countries with the largest area of production were Nigeria (601,000 ha), South Africa (150,000 ha), Uganda (144,000 ha), Malawi (68,000 ha) and Zimbabwe (61,000 ha). Other countries with sizeable areas are Rwanda (42,160 ha), DRC (30,000 ha) and Zambia (15,000 ha). The main producers of soybean are the United States (35%), Brazil (27%), Argentina (19%), China (6%) and India (4%) (World, 2010).

Soybean growth is influenced by climate and soil characteristics. Soybean performs well in the southern and northern Guinea savannas of Nigeria where rainfall is more than 700 mm (IITA, 2009).However, short-duration varieties can thrive in the much drier Sudan savanna when sown early and with an even distribution of rainfall throughout the growing period. The time for planting soybean depends upon temperature and day length. Soybean is a short-day plant and flowers in response to shortening days. It can be grown on a wide range of soils with pH ranging from 4.5 to 8.5. Soybean should not be planted

in sandy, gravelly, or shallow soils to avoid drought stress. It should not be grown in waterlogged soils or soils with surfaces that can crust, as this will lead to poor seedling emergence (IITA, 2009).

Soybean grows from sea level, up to 2000 meters from equator to latitudes 55 ^{o} N and 55^{0}S. It grows under a wide range of temperature, but the optimum for growth and development is 30^{0}C whilst for proper emergence of seedlings, a seedbed temperature of 25–33^{0}C is optimal. The crop requires 500–850 mm water during the growing season (IITA, 2009).

A good fertilizer for soybean production depends on a good soil test. Under normal conditions, soybean as a legume should provide itself with nitrogen through biological nitrogen fixation. Until nodulation occurs, the soybean plant depends on soil nitrogen for growth (Dugje *et al*., 2009). Phosphorus is often the most deficient nutrient. Fertilizer is usually incorporated into the soil at land preparation during harrowing and leveling the field (Dugje *et al*., 2009).Human sewage sludge can be used as fertilizer to grow soybean. Soybean can be grown organically, that is, without the use of synthetic pesticides and fertilizers (Dugje *et al*., 2009).

Soybean also improves soil fertility by adding nitrogen from the atmosphere, this is a major benefit in farming systems, where soils have become exhausted by the need to produce more food for increasing populations, and where fertilizers are hardly available and are expensive for farmers (IITA, 2009).

1.3 Composition and Uses of Soybean

In Nigeria, it is called soybean, but called soya bean in United States of America and soybean in United Kingdom (Multilingual Multiscript Plant Name Database, 2012). It is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classified as an oil-seed rather than a pulse by the United Nations, Food and Agricultural Organization (FAO) (2012).

Soybean is considered by many agencies to be a source of complete protein (Henkel, 2000). Complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body's inability to synthesize them. For this reason, soybean is a good source of protein, amongst many others, for vegetarians and vegans or for people who want to reduce the amount of meat they eat (United States of America Food and Drug Administration, 2012).

Soy-protein is essentially identical to the protein of other legume seeds and pulses (Wolf, 2012). Moreover, soybean can produce at least twice as much protein per acre than any other major vegetable or grain crop besides hemp, 10 times more protein per acre than land set aside for grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production (National Soybean Research Laboratory, 2012).

Soybean protein has great potential as a major source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol. A by-product from the oil production (soybean cake) is used as a high-protein animal feed in many countries (IITA, 2009).

It is good for food, soy-milk, soy-cheese, '*Dadawa'* and 'Tom Brown' (infant weaning food). It is the source of an excellent vegetable oil, it improves soil fertility and controls the parasitic weed, *Striga hermonthica* (Dugje *et al.,* 2009). Soybean cake is an excellent livestock feed, especially for poultry, the haulms provide good feed for sheep and goats (Dugje *et al.,* 2009). For human consumption, soybean must be cooked with "wet" heat to destroy the trypsin inhibitors (serine protease inhibitors). Raw soybean, including the immature green form, is toxic to humans, swine, chickens, and infact, all monogastric animals (Dugje *et al*., 2009).

A very small proportion of the crop is consumed directly by humans (Wolf, 2012). Soybean products do, however, appear in a large variety of processed foods. Though raw soybean flour is known to cause pancreatic cancer in rats (Dethloff *et al*., 2000), the cooked flour has not been found carcinogenic (Sarkar *et al*., 2007). Whether soybean might promote pancreatic cancer in humans is unknown because studies have not yet attempted to single out soybean intake and the incidence of pancreatic cancer in humans, and the amount of soybean fed to the rats is proportionately far larger than what humans would normally consume. However, the soybean isoflavone has been suggested as a chemo- preventive agent against pancreatic cancer, by interfering with the chemical pathways that promote the creation and growth of tumors (Sarkar *et al*., 2007).

In tropical Africa, fat-free (defatted) soybean meal is a significant and cheap source of protein for animal feeds and many pre-packaged meals. Soybean vegetable oil is another product of processing the soybean crops for example; soybean products such as textured vegetable protein (TVP) are ingredients in many meat and dairy analogues (Riaz, 2006).

Soybean produces significantly more protein per acre than most other uses of it (National Soya bean Research Laboratory, 2012).

However, in Nigeria soybean is used for “wara”, Soybean milk and the residues is used to feed animals. Traditionally, non-fermented food uses of soybean in the world include soy milk. Fermented foods include soybean sauce, fermented bean paste, among others (National Soya bean Research Laboratory, 2012). The oil is used in many industrial applications (World Market, 2012). The beans contain significant amounts of phytic acid, alpha-linoleic acid, and isoflavones.

1.4 Problems of Soybean Production

Soybean plants are vulnerable to a wide range of bacterial diseases, fungal diseases, viral diseases and parasites. The most common and destructive pest of soybean growth in Virginia is the corn earworm moth (Herbert *et al*., 2009).

Diseases such as rust, red leaf blotch, frog-eye leaf spot, bacterial pustule, bacterial blight, and soybean mosaic virus are problems to be resolved in soybean. Soybean rust (*Phytophthora pachishize*) particularly is the most destructive foliar disease of soybean in recent times, and can cause 50–60% yield loss. It is a major disease worldwide. This disease was first reported in 1998 in Uganda and Zimbabwe; and in 1999, its existence was reported in Nigeria, Cameroon, and Benin Republic. In Nigeria, the disease kept on recurring every season since 1999 (Dugje *et al*., 2009).

Seed production and distribution is also a major impediment in soybean– growing countries like Nigeria. Pod shattering especially in the hot dry savanna environments,

reduces seed longevity, and lodging are still constraints in soybean production (Dugje *et al*., 2009).

1.5 Statement of Research Problem

Average grain yield of soybean is low (<1 t/ha) in tropical Africa (Dugje *et al*., 2009).

Dual-purpose improved varieties of soybean have not reached many soybean growers to increase production (Dugje *et al*., 2009).

Among insect pests, pod sucking and defoliating insects are major constraints (Dugje *et al*., 2009).

Lack of varieties tolerant to midseason drought stress and high yielding varieties tolerant to low phosphorus are among the abiotic constraints (Dugje *et al*., 2009).

Research on seed quality such as protein, oil, carbohydrate, and anti-nutritional factors is meagre (Dugje *et al*., 2009).

There is too much dependence on soybean (*Glycine max* (L.)Merill) which has led to tremendous increase in market price.

In large parts of Sub-Saharan Africa particularly in Nigeria, smallholder agricultural production has remained consistently low and food security is catastrophically poor

1.6 Justification

This research will provide baseline information on the effects of gamma radiation and colchicine on soybean germination, morphology as well as the growth activity.

The treatment of soybean with gamma radiation and colchicine could possibly lead to attainment of beneficial genetic variability in the plant which might be useful to plant breeders.

The present study needs to be undertaken to investigate the mutagenic effects of gamma radiation and colchicine as a means of increasing the beneficial variability within cultivars and hence improve its productivity.

1.7 Aim

The aim of this research was to assess the mutagenic effects of Gamma radiation and Colchicine on the morphology and growth performance of soybean (*Glycine max*).

1.8 Objectives

These include: To determine

1. The effects of gamma radiation and colchicine concentration on the morphology and growth performance of soybean.

2. The effective dose of gamma radiation and colchicine concentration capable of inducing beneficial mutation with minimum level of lethality

3. The combined effects of gamma radiation and colchicine concentration on the growth and morphology of soybean plants.

1.9 Null Hypotheses

These include:

1. Gamma radiation and colchicine concentration have no effect on the morphology and growth performance of soybean plants.

2. Gamma radiation dosage and colchicine concentration have no beneficial effects on soybean.

3. Gamma radiation and colchicine concentration have no combined effects on the growth and morphology of soybean plants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Agricultural and Economic Importance of Legumes

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago. They were used as a food source during the pre-history of North and South America (more than 3,000 years ago) and by the Roman Empire as a food source and for soil improvement (Graham and Vance, 2003). Today, legumes are an increasingly invaluable food source not just for humans, accounting for 27% of the world's primary crop production, but also for farm animals (Graham and Vance, 2003). Legumes were grown on more than 13% of the total arable land under cultivation in the world in 2004 (Gepts *et al*., 2005). Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans, while soybeans (*Glycine max*) and peanut (*Arachis hypogaea*) provide more than 35% of the world's processed vegetable oil and a rich source of dietary protein for the poultry and pork industries (Graham and Vance, 2003).

Legumes produce nitrogen-containing protein in abundance, but they are deficient in sulfur containing amino acids and other nutrients needed by people and animals. For this reason, legumes and cereal crops are often raised together to account for the amino acids and other elements they are each deficient in (Gepts *et al*., 2005). The primary dietary legumes grown, such as bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicera rietinum*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna*

unguiculata), and lentils (Graham and Vance, 2003), include representatives of each of the four clades within papilionoids.

Many legumes form root nodules to fix atmospheric nitrogen in a symbiotic relationship with the soil bacteria 'rhizobia'. Legumes are extremely diverse in their abilities to nodulate. Not all species can and there is a wide variety of nodules that form, depending on the species in symbiosis. In addition to their uses as food, legumes are still used as tools in agriculture and forestry as the Romans did. The plants themselves or their parts like leaves and pods can be tilled into the soil as a nitrogen source and can be rotated with others for soil improvement. These techniques save farmers billions of dollars in the cost of nitrogen fertilizers (Graham and Vance, 2003).

Industrially, legumes have many uses in making biodegradable plastics, oils, dyes, and biodiesel fuel (Gepts *et al*., 2005). Legumes are used traditionally in folk medicines, but also demonstrate importance in modern medicine. Isoflavones commonly found in legumes are thought to reduce the risk of cancer and lower cholesterol and soybean phyto-estrogens are being studied for use in postmenopausal hormone replacement therapy (Graham and Vance, 2003). Legumes also produce a hypoglycemic effect when eaten, making them a recommended food for diabetics (Gepts *et al*., 2005).

2.2 Mutation

Mutation is a sudden random change in the genetic material of a cell, which is inheritable. Mutation occurs when a gene is damaged or changed in such a way to alter the genetic message carried by the gene (Gordon *et al.*, 2007). Similarly, mutation can be

natural and can be through human-made agents (physical or chemical) which can alter the structure or sequence of DNA (Montelone, 1998). Since their discovery, mutagens have been widely employed in several fields of Biology, Medicine and Agriculture (Ranch, 2001). A mutagen is an agent or substance that can bring about permanent alteration to the physical composition of a gene such that the permanent message is changed. The polypeptide made by translating the altered mRNA will now contain different sequence of amino acids. The function of protein made by folding this polypeptide will probably be changed or lost. The enzyme that is catalyzing the production of flower colour pigment have been altered in such a way that is no longer catalyzes the production of red pigments. Mutations, either spontaneous or artificially induced both in seeds and vegetatively propagated crops are of scientific and commercial interest. Mutations are the tools that geneticists used to study the nature and function of genes, which are the building blocks and basis of plant growth and development. Thus, producing new material for genetic improvement of economic crops (Adamu *et al*., 2004).

In general, the appearance of a new mutation is a rare event. Most mutations that were originally studied occurred spontaneously. This class of mutation is termed spontaneous mutations. Mutations can also be induced by several methods. The three general approaches used in order to generate mutations are; radiation, chemical and transposon insertion. Scientists are now using the power of transposable elements to create new mutations. Transposable elements are mobile pieces of DNA that can move from one location in genome to another. Often, when they move to a new location, the result is now a mutant. The mutant arises because the presence of a piece of DNA in a wild type gene disrupts the normal function of that gene (McClean, 1999).

The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in different selected plants by mutagens. Mutation breeding generates a knowledge base that guides future users of mutation technology for crop improvement.

2.2.1 Ionizing radiation

Several crop plants especially cereal varieties have been treated with a range of ionizing wavelength in the electromagnetic radiation spectrum. X-rays and gamma rays are the ones commonly used for this purpose due to their high penetrative power (Montelone, 1998). Ionizing radiations are easy to use but they are also disruptive to cell organelles, cell chemistry, chromosomes as well as DNA structure (Adamu, 1997). They are useful in restructuring the genome of an organism via chromosomal aberration (Neuffer, 1982). The most important aberration produced by ionizing radiations according to Odeje (2008), is translocation. They are useful in gene location to chromosome arm (Odeje, 2008). Gamma rays are more effective than X-rays as a mutagenic agent (Odeje, 2008). In like manner, neutron particles have also been employed at one time or another as mutagenic agent (Odeje, 2008).

2.2.2 Chemical mutagens

Chemical mutagens are those compounds that increase the frequency of mutations. They vary in their potency since this term reflects their ability to enter the cell, their reactivity with DNA, their general toxicity and the likelihood that the type of chemical change they introduce into the DNA will be corrected by a repair system. Most mutagens are used *in vivo* treatment but some of them can also be used *in vitro*. It is exceedingly difficult to

examine the actual mode of mutagenesis *in vivo*, because it is a very rare event (Robert, 2000). Even if it can be verified that a particular modified base can be formed *in vitro* by reaction with a mutagen and the modified base can give rise to a stable mutation *in vivo*, it cannot be assumed that this is the pathway of mutagenesis for the majority of the observed *in vivo* events (Robert, 2000). The source of such chemical are base analogs, the base pair with two different basis because of their lack of consistency in base pairing; alkylators include; ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), diethyl sulphate (DES), nitrosoguanidine (NG) (Robert, 2000).

2.2.3 The Chemical Induced Genetic Sterility

The chemical induces genetic sterility in rice without changes in vigor (Mensah *et al*., 2005). On the other hand, colchicine is both a polyploidizing and mutagenic agent (Bragal, 1955). This chemical has been used for a long time to produce polyploidy plants. The mutagenic effects of EMS and colchicine on plant morphology, chlorophyll, sterility and yield have earlier been confirmed by Ahoowalia, (2007). The influence of genotype on mutagenic variability in barley following colchicine treatment (Mensah *et al*., 2007). Another variety of *Sorghum bicolor* called 'Sodge' was found to mature earlier and developed a better shortness trait with enhanced lodging resistance better than the parent (IPS 001) after treatment with 300Gy of gamma rays. Similarly, a *Sorghum* grain phenotype with a unique modified endosperm texture that has near normal hardness and possesses superior nutritional quality traits of high digestibility and enhanced lysine content was developed from crosses between hard endosperm, normal nutritional *Sorghum* lines (Tesfaye *et al*., 2006).

2.3 The Concept of Polyploidy

Polyploidy organisms have more than two haploid sets of chromosomes which either by the duplication of chromosome number in somatic tissue with suppression of cyto-kinesis or by the formation of gametes with an unreduced number of chromosomes (Sidi, 2006). About 35% of the species of flowering plants are polyploids. The most common level of polyploidy is tetraploidy which means that they have four sets of chromosomes instead of two. Hybrids are often sterile because their chromosomes are unpaired at meiosis and thus cannot form viable gametes. Polyploidy can restore paired chromosomes and thus fertility (Uno *et al*., 2007).

Polyploidy has been recognized as a common phenomenon in the evolution of plants (Wenzel, 2000). According to Dean, (1959), polyploids generally, are often harder, more vigorous in growth, able to occupy less favorable habitats and or have larger flowers and fruits. They may be more adapted to temperature or water stress than their diploid relatives. Most of the easily observed effects of polyploids are associated with increased size usually due to induction by humans; by treating with chemicals like colchicine (Uno *et al*., 2007).

Colchicine is a mutagen which induces polyploidy in plants. It does not seem to have any visible effects on the chromatic material. Rather, it works on the cytoplasm and the spindle in mitosis, resulting in suppression of the new cell wall and the spindle formation (Bernard, 2005). In meiosis, a diploid nucleus is formed after the first meiotic division instead of a haploid nucleus. A tetraploid nucleus is therefore formed at the end of the next division, this means that the pollen grains formed under these conditions may be

provided with a double or quadruple their normal number of chromosomes (Bernard, 2005).

A fungicide, under the name "Granosan" has been used for doubling chromosomes number in plant cells. It has advantage over colchicine in that, seeds treated with it are not subjected to fungal infections (Bernard, 2005). Other chemicals such as acenaphtene and veratine induced polyploidy.

2.4 Effects of Mutation on Plant Morphology

Mutagenic effects on plant morphology have been confirmed (Mensah *et al*., 2007). Mensah*et al*., (2007) reported shortened and swollen internodes and deformed leaves in *Sesame indicum* treated with sodium azide and colchicine. Increases in number of branches and pods per plant have been reported by Biswas and Datta (1988) in *Trigonella foenum-fraeum* using 0.25% of ethyl methane sulphonate. Pathak (1991) also reported an increase in pod length in aphid resistant cowpea which was irradiated with 20 Krad of gamma rays. In some seeds of inbred maize line which were treated with aqueous solution of N-nitrosoethylurea, N-nitrosomethylurea, Hydroxylamine, Diethylsulphate and 1, 4-Bisdiazoacetyl, mutations were obtained in plant colouration, inflorescence structure, leaf and stem structure and endosperm quality (Mensah and Obadoni, 2007). Morphology aberrations including distorted leaf forms and swollen and or shortened internodes have been observed in *Arachis hypogaea* which were treated with sodium azide (Mensah and Obadoni, 2007).

2.5 Mutation Induced Seed Sterility

Seeds of khesari (*Lathyrus sativa*) when treated with gamma rays and with diethyl sulphate induced greater pollen seed sterility and lower survival percentages. Radiation induced sterility might be the result of chromosomal aberrations caused by cryptic deletions specific gene mutations. High frequencies of translocation leading to multivalent association involving varying number of chromosomes were induced in all the treatments with EMS (Kumar and Dubey, 1998)

2.6 Mutagenesis for Chlorophyll Deficient Mutants

Chlorophyll mutations are remarkable results of plant mutagenesis. They are very popular among gardeners as a component of ornamental periclinal chimeras with motely leaves ("variegate", marginata", media pieta",). According to Odeje (2008), such periclinal chimeras were induced when seeds of *Sorghum bicolor* were treated with solutions of N-methyl-N-nitrosourea (MNH), 0.02%., N-ethyl-N-nitrosourea (ENH), 0.05%., dimethyl-N-nitrosourea (dMNH) 0.1% for 18 hours. Similar results have also been induced in *Pinus montana* and *Pinus sylvestris* (Odeje, 2008).

Chlorophyll mutation according to Khan and Tyagi (2009) are a common occurrence after mutagenic treatments. In this research four different chlorophyll deficient mutants were induced. These include chlorina, striata, virescent and lustescent. Gamma rays and colchicine concentration differ with respect to the spectrum and the frequency of chlorophyll mutations induced. Giri and Apparao (2011) observed different chlorophyll mutants like chlorina, xantha, albina and striata in pigeon pea (*Cajanus cajan* L.) after treatments with EMS. Differential behaviour of the mutagens to produce specific types

of chlorophyll mutants may be attributed to their mutagenic effectiveness Adamu *et al*., (2002) and Neuffer (1988). The pigment that may be found in most cereals chloroplastids are chlorophyll and carotenoids. (Adamu *et al*., 2002). As such a breakdown in one or both of these pigments may result in the formation of any of the chlorophyll deficient mutants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This research was carried out in the laboratory, Department of Biological Sciences and in the Botanical Garden, Ahmadu Bello University (A.B.U.), Zaria (Altitude 610 meters above sea level, latitude 11˚ 12'N and longitude 07˚33'E) (Osuhor *et al*., 2004), Nigeria. Samaru lies in the Northern Guinea Savanna agro ecological zone of Nigeria with a mean annual rainfall of about 1100 mm. Rainfall is essentially between May - September and dry season between October - May. Hottest month is around March - April, and the mean daily Temperature is about 27^0C. The coldest Month is between November – January (Osuhor *et al*., 2004).

3.2 Plant Source and Mutagen Treatment

The seeds of two improved varieties of soybean *Glycine max* (L.) Merrill (*G. max* var. SAMSOY-2 and *G. max* var. TGX-1448-2E) were obtained from Department of Plant Science, Institute for Agricultural Research (I.A.R), Ahmadu Bello University, Zaria, and were taken to A.B.U Centre for Energy Research and Training, Samaru, Department of Radiology for irradiation with gamma ray (AmBe source) Fast Neutrons. Part of the seeds was treated with colchicine in the laboratory in Department of Biological Sciences, Ahmadu Bello University, Zaria (Altitude 667.88 meters above sea level, latitude 11˚ 04'N and longitude 7˚42'E).

3.3 Treatment of Seeds with the Mutagens

The seeds of two improved varieties of *Glycine max* (L.) Merrill (*G. max* var.Samsoy-2 and *G. max* var. TGX-1448-2E) were air –dried and divided into five sets, and were pre-soaked for 1 hour in distilled water. One set was the control and the remaining four sets of the seeds were soaked in various concentrations of colchicine (0, 0.5, 1.0, 1.5, and 2.0 mM) for two hours, respectively. The treated seeds were then rinsed thoroughly with distilled water, partly dried and sown in soil in polythene bags.

In the second sets of treatment, another four set of seeds were also irradiated with Gamma rays (0, 5, 10, 15 and 20 Krad).These seeds were also sown in soil in polythene bags.

In the third set of treatment, combined treatment where the seeds were irradiated with Gamma rays (0, 5, 10, 15 and 20 Krad) and the irradiated seeds were also soaked in all colchicine concentration for one hour, respectively. The seeds were washed with distilled water, partly dried and sown in polythene bags (Borkar and More., 2010).

3.4 Collection of Soil and Determination of its properties

Top soil was collected from uncultivated land within the Botanical Garden in Ahmadu Bello University, Zaria and used to fill the 900 polythene bags.

3.5 Sowing of the Seeds

Planting of the treated and the control seeds were done in Botanical garden, Ahmadu Bello University Zaria, using polythene bags; Nine hundred (900) polythene bags were filled with top soil and arranged randomly with three (3) replication per treatment and the

treatment was represented six times having (150) treatment per replicate for the two varieties. Four seeds were sown per polythene bag and thinned to two plants in each polythene bag at 2WAP.

3.6 Experimental Design

The seeds of two varieties of Soybean (TGX 1448-2E and SAMSOY-2) were exposed to five treatments (0, 5, 10, 15, and 20 Krad) of gamma rays and five colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0 mM). Combinations of these two mutagens were also used to treat the two soybean varieties. The experiment was replicated thrice and each replicate was represented with 6 bags. A total of 900 bags were used in this experiment. The bags were laid out in a complete randomized design.

3.7 Data Collection

One polythene bag was sampled per each replicate of treatment at 3 weeks interval starting from 3 WAP. One plant was selected from each polythene bag using simple random sampling method and the data was collected from the plant for the following parameters: germination percentage, plant height, survival at maturity, days to flowering, fresh weight and height at maturity.

3.7.1 Germination rate and percentage

The number of plants that came out was counted and divided by the number of plant sown, and multiplied by hundred. The estimate was carried out two weeks after planting (2 WAP).

3.7.2 Seedling height

Seedling heights were measured in centimeter at three weeks after planting (3 WAP). A ruler was used to measure seedlings from the ground level to the tip of the apical bud.

3.7.3 Seedling survival

The estimates of seedling survival were taken at three weeks after planting (3 WAP) to ascertain the relative emergence of seedling survival of the treatment population.

3.7.4 Survival to maturity

Plant were counted at six weeks after planting (6 WAP) and this was compared with the number of plant that germinated to determine whether there was lethality before maturity.

3.7.5 Days to flowering

This was determined by counting the number of days from planting to opening of flowers.

3.7.6 Heights at maturity

Plant height were measured six (6) weeks after planting (WAP) using a meter rule from the ground to the top of the apical bud on the main stem.

3.7.7 Fresh weight of the plant

Plants were uprooted six (6) weeks after planting (WAP), the bag of soil was carefully removed from the plant in order to recover most of the roots. The sampled plant was washed in water and then drained on clean table in the laboratory. The root nodules and leave number were counted then the shoot fresh weight, root fresh weight and the total fresh weight were measured using an electric weighing balance machine.

3.7.8 Chimera induction

Chlorophyll deficient mutant plant was observed during growth at 5 WAP and 6 WAP. Then it was captured using digital camera.

3.8 Yield Parameters

At harvest, the following yield components were determined: Number of pods, Pod weight, Total grain yield, 100 seed weight and crude protein content.

3.8.1 Number of pods

Number of pod per plant was determined.

3.8.2 Pod dry weight

The harvested pods were dried in the laboratory after which the Pod dry weight in each treatment was measured using an electric weighing machine.

3.8.3 Total grain yield

Total grain yield per treatment were determined using an electric weighing machine.

3.8.4 100 seed weight

Seeds (100) were counted from each treatment and weighed using an electric weighing machine.

3.8.5 Crude protein content

Crude protein content of each treatment were determined through proximate analysis procedure following (AOAC, 1980) method.

3.8.5.1 *Determination of crude protein content*

The samples were digested in sulphuric acid to break down organic matter and reduce nitrogenous compounds to ammonium compounds. Ammonia was liberated by boiling with sodium hydroxide, the steam was distilled into boric acid solution and determined titrimetrically.

During the digestion phase: tablets containing potassium sulphate and mercuric oxide were added. The potassium sulphate raises and control the temperature of the reaction, while the mercuric oxide, acted as a catalyst. During the digestion phase, the following reaction occurs:

$$CH_3NH^{-} + 3(H_2N)_2SO_4 \rightarrow CO_2 + SO_2 + 2NH_4HSO_4 + 3H_2$$

An aliquot of the diluted digest was reacted with sodium sulphide. The ammonia released was steam distilled into the boric acid solution and was titrated with standard sulphuric acid solution. During the distillation phase, the following reaction occurs.

$$NH_4HSO_4 + 2NaOH \rightarrow NH_3 + Na_2SO_4 + 2H_2O$$

Sodium thiosulphate was added to the distillation to break down mercury / ammonium complex formed during the digestion

Procedure: 0.5g of the samples were weighed each, into the digestion flask. 30cm^3 of concentrated sulphuric acid was added and two kjeltabs were also added. It was heated gently on a digestion apparatus by means of electric elements. At this stage of digestion, the reaction mixture was turn black due to the dehydrating action of the sulphuric acid and formation of free carbon. As the reaction of free carbon, oxidized, a clear, yellow

solution remained. After heating strongly for 2 hours, the sulphuric acid was seen by its condensation in the lower part of the neck of the flask. This was allowed to cool after digestion and small quantity of distill water was add to dissolve the crystals that was formed, and make it with distilled water in a 100cm^3 volumetric flask. 10ml of 2.0% boric acid solution and 2 drops of indicator was mix into a 100ml conical flask. While 10ml of the diluted sample was pipette into the Markham distiller with 10ml of 40% sodium Hydroxide. The mixed boric acid and indicator in the flask was placed at the bottom of the condenser to collect the ammonia, while all other possible channels for the escape of the ammonia closed. When about 50cm^3 of the distillate was collected in the row green boric acid and indicator solution, the distillate was then titrated with 0.02 normal Sulphuricacid which gave a grey-mauve end point.

Calculation:

Where: N_2 = is the factor, Sample weight = is the weight of the soybean, Sample titre = titre value of soybean, Blank titre = untitre value and 0.01N = normality of acid

$$\% N_2 = \frac{(\text{Sample titre} - \text{blank titre}) \times \text{Normality of acid} \times 0.001402 \times 10 \times 100}{\text{Weight of Sample (g)} \times \text{aliquot}}$$

% crude protein: $\%N_2 \times 6.25$ (i.e. there is an average of 16% N_2 proteins; therefore 100/16=6.25 Factor to convert N_2 to crude protein or generalized protein = 6.25).

3.9 Statistical Analysis

The data obtained from the above parameters were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2004). Duncan's multiple range test (DMRT) was used to compare significant differences between the treatment means.

CHAPTER FOUR

4.0 RESULTS

The results obtained from treating two varieties of Soybean with different mutagens were as follows:

4.1 Soil Properties

The properties of soil used in this experiment are as presented in Table 1. The soil contained 0.39 %N, 4.36 % organic carbon and the textural class was sandy-loam.

4.2 Colchicine Concentration

4.2.1 Germination percentage

The findings on Soybean varieties with Colchicine concentration (Table 2) indicated that the control 0 mM (77.14 %) resulted in the highest percentage germination. The lowest percentage germination was due to 2.0 mM (52.92 %). Generally, 1.0 mM (60.56 %) and 1.5 mM (59.63 %) are comparable with each other. Similarly, 0.5 mM (66.46 %) differ significantly with all other treatments (Table 2).

4.2.2 Plant survival

At 3 WAP, the control 0 mM (3.33 %) resulted in the highest rate of survival which is comparable with 0.5 mM (3.16 %). The lowest rate of survival was due to 2.0 mM (2.26 %). Generally, 1.0 mM (2.86 %) and 1.5 mM (2.63 %) treatment did not differ significantly with each other (Table 2).

At 6 WAP, the highest rate of survival was under 0.5 mM colchicine (3.03 %) was similar to that due to the control. The lowest survival rate was due to 2.0 mM (1.86 %) and 1.5 mM (2.10 %) (Table 2).

4.2.3 Plant height

At 3 WAP, the highest plant height resulted in 1.5 mM (10.88 cm) and 2.0 mM (10.73 cm) which is comparable with 1.0 mM (10.57 cm) treatment. The lowest plant height resulted in the control 0 mM (10.15 cm) which is comparable with 0.5 mM (10.28 cm) (Table 2). At 6 WAP, the highest plant height was due to 1.0 mM (23.51 cm) and 2.0 mM (23.30 cm) colchicine treatments. Generally, all the treatments were comparable except 1.5 mM that is comparable with only 0.5 mM and the control. The lowest plant height was due to 1.5 mM (21.90 cm) colchicine treatment (Table 2).

4.3 Gamma Radiation

4.3.1 Germination percentage

The highest percentage germination resulted from the control (70.78 %). This was followed by that due to 5 Krad (65.33 %) and 10 Krad (63.36 %) were comparable then, 10 Krad (63.36 %) and 15 Krad (61.14 %) are also comparable with each other. The lowest percentage germination was due to 20 Krad (56.09 %) (Table 2).

4.3.2 Plant survival

At 3 WAP, the highest rate of survival occurred under the control (3.30 %). This was followed by that due to 5 Krad (2.93 %), 10 Krad (2.90 %) and 15 Krad (2.70 %) which were comparable with each other. The lowest rate of survival was due to 20 Krad (2.43 %) (Table 2). At 6 WAP, the highest rate of survival resulted from the control (0 Krad)

(2.70 %). This was followed by that due to 5 Krad (2.40 %), 10 Krad (2.40 %) and 20 Krad (2.63 %) were comparable with each other. The lowest rate of survival was due to 15 Krad (2.33 %) (Table 2).

4.3.3 Plant height

At 3 WAP, the highest plant height attained was under the control (0 Krad) (10.80 cm) and 15 Krad (10.78 cm) treatment. Following this was that of 5 Krad (10.12 cm), 10 Krad (10.47 cm) and 20 Krad (10.43 cm) which were comparable with each other. The lowest plant height was due to 5 Krad (10.12 cm) (Table 2). At 6 WAP, the highest plant height attained was due to 15 Krad (23.46 cm) treatment. Generally, all the treatments resulted in comparable plant height at 6 WAP. The lowest height attained was due to 10 Krad (22.41 cm) (Table 2).

4.4 Varieties

The germination percentage was significantly ($p \leq 0.05$) better in TGX 1448-2E (69.65 %) than SAMSOY-2 (57.93 %). In the same vein, plant survival (3 and 6 WAP) was better in TGX 1448-2E than SAMSOY-2. In contrast, plant height (3 WAP) (Table 2).

4.5 Interaction effects of Gamma Radiation and Colchicine Concentrations

The effect of interaction of C×G and C×V on plant germination were highly significant ($p \leq 0.01$), while G×V was significant ($p < 0.05$). In another development, plant survival (6 WAP) did not had significant ($p > 0.05$) effect except G×V interaction that was highly significant ($p \leq 0.01$). There was significant interaction only at C×V for plant height (3 and 6 WAP) (Table 2).

Table 1: Physico-chemical parameters of the Soil used for the Experiment.

Soil Parameters	Value
pH in H_2O (1:2.5)	7.2
pH in 0.01m$CaCl_2$ (1:2.5)	6.65
Organic carbon (%)	4.36
Percentage Available Phosphorus in (ppm)	7.53
Total Nitrogen (%)	0.385
Exchange Base (Cmol/Kg)	
Ca	6.71
Mg	0.52
K	1.59
Na	0.63
CEC	12.5
Particle Size (%)	
Clay	20
Silt	26
Sand	54
Textural Class	Sandy-Loam.

Table 2: Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Germination, Plant Survival and Plant Height of two Soybean Varieties.

Treatment	Germination Percentage (%)	Plant Survival (3 WAP) (%)	Plant Survival (6 WAP) (%)	Plant Height (3 WAP) (cm)	Plant Height (6 WAP) (cm)
Colchicine (C)					
Control	77.14[a]	3.33[a]	2.83[ab]	10.15[c]	23.15[ab]
C1 (0.5mM)	66.46[b]	3.16[ab]	3.03[a]	10.28[bc]	23.03[ab]
C2 (1.0mM)	60.56[c]	2.86[bc]	2.63[b]	10.57[ab]	23.51[a]
C3 (1.5mM)	59.63[c]	2.63[c]	2.10[c]	10.88[a]	21.90[b]
C4 (2.0mM)	52.92[d]	2.26[d]	1.86[c]	10.73[a]	23.30[a]
SE±	1.97	0.15	0.12	0.19	0.60
Gamma Radiation (G)					
Control	70.78[a]	3.30[a]	2.70[a]	10.80[a]	23.05[a]
G1 (5Krad)	65.33[b]	2.93[b]	2.40[bc]	10.12[b]	23.01[a]
G2 (10Krad)	63.36[bc]	2.90[b]	2.40[bc]	10.47[ab]	22.41[a]
G3 (15Krad)	61.14[c]	2.70[bc]	2.33[c]	10.78[a]	23.46[a]
G4 (20Krad)	56.09[d]	2.43[c]	2.63[ab]	10.43[ab]	22.94[a]
SE±	1.97	0.15	0.12	0.19	0.60
Variety					
TGX	69.65[a]	3.02[a]	2.64[a]	9.88[b]	22.94[a]
SAM	57.03[b]	2.68[b]	2.34[b]	11.16[a]	23.01[a]
SE±	1.24	0.09	0.07	0.12	0.38
Interaction					
C×G	**	NS	**	NS	NS
C×V	**	NS	NS	**	*
G×V	*	*	**	NS	NS
CV (%)	12.07	20.79	19.35	7.17	10.25

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%).
Keys: TGX=TGX 1448-2E, SAM= SAMSOY=2, CV= Coefficient of Variance, SE±= Standard Error Mean.

4.6 Colchicine Concentration

4.6.1 Root nodules

The highest root nodules resulted in 2.0 mM (13.83). Generally, the control (0 mM) (4.46), 0.5 mM (5.03), 1.0 mM (5.26) treatments resulted in lower but comparable root nodule numbers (Table 3).

4.6.2 Number of leaves

The highest leaf number was produced under 0 mM (6.76). Generally, all the treatments produced comparable leaf number. The lowest leaf number was due to 1.0 mM colchicine (6.43) (Table 3).

4.6.3 Root weight

The highest root weight resulted from 1.5 mM colchicine (1.63 g). Generally, the control 0 mM (1.33 g), 0.5 mM (1.50 g) and 1.0 mM (1.31 g) produced comparable root weight with each other. The lowest root weight was due to 2.0 mM (1.15 g) (Table 3).

4.6.4 Shoot weight

The highest shoot weight was due to 1.5 mM colchicine (3.15 g). Generally, all the treatments produced comparable shoot weight. The lowest shoot weight was due to the control (2.96 g) (Table 3).

4.6.5 Total fresh weight

The highest fresh weight total was produced under 1.5 mM colchicine treatment (4.78 g). Generally, all the treatments produced comparable total fresh weight with all other

treatments. The lowest fresh weight total was due to the control (4.29 g) and 2.0 mM colchicine treatments (4.29 g) (Table 3).

4.7 Gamma Radiation

4.7.1 Root nodules

The highest number of root nodules was due to 20 Krad (8.90) treatment. Generally, 5 Krad (6.66), 10 Krad (6.83) and 15 Krad (7.63) produced comparable number of root nodules with each other. The lowest root nodule was due to the control (6.50) (Table 3).

4.7.2 Number of leaves

The highest number of leaf resulted from 5 Krad (6.76) treatment. Generally, all the treatments resulted in comparable number of leaves. The lowest number of leaf was due to 15 Krad treatment (6.43) (Table 3).

4.7.3 Root weight

The highest root weight resulted from 20 Krad (1.59 g) treatment. Generally, all the treatments produced comparable root weight except the highest due to 20 Krad (1.59 g). The lowest root weight was due to the control (1.27 g) and 5 Krad (1.29 g) treatments (Table 3).

4.7.4 Shoot weight

The highest shoot weight resulted from 20 Krad (3.23 g) treatment. All the treatments produced comparable shoot weight. The lowest shoot weight was due to the control (2.88 g) (Table 3).

4.7.5 Total fresh weight

The highest fresh weight total resulted from 20 Krad (4.82 g) treatment. The 5 Krad (4.30 g), 10 Krad (4.50 g) and 15 Krad (4.44 g) produced comparable total fresh weight with each other. The lowest fresh weight total was due to the control (4.15 g) (Table 3).

4.8 Varieties

There was significant ($p \leq 0.05$) increase in the number of root nodules in variety TGX 1448-2E than SAMSOY-2.Similar level of significant ($p \leq 0.05$) increase was observed in number of leaves in TGX 1448-2E than the other variety. There was no significant ($p > 0.05$) difference in root weight of the two varieties. Increase in shoot weight and total weight was significantly ($p \leq 0.05$) higher in TGX 1448-2E than the other variety (Table 3).

4.9 Interaction effects of Gamma Radiation and Colchicine Concentrations

Root nodules was determined significantly ($p < 0.01$) by C×G and C×V. In contrast, interaction of C×G, C×V and G×V did not influence number of leaves significantly ($p > 0.05$). Root weight and total fresh weight were determined by the interaction of C×G and C×V, while shoot weight was highly significant ($p \leq 0.01$) at interaction C×G as well (Table 3).

4.10 Colchicine Concentration

4.10.1 Days to flowering

The highest day to flowering was under 2.0 mM colchicine treatment (46.03). Generally, all the treatments resulted in comparable days to flowering in soybean varieties. The lowest day to flowering was due to 1.5 mM colchicine treatment (44.20) (Table 4).

Table 3: Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Root Nodules, Number of Leaves and Total fresh weight of two Soybean Varieties.

Treatment	Root Nodules	No. of Leaves	Root weight (g)	Shoot weight (g)	Total fresh weight (g)
Colchicine (C)					
Control	4.46^{c}	6.76^{a}	1.33^{bc}	2.96^{a}	4.29^{a}
C1 $_{(0.5mM)}$	5.03^{c}	6.73^{a}	1.50^{ab}	3.00^{a}	4.50^{a}
C2 $_{(1.0mM)}$	5.26^{c}	6.43^{a}	1.31^{bc}	3.03^{a}	4.34^{a}
C3 $_{(1.5mM)}$	7.93^{b}	6.50^{a}	1.63^{a}	3.15^{a}	4.78^{a}
C4 $_{(2.0mM)}$	13.83^{a}	6.56^{a}	1.15^{c}	3.14^{a}	4.29^{a}
SE±	0.49	0.22	0.13	0.23	0.27
Gamma Radiation (G)					
Control	6.50^{c}	6.53^{a}	1.27^{b}	2.88^{a}	4.15^{b}
G1 $_{(5Krad)}$	6.66^{bc}	6.76^{a}	1.29^{b}	3.01^{a}	4.30^{ab}
G2 $_{(10Krad)}$	6.83^{bc}	6.73^{a}	1.44^{ab}	3.05^{a}	4.50^{ab}
G3 $_{(15Krad)}$	7.63^{b}	6.43^{a}	1.33^{ab}	3.11^{a}	4.44^{ab}
G4 $_{(20Krad)}$	8.90^{a}	6.53^{a}	1.59^{a}	3.23^{a}	4.82^{a}
SE±	0.49	0.22	0.13	0.23	0.27
Variety					
TGX 1448-2E	10.12^{a}	6.74^{a}	1.30^{a}	3.34^{a}	4.65^{a}
SAMSOY-2	4.49^{b}	6.45^{b}	1.46^{a}	2.77^{b}	4.23^{b}
SE±	0.31	0.14	0.08	0.14	0.17
Interaction					
C×G	**	NS	*	**	**
C×V	**	NS	**	NS	**
G×V	NS	NS	NS	NS	NS
CV $_{(\%)}$	26.22	13.06	38.65	29.48	23.91

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).
Keys: TGX=TGX 1448-2E, SAM= SAMSOY=2, CV= Coefficient of Variance, SE±= Standard Error Mean.

4.10.2 Number of pods

The highest number of pods was obtained from 2.0 mM colchicine treatment (8.13). Generally, all the treatments produced comparable number of pods. The lowest number of pods was due to 0.5 mM (7.70) and 1.5 mM colchicine treatments (7.70) (Table 4).

4.10.3 Pod weight

The highest pod weight was produced under 2.0 mM colchicine treatment (3.41 g). The control 0 mM (2.89 g), 1.0 mM (2.86 g) and 1.5 mM colchicine treatments (3.01 g) produced comparable pod weight. The lowest pod weight was due to 0.5 mM (2.66 g) and 1.5 mM colchicine treatments (7.70) (Table 4).

4.10.4 100 Seeds weight

The highest 100 Seeds weight resulted from 2.0 mM colchicine treatment (11.42 g). The control (10.82 g), 0.5 mM (10.97 g) and 1.5 mM colchicine treatments (10.74 g) produced comparable 100 seed weight. The lowest 100 Seeds weight was due to 1.0 mM colchicine treatment (10.22 g) (Table 4).

4.10.5 Grain Yield

The highest grain yield resulted from 2.0 mM colchicine treatment (2.11 g). All the treatments resulted in comparable grain yield except 2.0 mM colchicine treatment (2.11 g). The lowest grain yield was due to 1.0 mM colchicine treatment (1.68 g) (Table 4).

4.10.6 Crude protein content

The highest crude protein in soybean seed was due to 1.0 mM colchicine treatment (35.51 %). All the treatments resulted in comparable seed crude protein content. The lowest crude protein was due to 2.0 mM colchicine treatment (35.14 %) (Table 4).

4.11 Gamma Radiation

4.11.1 Days to flowering

The highest day to flowering resulted from 20 Krad (46.20) treatment. Generally, all the treatments resulted in comparable number of days to flowering. The lowest day to flowering was due to 10 Krad (44.73) (Table 4).

4.11.2 Number of pods

The highest pods number was produced from 5 Krad (8.13). Generally, all the treatments produced comparable number of pods in soybean varieties. The lowest pod number was due to the control (0 Krad) (7.43) (Table 4).

4.11.3 Pod weight

The highest pod weight resulted from the control 0 Krad (3.15 g). Generally, all the treatments produced comparable pod weight except 15 Krad (2.77 g). The lowest pod weight was due to 15 Krad (2.77 g) (Table 4).

4.11.4 100 Seeds weight

The highest 100 seeds weight resulted from 20 Krad (10.96 g). Generally, all the treatments produced comparable 100 seeds weight. The lowest 100 seeds weight was due to 15 Krad (10.68 g) (Table 4).

4.11.5 Grain yield

The highest grain yield resulted from the control 0 Krad (1.87 g), 5 Krad (1.88 g) and 20 Krad (1.86 g) treatments. Generally, the control (1.87 g), 5 Krad (1.88 g) and 20 Krad (1.86 g) produced comparable grain yield with each other. The lowest grain yield was due to 10 krad (1.72 g) and 15 Krad (1.69 g) treatments (Table 4).

4.11.6 Crude protein content

The highest crude protein content resulted from 5 Krad (35.46 %) treatment. Generally, all the treatments produced comparable crude protein content. The lowest content of protein was due to 20 Krad (35.19 %) treatment (Table 4).

4.12 Varieties

Days to flowering was significantly ($p \leq 0.05$) higher in SAMSOY-2 than other variety, SAMSOY-2 variety produced significant ($p < 0.05$) number of pods than the TGX 1448-2E (7.52). The pod weight, 100 seeds weight, grain yield and crude protein were significantly ($p < 0.05$) higher in TGX 1448-2E variety than the other variety (Table 4).

4.13 Interaction effects of Gamma Radiation and Colchicine Concentrations

Days to flowering was significantly ($p<0.05$) interacted only at G×V, the number of pods were controlled significantly ($p<0.05$) by the effect of C×G. Pod weight, 100 seeds weight and crude protein were not control by the variables. There was high significant ($p\leq0.01$) influence of C×G and C×V on the grain yield (Table 4).

4.13.1 Effect of Gamma Radiation and Colchicine Interaction on Percentage Germination of two Soybean Varieties at 2 WAP.

Application of gamma radiation and colchicine reduced the percentage germination at 2 WAP. The interaction of G × C on percentage germination data from the two varieties showed that, 0 – 15 Krad × 0 mM) resulted in significantly higher percentage germination than the other treatments. This was followed by 0 – 10 Krad × 0.5 mM G × C interaction (Table 5). The lowest percentage germination at 2 WAP due to (2.0 mM × 20 Krad) was significantly lower than that of all other treatments (Table 5).

4.13.2 Effects of Gamma Radiation and Colchicine Interaction on Plant survival of two Soybean Varieties at 6 WAP.

At 6 WAP, application of gamma radiation and colchicine reduced the plant survival significantly, the interaction data of G × C on the two varieties at 6 WAP also showed that, the control (0 mM × 0 Krad) resulted in the highest plant survival. Generally, there was higher plant survival at 0 – 20 Krad × 0 – 1.0 mM than the other treatments (Table 6). The lowest plant survival was due to (2.0 mM × 20 Krad) treatment (Table 6).

Table 4: Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Yield Parameters and Crude Protein Content of two Soybean Varieties.

Treatment	Days to Flowering	Number of Pods	Pod weight (g)	100 Seeds weight (g)	Grain yield (g)	Crude protein (%)
Colchicine (C)						
Control	45.50^{a}	7.93^{a}	2.89^{bc}	10.82^{b}	1.76^{b}	35.29^{a}
C1 $_{(0.5mM)}$	45.03^{a}	7.70^{a}	2.66^{c}	10.97^{ab}	1.74^{b}	35.38^{a}
C2 $_{(1.0mM)}$	45.60^{a}	7.96^{a}	2.86^{bc}	10.22^{c}	1.68^{b}	35.51^{a}
C3 $_{(1.5mM)}$	44.20^{a}	7.70^{a}	3.01^{b}	10.74^{bc}	1.74^{b}	35.31^{a}
C4 $_{(2.0mM)}$	46.03^{a}	8.13^{a}	3.41^{a}	11.42^{a}	2.11^{a}	35.14^{a}
SE±	0.89	0.31	0.18	0.27	0.12	0.26
Gamma Radiation (G)						
Control	45.03^{a}	7.43^{a}	3.15^{a}	10.92^{a}	1.87^{a}	35.31^{a}
G1 $_{(5Krad)}$	45.53^{a}	8.13^{a}	3.04^{ab}	10.75^{a}	1.88^{a}	35.46^{a}
G2 $_{(10Krad)}$	44.73^{a}	7.80^{a}	2.94^{ab}	10.86^{a}	1.72^{b}	35.29^{a}
G3 $_{(15Krad)}$	44.86^{a}	8.03^{a}	2.77^{b}	10.68^{a}	1.69^{b}	35.38^{a}
G4 $_{(20Krad)}$	46.20^{a}	8.03^{a}	2.92^{ab}	10.96^{a}	1.86^{a}	35.19^{a}
SE±	0.89	0.39	0.12	0.28	0.05	0.27
Variety						
TGX	43.81^{b}	7.52^{b}	3.15^{a}	12.91^{a}	1.93^{a}	35.63^{a}
SAM	46.73^{a}	8.25^{a}	2.77^{b}	8.75^{b}	1.68^{b}	35.02^{b}
SE±	0.56	0.24	0.08	0.18	0.03	0.17
Interaction						
C×G	NS	*	NS	NS	**	NS
C×V	NS	NS	NS	NS	**	NS
G×V	*	NS	NS	NS	NS	NS
CV $_{(\%)}$	7.65	19.18	16.88	10.25	12.42	3.06

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).
Keys: TGX=TGX 1448-2E, SAM= SAMSOY=2, CV= Coefficient of Variance, SE±= Standard Error Mean.

Table 5: Effect of Gamma Radiation and Colchicine Concentration Interaction on the Percentage Germination of two Soybean Varieties at 2 WAP.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Percentage Germination (%) 0	0.5	1.0	1.5	2.0
0	92.52±1.78^{a}	78.60±1.84^{bc}	65.80±3.13^{d-h}	54.47±3.16^{ij}	62.53±4.41^{e-j}
5	84.70±0.89^{ab}	68.07±0.86^{def}	58.70v3.41^{f-j}	58.68±7.74^{f-j}	56.50±4.77^{hij}
10	71.37±5.61^{cde}	66.73±3.86^{d-g}	61.55±4.88^{f-j}	60.48±5.47^{f-j}	56.67±5.34^{g-j}
15	73.22±4.28^{cd}	60.65±2.91^{f-j}	59.60±4.49^{f-j}	59.77±4.22^{f-j}	52.50±4.98^{j}
20	63.90±4.22^{d-i}	58.27±4.67^{f-j}	57.15±6.64^{g-j}	64.75±4.41^{d-h}	36.42±3.02^{k}
SE±	2.96				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

Table 6: Effects of Gamma Radiation × Colchicine Concentration Interaction on the Plant survival of two Soybean Varieties at 6 WAP.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Plant survival (%) 0	0.5	1.0	1.5	2.0
0	3.83±0.17^{a}	2.83±0.40^{bcd}	2.17±0.31^{d-g}	2.50±0.22^{c-f}	2.17±0.31^{d-g}
5	2.17±0.17^{d-g}	3.33±0.42^{ab}	2.50±0.22^{c-f}	2.00±0.00^{efg}	2.00±0.00^{efg}
10	2.17±0.17^{d-g}	3.33±0.21^{ab}	2.67±0.21^{b-e}	2.00±0.00^{efg}	1.83±0.17^{fg}
15	2.67±0.21^{b-e}	2.67±0.21^{b-e}	2.50±0.22^{c-f}	2.00±0.00^{efg}	1.83±0.17^{fg}
20	3.33±0.21^{ab}	3.00±0.00^{bc}	3.33±0.21^{ab}	2.00±0.00^{efg}	1.50±0.22^{g}
SE±	0.21				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), ∗ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

4.13.3 Effects of Gamma Radiation and Colchicine Interaction on the Root Nodule of two Soybean Varieties at 6 WAP.

The interaction of G × C data from the two varieties also showed that, (2.0 mM × 20 Krad) treatment resulted in the highest root nodule (Table 7). Generally, (0 – 20 Krad × 2.0 mM) G × C interaction resulted in higher root nodules than the other treatments.The lowest root nodule was due to the control (0 mM × 0 Krad) treatment (Table 7).

4.13.4 Effects of Gamma Radiation and Colchicine Interaction on the Root Fresh Weight of two Soybean Varieties at 6 WAP.

The interaction of G × C data from the two varieties at 6WAP also showed that, 0 – 20 Krad × 0 – 1.5 mM generally, produced comparable root fresh weight while lower root fresh weight at 6 WAP was due to (2.0 mM × 0 - 5 Krad) and (2.0 mM × 15 - 20 Krad) (Table 8).

4.13.5 Effects of Gamma Radiation and Colchicine Interaction on the Shoot Fresh Weight of two Soybean Varieties at 6 WAP.

The interaction of G × C data from the two varieties at 6 WAP also showed that, (2.0 mM × 15 Krad) treatment resulted in the highest shoot fresh weight (Table 9). However, the shoot fresh weight produced by G × C interaction was generally comparable for all treatments (Table 9).

Table 7: Effects of Gamma Radiation × Colchicine Concentration Interaction on the Root Nodule number of two Soybean Varieties at 6 WAP.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Root Nodule Number 0	0.5	1.0	1.5	2.0
0	3.50±0.22^{g}	4.67±0.49^{fg}	5.00±0.77^{fg}	5.50±0.76^{f}	13.83±4.61^{b}
5	4.67±0.49^{fg}	5.67±0.49^{f}	5.67±0.49^{f}	5.00±0.52^{fg}	12.33±3.73^{c}
10	4.50±0.43^{fg}	4.67±0.49^{fg}	5.17±0.54^{fg}	9.17±2.18^{e}	10.67±3.29^{cd}
15	4.50±0.34^{fg}	4.67±0.49^{fg}	5.67±0.92^{f}	8.83±2.50^{e}	14.50±2.26^{b}
20	5.17±0.48^{fg}	5.50±0.43^{f}	4.83±0.83^{fg}	11.17±3.23^{cd}	17.83±3.34^{a}
SE±	0.52				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

Table 8: Effects of Gamma Radiation × Colchicine Concentration Interaction on the Root Fresh Weight of two Soybean Varieties at 6 WAP.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Root Fresh Weight (g) 0	0.5	1.0	1.5	2.0
0	1.07±0.14^{def}	1.47±0.23^{b-f}	1.27±0.16^{b-f}	1.68±0.36^{a-d}	0.90±0.15^{f}
5	1.02±0.06^{ef}	1.18±0.08^{b-f}	1.15±0.08^{b-f}	2.18±0.46^{a}	0.92±0.20^{f}
10	1.58±0.36^{a-e}	1.37±0.24^{b-f}	1.32±0.20^{b-f}	1.22±0.10^{b-f}	1.73±0.27^{abc}
15	1.35±0.06^{b-f}	1.73±0.27^{abc}	1.18±0.09^{b-f}	1.28±0.17^{b-f}	1.10±0.13^{c-f}
20	1.65±0.36^{a-e}	1.77±0.41^{ab}	1.65±0.35^{a-e}	1.78±0.24^{ab}	1.10±0.16^{c-f}
SE±	0.18				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

Table 9: Effects of Gamma Radiation × Colchicine Concentration Interaction on the Shoot Fresh Weight of two Soybean Varieties at 6 WAP.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Shoot Fresh Weight (g) 0	0.5	1.0	1.5	2.0
0	2.85±0.40^{bcd}	2.42±0.25^{bcd}	3.57±0.57^{abc}	3.00±0.32^{a-d}	2.57±0.30^{bcd}
5	2.13±0.24^{d}	3.22±0.43^{a-d}	3.40±0.32^{a-d}	3.62±0.21^{ab}	2.70±0.42^{bcd}
10	2.97±0.89^{a-d}	2.97±0.36^{a-d}	3.30±0.19^{a-d}	3.10±0.18^{a-d}	2.95±0.28^{a-d}
15	3.43±0.46^{abc}	3.02±0.22^{a-d}	2.30±0.52^{cd}	2.67±0.33^{bcd}	4.13±0.63^{a}
20	3.42±0.33^{a-d}	3.38±0.38^{a-d}	2.60±0.20^{bcd}	3.40±0.14^{a-d}	3.37±0.38^{a-d}
SE±	0.37				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), ∗ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

4.13.6 Effects of Gamma Radiation and Colchicine Interaction on the Total Fresh Weight of two Soybean Varieties at 6 WAP.

The interaction of G × C data from the two varieties at 6 WAP also showed that, (1.5 mM × 5 Krad) resulted in the highest total fresh weight of soybean (Table 10). The lowest total fresh weight of soybean was due to (0 mM × 5 Krad) which is significantly lower than that of all other treatments (Table 10). The G × C interaction resulted in generally comparable total fresh weight in the soybean varieties at 6 WAP.

4.13.7 Effects of Gamma Radiation and Colchicine Interaction on the Number of Pod of two Soybean Varieties.

The interaction of G × C data from the two varieties at harvest also showed that, (1.0 mM × 5 Krad) treatment resulted in the highest number of pod (Table 11). The 5 – 20 Krad × 0 – 2.0 mM generally, produced comparable number of Pod. The lowest number of pod was due to (0.5 mM × 0 Krad) treatment (Table 11).

4.13.8 Effects of Gamma Radiation and Colchicine Interaction on the Total Grain Yield of two Soybean Varieties.

The interaction of G × C data from the two varieties at harvest also showed that, (2.0 mM × 0 - 20 Krad) treatment resulted in higher total grain yield than the other treatments (Table 12). The lowest total grain yield was due to (0 mM × 15 Krad) treatment (Table 12).

Table 10: Effects of Gamma Radiation and Colchicine Concentration Interaction on the Total Fresh Weight of two Soybean Varieties at 6 WAP.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Total Fresh Weight (g) 0	0.5	1.0	1.5	2.0
0	3.92±0.44^{bcd}	3.88±0.17^{bcd}	4.83±0.49^{abc}	4.68±0.42^{abc}	3.47±0.41^{cd}
5	3.15±0.28^{d}	4.40±0.38^{a-d}	4.55±0.39^{a-d}	5.80±0.53^{a}	3.62±0.60^{cd}
10	4.55±1.14^{a-d}	4.33±0.54^{bcd}	4.62±0.16^{abc}	4.32±0.24^{bcd}	4.68±0.27^{abc}
15	4.78±0.45^{abc}	4.75±0.41^{abc}	3.48±0.45^{cd}	3.95±0.43^{bcd}	5.23±0.59^{ab}
20	5.07±0.24^{ab}	5.15±0.63^{ab}	4.25±0.39^{bcd}	5.18±0.32^{ab}	4.47±0.46^{a-d}
SE±	0.42				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

Table 11: Effects of Gamma Radiation and Colchicine Concentration Interaction on the Number of Pod of two Soybean Varieties.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Number of Pod 0	0.5	1.0	1.5	2.0
0	8.50±1.31^{abc}	6.50±0.22^{c}	7.17±0.79^{bc}	7.17±0.54^{bc}	7.83±0.17^{abc}
5	8.67±0.61^{abc}	7.50±0.50^{abc}	9.67±1.89^{a}	8.00±0.52^{abc}	6.83±0.17^{c}
10	7.83±0.31^{abc}	7.83±0.31^{abc}	7.67±0.21^{abc}	7.33±0.33^{bc}	8.33±0.56^{abc}
15	7.67±0.42^{abc}	8.33±0.76^{abc}	8.00±0.45^{abc}	7.83±0.48^{abc}	8.33±0.49^{abc}
20	7.00±0.00^{c}	8.33±0.33^{abc}	7.33±0.33^{bc}	8.17±0.31^{abc}	9.33±0.33^{ab}
SE±	0.65				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), ∗ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

Table 12: Effects of Gamma Radiation × Colchicine Concentration Interaction on the Total Grain Yield of two Soybean Varieties.

Gamma Radiation (Krad)	Colchicine Concentration (mM) Total Grain Yield (g) 0	0.5	1.0	1.5	2.0
0	2.18±0.10^{ab}	1.82±0.05^{d-h}	1.65±0.09^{f-i}	1.65±0.16^{f-i}	2.08±0.11^{a-d}
5	1.82±0.09^{d-h}	1.70±0.10^{e-i}	1.90±0.22^{c-f}	1.72±0.11^{e-i}	2.28±0.07^{a}
10	1.55±0.10^{ghi}	1.82±0.10^{d-h}	1.53±0.06^{hi}	1.58±0.07^{ghi}	2.13±0.13^{abc}
15	1.52±0.09^{i}	1.72±0.06^{e-i}	1.63±0.09^{f-i}	1.78±0.08^{e-i}	1.83±0.09^{d-g}
20	1.77±0.08^{e-i}	1.65±0.18^{f-i}	1.70±0.14^{e-i}	1.98±0.16^{b-e}	2.22±0.13^{ab}
SE±	0.09				

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT (P≤ 0.05), ∗ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at (P≥ 0.05%).

4.14 Effects of Colchicine Concentration and variety Interaction on Germination Percentage, Plant Height 3 and 6 WAP, Root Nodules, Root Fresh Weight, Total Fresh Weight and Total Grain Yield of two Soybean Varieties.

4.14.1 Germination percentage

The germination percentage of TGX 1448-2E was significantly ($P \leq 0.05$) highest at control (0 mM) in both varieties, followed by 81.93 % at concentration 0.5 mM; while concentration 1.0 mM (70.80 %) and 2.0 mM (70.87 %) did not differ significantly ($p > 0.05$) from each other and concentration 0.5 mM. On the other hand, dose 1.5 was significantly lowest. Control (94.77 %) performed significantly ($p \leq 0.05$) good from all the test experiment of SAMSOY-2. At dose 0.5 mM (75.27 %), the germination percentage was significantly ($p \leq 0.05$) different from the remaining doses. Concentration 1.0 mM (60.80 %), 1.5 mM (56.13 %) and 2.0 mM (54.20 %) did not differ significantly ($p \geq 0.05$) from each other (Table 13). Both varieties had higher germination percentage at 0 and 0.5 mM colchicine.

4.14.2 Plant height

At 3 WAP, both varieties showed similar plant height at all colchicine treatments. The plant height of TGX 1448-2E showed that the control and all the test concentration were significantly ($p \geq 0.05$) the same. In the same way, SAMSOY-2 did not differ significantly ($p \geq 0.05$) between control and all the test experimental concentration. This showed that both varieties are comparable with each other (Table 13).

The plant height at 6 WAP was similar in both varieties under all colchicine treatments except at 0 and 1.5 mM colchicine treatments in TGX 1448-2E. Similarly, SAMSOY-2

plant height at 6 WAP did not show significant ($p \geq 0.05$) variation in all the test experiment and the control (Table 13).

4.14.3 Root nodules

The number of root nodules under most colchicine treatments was comparable in SAMSOY-2 while TGX 1448-2E showed the highest number of root nodules at 2.0 mM and the lowest under the control. SAMSOY-2 did not show significant ($p \leq 0.05$) variation in roots nodules for both control and test experiment (Table 13).

4.14.4 Root fresh weight

Variety TGX 1448-2E showed comparable root fresh weight under all colchicine treatments. SAMSOY-2 under 1.5 mM colchicine (2.47 g) recorded the highest root fresh weight compared with other treatments (Table 13).

4.14.5 Total fresh weight

The total fresh weight at 6 WAP of variety TGX 1448-2E did not exhibit significant ($p \geq 0.05$) increase in all the test concentration including control. This showed that all treatments in Variety TGX 1448-2E are comparable. However, in Variety SAMSOY-2 there was higher total fresh weight at 0.5 – 1.5 mM colchicine than the other treatments (Table 13).

4.14.6 Total grain yield

The total grain yield did not vary significantly ($p \geq 0.05$) at all concentrations and control for variety TGX 1448-2E. Generally, the result showed that all the treatments in variety TGX 1448-2E are comparable while in SAMSOY-2 variety, total grain yield at control (2.23 g), 0.5 mM (1.77 g) and 2.0 mM (2.13 g) were significantly ($p \leq 0.05$) higher than the other treatments (Table 13).

4.15 Effects of Gamma Radiation × Variety Interaction on Germination Percentage, Plant Survival 3 and 6 WAP and Days to Flowering of two Soybean Varieties.

4.15.1 Germination percentage

Table 14 is showed that there was decrease in germination percentage as the gamma radiation increase in both varieties. In variety TGX 1448-2E it ranged from (69.43 %) at 10 Krad to (90.27 %) at control but did not differ significantly ($p \leq 0.05$) from each other. In SAMSOY-2 germination percentage were in significant ($p \leq 0.05$) decreased in this order: control (94.77 %) > 5 Krad (84.70 %) > 10 Krad (73.30 %) > 15 Krad (64.50 %) > 20 Krad (55.56 %) respectively (Table 14).

4.15.2 Plant survival

The survival of plant at 3 WAP of variety TGX 1448-2E after exposure to the doses varied significantly being the highest under the control 0 Krad (4.00 %) and the lowest at 15 Krad (2.67 %). Alternatively, doses 5 Krad (3.67 %), 10 Krad (3.67 %) and 20 Krad (3.33 %) did not differ significantly ($p > 0.05$) from that of the control. The effect of exposure was slightly observed on SAMSOY-2 variety with the control recording the highest value of (4.00 %) and the lowest was at 15 Krad (2.67 %) but did not differ significantly ($p > 0.05$) (Table 14). Control survived better at 6 WAP under both varieties and was significantly ($p \leq 0.05$) higher than all the doses except dose 20 Krad (3.33 %) for variety TGX 1448-2E. The effect of radiation at doses 5 Krad (2.33 %), 10 Krad (2.67 %) and 15 Krad (2.67 %) did not varied significantly ($p > 0.05$) from each other as well as from dose 20 Krad. However, in SAMSOY-2 the control 0 Krad and 20 Krad showed the highest plant survivals while 5, 10 and 15 Krad did show significantly lower plant survival (Table 14).

Table 13: Effects of Colchicine Concentration × Variety Interaction on Germination Percentage, Plant Height 3 and 6 WAP, Root Nodules, Root Fresh Weight, Total Fresh Weight and Total Grain Yield of two Soybean Varieties.

	Colchicine Concentration (mM)				
Variety	0	0.5	1.0	1.5	2.0
Germination Percentage (%)					
TGX 1448-2E	90.27±2.77^{a}	81.93±1.37^{ab}	70.80±4.79^{b}	52.80±6.05^{c}	70.87±4.17^{b}
SAMSOY-2	94.77±0.18^{a}	75.27±1.97^{b}	60.80±0.98^{c}	56.13±3.26^{c}	54.20±3.25^{c}
Plant Height 3 WAP (cm)					
TGX 1448-2E	9.40±0.06^{a}	10.83±0.32^{a}	10.07±0.20^{a}	10.43±0.73^{a}	10.50±0.49^{a}
SAMSOY-2	11.20±1.06^{a}	10.40±0.64^{a}	10.73±0.52^{a}	11.83±0.38^{a}	11.67±0.24^{a}
Plant Height 6 WAP (cm)					
TGX 1448-2E	21.00±1.53^{bc}	22.50±0.76^{abc}	23.67±0.33^{ab}	20.00±0.58^{c}	26.00±1.53^{a}
SAMSOY-2	25.33±2.03^{a}	22.00±1.53^{a}	22.67±2.40^{a}	23.33±0.88^{a}	24.00±1.15^{a}
Root Nodules					
TGX 1448-2E	3.67±0.33^{c}	5.00±0.58^{bc}	6.67±0.33^{b}	7.00±0.58^{b}	24.00±1.53^{a}
SAMSOY-2	3.33±0.33^{a}	4.33±0.88^{a}	3.33±0.33^{a}	4.00±0.58^{a}	3.67±0.88^{a}
Root Fresh Weight (g)					
TGX 1448-2E	1.37±0.09^{a}	1.07±0.19^{a}	1.03±0.23^{a}	0.90±0.06^{a}	1.20±0.06^{a}
SAMSOY-2	0.77±0.03^{c}	1.87±0.28^{b}	1.50±0.12^{b}	2.47±0.22^{a}	0.60±0.12^{c}
Total Fresh Weight (g)					
TGX 1448-2E	4.50±0.61^{a}	3.73±0.30^{a}	4.93±0.88^{a}	4.07±0.68^{a}	4.07±0.47^{a}
SAMSOY-2	3.33±0.50^{bc}	4.03±0.17^{abc}	4.73±0.63^{ab}	5.30±0.12^{a}	2.87±0.50^{c}
Total Grain Yield (g)					
TGX 1448-2E	2.13±0.09^{a}	1.87±0.03^{a}	1.73±0.09^{a}	1.87±0.29^{a}	2.03±0.18^{a}
SAMSOY-2	2.23±0.19^{a}	1.77±0.09^{ab}	1.57±0.17^{b}	1.43±0.03^{b}	2.13±0.18^{a}

Note: Means followed by the same letter(s) in each row, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

4.15.3 Days to flowering

The days to flowering for variety TGX 1448-2E ranged from (42.33) at control and dose 20 Krad to (49.33) at 10 Krad but did not differ significantly ($p \geq 0.05$) from each other. Similarly, the variation in days to flowering of SAMSOY-2 variety ranged from (44.67) at to 10 Krad to (50.33) at 20 Krad but they were significantly ($p > 0.05$) similar. Hence, all the treatments in both the two varieties are comparable with each other (Table 14).

4.16 Chimera Induction

The mutations manifested as a result of the combined use of gamma radiation and colchicine was mostly noticed at the seedling stage. Consequently some were affected and some abnormalities were manifested. The chlorophyll mutants observed were Chlorina, Striata, Virescent, and Lustescent.

Chlorina was characterized by yellow green colouration of the leaves, these stripes may be yellow or white in colour (Plate1). Striata is a chlorophyll deficient mutant characterized by a persistent, yellow longitudinal stripe (Plate 2).There were also the Virescent whose leaves appeared first as yellow and gradually turned green (Plate 3 and 6) and finally Lustescent with green colour initially but the pigment got destroyed or dried with time (Plate 4 and 5) Among the chlorophyll deficient mutants manifested, Chlorina and Lustescent were found to be most frequent generally.

It was also observed that the combination of 2 mM and 20 krad manifest the most frequent number of the chlorophyll deficient mutation in TGX 1448-2E and SAMSOY-2 (13mutant and 11mutant). The least number was produced by 1.5 mM and 15 krad (8 and 7).

Table 14: Effects of Gamma Radiation × Variety Interaction on Germination Percentage, Plant Survival 3 and 6 WAP and Days to Flowering of two Soybean Varieties.

	Gamma Radiation (Krad)				
Variety	0	5	10	15	20
Germination Percentage (%)					
TGX 1448-2E	90.27±2.77^{a}	84.70±1.40^{a}	69.43±12.36^{a}	81.93±3.67^{a}	72.23±3.67^{a}
SAMSOY-2	94.77±0.18^{a}	84.70±1.40^{b}	73.30±0.93^{c}	64.50±1.51^{d}	55.57±2.49^{e}
Plant Survival 3 WAP (%)					
TGX 1448-2E	4.00±0.00^{a}	3.67±3.33^{ab}	3.67±3.33^{ab}	2.67±3.33^{b}	3.33±3.33^{ab}
SAMSOY-2	4.00±0.00^{a}	3.00±0.58^{a}	3.00±0.58^{a}	2.67±3.33^{a}	3.33±3.33^{a}
Plant Survival 6 WAP (%)					
TGX 1448-2E	4.00±0.00^{a}	2.33±3.33^{b}	2.33±3.33^{b}	2.67±3.33^{b}	3.33±3.33^{ab}
SAMSOY-2	3.67±0.33^{a}	2.00±0.00^{c}	2.00±0.00^{c}	2.67±3.33^{bc}	3.33±3.33^{a}
Days to Flowering					
TGX 1448-2E	42.33±0.33^{a}	43.67±0.33^{a}	49.33±4.33^{a}	44.67±1.45^{a}	42.33±0.33^{a}
SAMSOY-2	46.33±0.67^{a}	45.33±0.88^{a}	44.67±0.88^{a}	46.00±2.52^{a}	50.33±2.91^{a}

Note: Means followed by the same letter(s) in each row, are not significantly different using DMRT ($P \leq 0.05$), $*$ - Significant at (5%), **- Highly Significant at (1%), ns- non significant at ($P \geq 0.05$%).

In SAMSOY-2 treated seeds, the chlorophyll deficiency was slightly different. lustescent were found to have higher frequency than Virescent furthermore, lustescent was most frequently manifested in SAMSOY 2 variety. There was no Chlorina and Striata observed out of mutants manifested. Similarly, TGX 1448 2E treated seeds, Chlorina, Lustescent and Striata were observed.

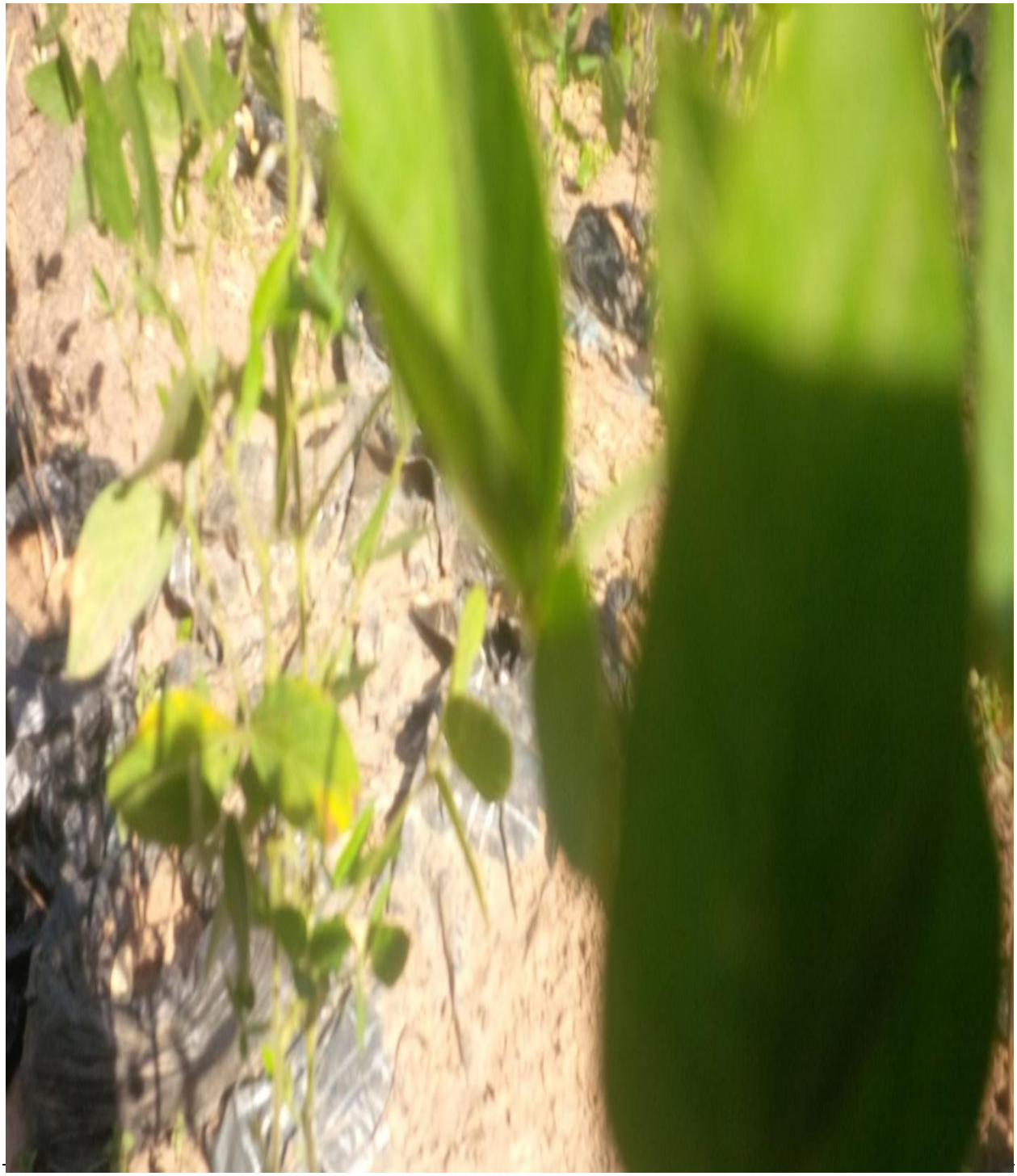


Plate 1: Chlorophyll deficient leaves of Soybean variety TGX 1448-2E plants at 6WAP under 2 mM + 20 Kradtreatment.

TGX 1448-2E: Chlorina- A chlorophyll deficient mutant, characterized by a yellow-green colouration of the leaves



Plate 2: Chlorophyll deficient leaves of Soybean variety TGX 1448-2E plants at 5WAP under 1.5 mM + 15 Kradtreatment

TGX 1448-2E: Striata- A chlorophyll deficient mutant, characterized by a persistent, yellow longitudinal stripe.



Plate 3: Chlorophyll deficient leaves of Soybean variety TGX 1448-2E plants at 6WAP under 2 mM + 20 Kradtreatment.
TGX 1448-2E: Virescent- A chlorophyll deficient mutant in which leaves appear first as yellow and gradually turned green.



Plate 4: chlorophyll deficient leaves of Soybean variety TGX 1448-2E plants at 5WAP under 1.5 mM + 15 Kradtreatment.

TGX 1448-2E: Lustescent- A chlorophyll deficient mutant in which leaves appear green initially but the pigments got destroyed or dried with time.



Plate 5: Chlorophyll deficient leaves of Soybean variety SAMSOY 2 plants at 6WAP under 2 mM + 20 Kradtreatment.

SAMSOY 2:Lustescent- A chlorophyll deficient mutant in which leaves appear green initially but the pigments got destroyed or dried with time.



Plate 6: Chlorophyll deficient leaves of Soybean variety SAMSOY 2 plants at 5WAP under 1.5 mM + 15 Kradtreatment.

SAMSOY 2: Virescent- A chlorophyll deficient mutant in which leaves appear first as yellow and gradually turned green.

CHAPTER FIVE

5.0 DISCUSSION

Variability in crop plants is brought about by different factors, which may include spontaneous and manifest mutations. The effects are observable morphologically or cytologically. The present study took into cognizance, such traits induced by some mutagens (gamma ray, colchicine concentration and a combination of the two).

Percentage germination was highly significant in all the treatment studied ($P \leq 0.01$) except in the interaction of V×G was significant at ($P \leq 0.05$). Percentage germination was reduced by colchicine and gamma radiation doses in the two varieties of Soybean (TGX 1448-2E and SAMSOY-2) used in this study. This reduction was found to be dose-dependent. As the dose increased, seed germination decreased. This may be attributed to high frequency of induced deletions or translocations (Adamu *et al*., 2004; Mensah *et al*., 2007; Adelanwa *et al.*, 2012 and Mathew, 2014). The result of the present study agrees with those of Khan and Wani (2005), who also reported adverse effects of radiation and chemical mutagens on seed germination in Mungbean.

Plant survival was highly significant in all the treatment studied ($P \leq 0.01$) except in the interaction of V×C, C×G and V×G was significant at ($P \leq 0.05$). Plant survivals were also found to decrease as the treatment dose increased in both varieties treated with gamma rays and colchicine. The result was in conformity with the work of Mensah *et al.* (2007), Shugufta (2012) and Mathew (2014) who reported that, there were dose related effects of mutagenic treatment on qualitative traits resulting in reductions of survival percentage. Adamu *et al*. (2002) made the same observation in groundnut.

The height of Soybean varieties is not significant to all the treatment except in variety, C, G, V×C in plant height at 3 WAP which is highly significant ($P \leq 0.01$) and V×C in plant height at 6 WAP which is significant at ($P \leq 0.05$), the height of the plant increased considerably with dose of gamma rays, colchicine and their combination. However, there were significant increases in the height of the treated plants than their respective controls. The increase in plant height in the present study might be due to the injurious effects of colchicine concentration combined with the gamma doses which led to increased cellular proliferation or cell division at cellular level. This is in conformity with the work of Lysenkov (1989) who induced a wide range of viable mutants and observed increase in wheat height. A similar result was reported by Adamu *et al*. (2002) when they irradiated the seeds of five varieties of groundnut (*Arachis hypogaea*) with gamma rays Cobalt 60 (^{60}Co) source.

Days to flowering and leaf number are not significantly different to all the treatments except variety that is highly significant in days to flowering and significant in interaction of V×G, where as, leaf number is significant at variety level. The application of gamma radiation and colchicine increased days to flowering and leaf number slightly higher in each variety compared with the control. This finding is in contrast to what Mensah *et al*. (2007) and Mathew (2014) reported when they observed that increase in colchicine concentration and para-dichlorobenzene decreased days to flowering and increase leaf number. The increase in days to flowering and leaf number in the present study might be due to inhibitory effects of the mutants and physiological changes in the plant as a result of delayed cellular division prior to flowering and reproductive stage especially at higher doses and concentration of gamma radiation and colchicine respectively. This type of

contradiction might be due to either difference in the genetic material used or difference in agro-climatic condition under which the experiment was conducted

Applications of gamma radiation and colchicine increased the number of root nodules slightly higher in each variety suggesting that this trait was also dose dependent. This may be as a result of the effectiveness of the radiation dose, which usually has adverse effect on biological materials as the radiation intensity increases (Khan and Wani, 2005).

Application of gamma radiation and colchicine led to slight increase in root fresh weight, shoot fresh weight and fresh weight of soybean compared with the control. This gives an indication of proper adaptation to the effect of the mutagens, though the root fresh weight, shoot fresh weight and fresh weight was observed to be higher than the control. This finding is in consonan with earlier findings of Singh *et al*. (2001) who reported that increased in fresh weight was also dose dependent.

Applications of gamma radiation and colchicine generally had no significant effect on numbers of pod but highly significant in variety at ($P \leq 0.01$) were in combination of C×G is significant at ($P \leq 0.05$) and 100 seed weight is not significant but highly significant in variety and colchicine compared with the control. The pod dry weight was not significant in the interaction but highly significant at variety and colchicine ($P \leq 0.01$) were gamma radiation at ($P \leq 0.05$). The Increase in colchicine concentration and gamma radiation led to increase in pod number and 100 seed weight in Soybean varieties in most cases than their respective controls. This is in conformity with the work of Abdi *et al*. (2007), Mathew (2014), Wani and Khan (2006) who reported similar increase in number of pod,

100 seed weight, pod dry weight and also decrease in pod dry weight in *Vigna radiata* (L).

Applications of gamma radiation and colchicine generally had no significant effect in all the treatment except on variety that is highly significant on the crude protein content compared with the control. However, application of gamma radiation and colchicine is highly significant on all the treatment ($P \leq 0.01$), the interaction of V×G and C×G is not significant on the total grain yield compared with the control. The findings of Mathew (2014) and Wani and Khan (2006) were in agreement with those of this study when they stated that with increase in Sodium azide and gamma radiation on *Cajanus cajan* crude protein was not significantly effected, unlike grain yield.

Chlorophyll mutations, according to Khan and Tyagi (2009), are common occurrence after mutagenic treatments. In this research, four different chlorophyll-deficient mutants were manifested. These include chlorina, striata, virescent and lustescent. The Gamma rays doses and colchicine concentrations differed with respect to the spectrum and frequency of chlorophyll mutations induced in this study. Giri and Apparao (2011) observed different chlorophyll mutants like chlorina, xantha, albina and striata in pigeon pea (*Cajanus cajan*L.) after treatments with Ethyl-methyl sulphate. Differential behaviour of the mutagens to produce specific types of chlorophyll mutants may be attributed to their mutagenic effectiveness (Neuffer, 1982 and Adamu *et al*., 2002). The pigment that may be found in most cereal chloroplastids are chlorophyll and carotenoid. (Adamu *et al*., 2002). As such, a breakdown in one or both of these pigments may result in the formation of any of the chlorophyll deficient mutants.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The seeds of two varieties of Soybean (TGX 1448-2E and SAMSOY-2) were exposed to five treatments (0, 5, 10, 15, and 20Krad) of gamma rays and five colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0mM). Combinations of these two mutagens were also used to treat the two soybean varieties. The treatments were laid out in a complete randomized design. The plants were sampled and the results obtained indicated a varying degree of effects in response to the mutagenic treatments. The higher doses of gamma rays (15Krad and 20Krad) were particularly lethal, as there were no seedling emergence in all the two varieties used in this research. Similarly, there was decrease in percentage germination with increase in colchicine concentrations observed in treated seeds. The combination of the two mutagens gave a significant decrease in percentage germination, plant survival, shoot fresh weight, fresh weight and pod dry weight.

The results also revealed that the treatment doses in the gamma rays were effective for seedling percentage germination, survival rate and height of the plant. The result was highly significant for percentage germination and survival rate.

The results obtained also revealed that some chlorophyll deficient mutants were manifested. Among these are chlorina, striata, virescent, lustescent are produced by TGX 1448-2E and SAMSOY-2 produced Lustescent and virescent types. In conclusion, the impact of mutagens on both vegetative and yield parameters of soybean varieties varied

with the level of mutagens. TGX 1448-2E was shown to be the most responsive to the treatment doses than SAMSOY-2 variety.

6.2 Conclusions

The findings of this study give insight into the usefulness of gamma rays and colchicine concentration in inducing mutants that are both beneficial and deleterious or even lethal. The conclusions drawn from this study therefore are as follows:

- Seed germination in soybean varieties decreases with increase in colchicine concentration and gamma radiation doses with 2.0mM concentration and 20Krad dose, respectively, as the most deleterious.

- Low doses or concentrations of the mutagens (0.5 and1.0 mM; 5 and 10 Krad) were highly effective in inducing mutant improvements in traits such as percentage germination and survival rate.

- Chlorophyll deficient mutants: Chlorina, striata, Iustescent and virescent were manifested by both gamma radiation doses and colchicine concentrations.

6.3 Recommendations

Based on the results obtained, the following recommendations are put forward, since the overall results suggest that low doses of both the gamma rays and colchicine concentrations gave superior performance in the two Soybean varieties when compared to the higher doses. In view of this result, it is recommended that:

- The low doses of the two treatments can be employed to induce high grain yield and survival rate in this crop plant and give superior performance.
- It is also recommended that further work be carried out using low doses and concentrations of gamma rays and colchicine concentration, respectively, to determine their effects in inducing resistance against diseases in Soybean.
- The outcome of this study could be beneficial to the scientific community especially in the application of the knowledge in the production of improved varieties of soybean with increased grain yield and superior performance of the varieties to the local farmers.

REFERENCE

Abdi, S., Fayaz, M. A. and Chadimzade, M. (2007). Effect of Different levels of Mutagens at Reproductive stage on grain yield and oil percent of two hybrid Sunflower. *Agricultural Natural Resources, Science and Technology*, **11**:225-245.

Adamu, A. K. (1997). Preliminary investigation on the influence of Gamma cobalt 60 (^{60}Co) and thermal Neutrons irradiation on tiller, ear production, male sterility and maturity in pop corn (*Zea mays* L. cultivars praecox) *Nigerian Journal of Botany*, **10**: 51-59.

Adamu, A. K., Olorunju, P. E., Bate, J. A. and Ogunlade, O. T. E. (2002). Radio sensitivity and effective dose determination in Groundnut (*Arachis hypogaea* L.) Irradiated with Gamma Rays (Cobalt 60) sources. *Journal of Agricultural and Environment*, **3**(1): 71-84.

Adamu, A. K. (2004). Gamma rays (^{60}CO) and thermal neutrons induced mutants in popcorn (*Zea mays* L. variety *praecox sturt*). *Nigerian Journal of Scientific Research*, **4**:52-63.

Adamu, A. K., Chung, S. S. and Abubakar, S. (2004). The effects of ionizing radiation (Gamma-Rays^{60}Co) on Tomato (*Lycopersicon esculentum* L.). *Nigerian Journal of Applied Biology*, **5** (2): 85-93.

Adelanwa, M. A., Habeeb, M. L. and Adelanwa, E. B. (2012) Morphological studies of the effect of colchicine and paradichlorobenzene on Tomato (*Lycopersicon esculentum* L.). *Journal of Environmental Issues and Agriculture in Developing Countries*, **3** (2):122-127

Ahloowalia, B. S. (2007). Induced mutation –A new paradigm in plant breeding, *International Journal of Toxicology and Applied Pharmacology*, **118**:167-173.

Association of Official Analytical Chemists (A.O.A.C.) (1980). *Official methods of analysis*. 13th edition Association Official Analysis Chemist, Washington D.C.

Baoge, Z., Aigiu, G., Xiangdoug, D., Yuxuan, G. and Zixian, L. (1995). Effects of caffeine post treatment on Ethyl-MethanneSulphate mutagenesis in soybean mutation resources, *Frontier Agriculture in China*, **334**: 157-159.

Baohui, S., Mary, A. M. and Shuang, X. (2006)."Competitive Analysis of Chinese Soybean Import Suppliers-U.S., Brazil, and Argentina." .*American Agricultural Economics Association Annual Meetings*, **2**: 17-29.

Bate, J. A. (1997). The effects of ionizing radiation on groundnut (*Arachis hypogaea*) in northern Nigeria. Unpublished B.Sc. degree project, Department of Biological Sciences. Ahmadu Bello university, Zaria Nigeria, 23p$_{p.}$

Bernard, R. L. (2005). Microsoft Encarta Encyclopedia (Soybean) 2008. *Journal of Environmental Issues and Agriculture in Developing Countries*, **3** (2):122-130.

Biswas, A. K. and Datta, A. K. (1988). Induced mutation in two *Trigonella* species Bangladesh, *Journal of Botany*, **17**: 221-214.

Borkar, A. T. and More, A. D. (2010). Induced flower colour mutation in *Phaseolus vulgaris* (L.) through physical and chemical mutagens. Advances in Biological Research, **1**: 22-28.

Bragal, S. E. (1955) Dry Matter Accumulation and yield in Potato as affected by Mutagens. *Journal of the Indian Potato Association*, **28**: 251-255

Dean, I. (1959). Hybridization between amphiploids and the evolution of polyploids in the spiderwort plant (*Tradescantia ohioensis*). Evolution, **6**: 56-59

Dethloff, L., Barr, B., Bestervelt, L., Bulera, S., Sigler, R., Lagattuta, M. and De La Iglesia, F. (2000)."Gabapentin-Induced Mitogenic Activity in Rat Pancreatic Acinar Cells". *Toxicological Sciences*, **55** (1): 52–59.

Dugje, I. Y., Omoigui, L. O., Ekeleme, F., Bandyopadhyay, R., Kumar1, P. L. and Kamara, A.Y. (2009).Soybean Production in Northern Nigeria. *Journal of Agriculture and Environmental Issues*, **7** (9): 324-337.

Food Agricultural Organization (2011). Production of Crops in Vienna. https:www.google.com/Values of Soybean. Retrieved February 18, 2015.

Food Agricultural Organization (2005). Nuclear Techniques and invitro culture for plant Improvement. *Proceedings of Plant Mutation for Crop Improvement*, Vienna, 529-698Pp.

Food Agricultural Organization (2012). *Cassava Production Statistics*. Vienna. https:www.google.com/uses of Soybean crop. Retrieved February 18, 2015.

Gepts, P., Beavis, W. D., Brummer, E. C., Shoemaker, R. C., Stalker, H. T., Weeden, N. F. and Young, N. D. (2005). Legumes as a model plant family. Genomics for food and feed report of the cross-legume advances through genomics conference. *Plant Physiology*, **137**: 1228–1235.

Giri, S. P. and Apparao, B. J. (2011). Studies on effectiveness and efficiency of ethyl-methannesulphate in pigeon pea. *Bioscience Discovery*, **2** (1): 55-98.

Girija, M. and Dhanavel, D. (2009). Mutagenic effectiveness and efficiency of gamma rays, ethylmethanesulphonate and their combined treatments in cowpea (*Vigna unguiculata* (L.)Walp) *Global Journal of Molecular Science*, **4**: 68–75.

Gordon, V., Richard, S. and Randy, M. (2007). *The principles of Botany*. Mc Graw Hill Campanus, 460p$_{p.}$

Graham, P. H., and Vance, C. P. (2003). Legumes: importance and constraints to greater use. *Journal of Plant Physiology,* **131**: 872 – 877.

Henkel, J. (2000). "Soya Health Claims for Soy Protein, Question About Other Components". *FDA Consumer* (Food and Drug Administration), **34** (3): 18–20.

Herbert, A., Cathy, H. and Eric D. (2009)."Corn Earworm Biology and Management in Soyabeans."Virginia Cooperative Extension, Virginia State University, Virginia.

International Institute of Tropical Agriculture (IITA) (2009). Soybean Production in Northern Nigeria. *Farmers' Guide to Nigeria*, **29**: 32-46.

Khan, S. and Wani, M. R. (2005). Induced variation for quantitative traits in mungbean. *India Journal of Applied and Pure Biological sciences*, **20**: 55-58.

Khan, S., Wani, M. R., Bhat, M. and Parveen, K. (2005). Induced chlorophyll mutations in chickpea (*Cicera rietinum* L.). *International Journal of Agriculture and Biological Sciences*, **7**: 764-767.

Khan, M. H. and Tyagi, S. D. (2009). Cytological effects of different mutagens in soybean (*Glycine max* (L.) Merrill). *Frontier Agriculture in China*, **3**: 397- 401.

Khan, Z., Gupta, H., Ansari, M. Y. K. and Chaudhary, S. (2009). Methyl-methane sulphonate induced chromosomal variations in a medicinal plant *Chichorium intybus* (L.) during microsporogenesis. *Biology and Medicine,* **1** (2): 66-69.

Khan, M. H. and Tyagi, S. D. (2010). Studies on effectiveness and efficiency of gamma rays, Ethyl-methannesulphate and their combination in soybean (*Glycine max* (L.) Merrill). *Journal of Plant Breeders Crop Science*, **2**: 55-58.

Khejahl method (1990). Routine Analysis of Soil. *Journal of Agronomy,* **43**:434-438.

Kumar, S. and Dubey, D.K. (1998). Influence of separate and simultaneous application of gamma rays and Ethyl-methannesulphate on meiosis in khesari (*Lathtyrus sativus* (L.)) *Journal Genetic and Breeding*, **52**:295-300.

Lim, T. K. (2012). "*Glycine max*". *Edible Medicinal and Non-Medicinal Plants*. Dordrecht Springer, China, 714p_p.

Lysenkov, V. I. (1989). Experimental production of mutants of winter breeds wheat, as breeding material. *Vavilour,* **187**: 17-20

Mathew, B. A. (2014). Morphological and Cytogenetics studies on the effect of Sodium Azide and Gamma irradiation treatments on Pigeon pea (*Cajanus cajan* (L.) Millspaugh). Unpublished MSc. thesis, Department of Biological Sciences. Ahmadu Bello University, Zaria, Nigeria, 78$p_{p.}$

McBride, M. B., Richards, B. K., Steenhuis, T. and Spiers, G (2000)."Molybdenum Uptake by Forage Crops Grown on Sewage Sludge-Amended Soils in the Field and Greenhouse" (PDF). *Journal of Environmental Quality*, **29** (3): 848–854.

McClean, P. (1999). Gene and Mutation. *Journal of Agricultural Science,* **12**: 200-225.

Mensah, J. K.,Akomeah, P. A. and Obadoni, B. (2005). Effects of colchicine on yield parameters of Guinea corn (*Sorghum bicolor* (L.) Moench). *African journal of Biotechnology*, **4**: 48-61.

Mensah, J. K. and Obadoni, B. (2007). Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.) *African Journal of Biotechnology*, **6** (6): 668-671.

Mensah, J. K., Obadoni, B. O., Akomeah, P. A., Ikhajiagbe, B. and Ajibohi, I. (2007). The effects of sodium azide and colchicine treatments on morphological and yield traits of *Sesame* seed (*Sesame indicum* L.). *African Journal of Biotechnology*, **6** (5): 534-538.

Montelone, B. A. (1998). *Mutation, Mutagens and D N A Repairs*. PhD Dissertation Division of Biology, Kansas State University, Kansas, Pp 11.

Multilingual (2012). Multiscript Plant Name Database. *Texas Agilife Extension Service*. https:www.wikipedia.com/Data of Soybean. Retrieved February 16, 2015.

National Soyabean Research Laboratory (2012). Soybean Benefits. https:www.google.com/Benefit of Soybean. Retrieved February 16, 2015.

Neuffer, M. C. (1982). Mutant induction in maize. In: Shenden, W.F. (Ed.) Maize for Biological research. *Plant Molecular Biology Association*. Charlottes Ville, CRC Press, **5**: 61-64.

Neuffer, M. C. (1988). The role of induced mutation in the improvement of common beans (*Phaseolus vulgaris* L.). *Mutation breeding newsletter*, **44**: 6-8.

Odeje, S. C. (2008). Effects of Gamma rays and Sodium Azide on three varieties of Guinea corn (*Sorghum bicolor* (L.) Moench).Unpublished M.Sc. thesis, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, 124$p_{p.}$

Osuhor, C. U., Tanko, R. J., Dung, D. D., Mohammed, I. R. and Odunze, A. C. (2004). Water consumption of Yankasa. Ramsal Federal and basal diet of maize stower-lab mixture. *Pakistani Journal of Nutrition* **3** (3): 154-157.

Pathak, R. S. (1991). Generic evaluation of two aphid-resistant cowpea mutants. *Kenya IAEA Symposium on Plant Mutation Breeding for Crop Improvement*, Nairobi. Pp. 17-24.

Ranch, J. (2001). *Mutation Breeding*. GENTECH Archives India, Pp.50.

Reddy, T. Y., Reddy, V. R. and Anbumozhi, V. (2003). Physiological responses of Groundnut (*Arachis hypogaea* (L.)) a critical review. *Plant Growth Regulation*, **41**:75-88

Riaz, M. N. (2006). *Soya Applications in Food*. CRC Press Boca Raton, FL.

Robert, G. (2000). *Chemical mutagens*. Blackwell Science, United Kingdom, p_p179.

Sarkar, F., Banerjee, S. and Li, Y. (2007)."Pancreatic Cancer: Pathogenesis, Prevention and Treatment". *Toxicology and Applied Pharmacology*, **224** (3): 326–336.

SAS Institute, (2004). SAS/STAT User's Guide Version 8.2 edition: Statistics. SAS Institute Incorporation., Cary, NC.

Shugufta, B. (2012). Studies on the Induction of Mutations in Fenugreek (*Trigonella foenum-graecum* (L.) PhD. Dissertation, University of Kashmir, India.

Sidi, H. (2006). Effect of Ethyl-methannesulphate and sodium azide on legume. Karachi Press, Karachi, pp 13.

Sidi, H., Tila, M. and Jabbar, A. (2006). Effect of Gamma rays and sodium azide on some yield components of wheat. Karachi, **2**: 27-28.

Singh, M., Joshi, A. K. and Singh, V. P. (2001). Genetic analysis of certain mutant lines of urdbean for yield and quality traits in M4 generation. *Indian Journal of Pulses Research*, **14** (1): 60-62.

Singh, S., Richharia, A. K. and Joshi, A. K. (2006). An assessment of gamma ray induced mutations in rice (*Oryza sativa* L.). *The Indian Journal of Genetics and Plant Breeding*, **58** (4): 455-463.

Singh, V. P. and Chaturvedi, S. N. (1980). Mutagenic efficiency of Ethyl-methannesulphate and gamma rays and their combined treatments in *Vigna radiata* (L.) *Journal of Plant Science*, **34**: 331-348.

Taylor, D. J., Green, N. P. O., Stout, G. W. (1997). *Biological Sciences*. Mc Graw Hill, United Kingdom, Pp. 890-897.

Tesfaye, T., Gebisa, E., Arun, C., Chia-Ping, H., Argung, T., Mammadou, L., Axtell, J. D. and Hamaker, B. R. (2006). A Novel Modified Endosperm Texture in a Mutant High Protein Digestibility/ High Lysine Grain Sorghum (*Sorghum bicolor* (L.) Moench).*Cereal Chemistry*, **83** (2): 194-201.

UNO/FAO (2000). Improvement and Production of maize, *Sorghum* and millet. *Journal of Genetic Breeding*, **10**: 245-256.

United Nation Food Agricultural Organization (2012). Improvement and Production of maize, *Sorghum* and millet. *Journal of Applied and Pure Sciences* California, **10**: 245-256.

United States of America Food and Drug Administration (USAFDA) (2012). *Soybean Production*. United States Department Agency, Washington DC, Pp157.

Uno, I., Odeigah, P. G., Osaryinpeju, A. O. and Myers, G. O. (2007). Induced male Sterility in cowpea (*Vigna unguiculata* L. walp). *Journal of Genetic Breeding*, **50**: 171-176.

Wani, M. R., Khan, S. and Parveen, K. (2005). Induced variation for quantitative traits in mungbean. *India Journal of Applied and Pure Biological Sciences,* **20**: 55-58.

Wani, M. R. and Khan, S. (2006). Estimates of genetic variability in mutated populations and the scope of selection for yield attributes in (*Vigna radiate* (L.) Wilczek). *Egyptian Journal Biological sciences*, **8**: 1-6.

Wani, M. R. (2007). *Studies on the Induction of Mutations in Mungbean* (*Vigna radiata* (L.) Wilczek). Ph.D. Thesis, Aligarh Muslim University, Aligarh.

Weiss, E. A. (2012). *Oil seed crops*. In: Willey, A. (Ed.). Requirement for Soybean growth. Blackwell Science, London. pp. 384.

Wenzel, G. O. (2000). Comparison of cells. *Allium sativum* L. *Journal of Applied genetics*. **27**:23-28.

Wenzel, G. O., Schieder, T., Prezewonzny, Y. K., Sopory, Y. and Melchers, G. (2000). Comparison of single cell culture derived. *Solanum tuberosum* L. *Indian Journal of Agricultural Sciences,* **55**:49-55

Wolf, W. J. (2012). "Legumes: Seed Composition and Structure, Processing Into Protein Products and Protein Properties." *World Journal of Agricultural Sciences,* **6** (10): 291–314.

World, S. P. (2010). World Soybean Production. *World Journal of Agricultural Sciences*, **4** (8): 896-900.

World Market (2012). Growing Crush Limits India's Soya Oil Imports. *World Markets and Trade*. United States Department of Agriculture. https:www.google.com/Oil values of Soybean. Retrieved February 17, 2015.

Zargar, G. H., Accra, N. A., Hanger, H. U and Wan, S. A. (1994). Mutagenic effects of gamma rays, ethyl-methannesulphate and their combination in top-onion (*Allium cepa* variety visiparum). *Journal of Nuclear Agricultural Biology,* **23** (4): 249-250.

Appendix 1: Analysis of variance for the Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Growth Parameters of two Soybean Varieties.

SOV	DF	Germination Percentage (%)	Plant Survival (3 WAP) (%)	Plant Survival (6 WAP) (%)	Plant Height (3 WAP) (cm)	Plant Height (6 WAP) (cm)
Variety	1	5974.93**	4.50**	3.22**	60.92**	NS
$C_{(mM)}$	4	2476.45**	5.41**	7.30**	2.75**	NS
$G_{(Krad)}$	4	874.80**	3.06**	0.79**	2.36**	NS
V×C	4	191.53**	NS	NS	1.79**	15.37*
V×G	4	163.10*	0.87*	0.81**	NS	NS
C×G	16	253.54**	NS	1.19**	NS	NS
Error	116					

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).
Keys: SOV= Source of Variation, DF= Degree of Freedom.

Appendix 2: Analysis of variance for the Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Growth Parameters of two Soybean Varieties.

SOV	DF	Root Nodules	No. of Leaves	Root weight (g)	Shoot weight (g)	Total weight (g)
Variety	1	1187.22**	3.22*	NS	12.32**	6.44**
$C_{(mM)}$	4	452.89**	NS	1.02**	NS	NS
$G_{(Krad)}$	4	29.47**	NS	NS	NS	NS
V×C	4	259.87**	NS	NS	NS	5.52**
V×G	4	NS	NS	NS	NS	NS
C×G	16	14.79**	NS	0.55*	1.75**	2.78**
Error	116					

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).
Keys: SOV= Source of Variation, DF= Degree of Freedom.

Appendix 3: Analysis of variance for the Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Yield Parameters of two Soybean Varieties.

SOV	DF	Days to Flowering	Number of Pods	Pod weight (g)	100 Seeds weight (g)	Grain yield (g)	Crude protein (%)
Variety	1	319.74**	20.16**	5.45**	647.71**	2.33**	13.95**
$C_{(mM)}$	4	NS	NS	2.28**	5.63**	0.87**	NS
$G_{(Krad)}$	4	NS	NS	0.58*	NS	0.24**	NS
V×C	4	NS	NS	NS	NS	0.27**	NS
V×G	4	33.14*	NS	NS	NS	NS	NS
C×G	16	NS	3.97*	NS	NS	NS	NS
Error	116						

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).
Keys: SOV= Source of Variation, DF= Degree of Freedom.