

**UNIVERSITY FOR DEVELOPMENT STUDIES**

**FACULTY OF AGRICULTURE**

**DEPARTMENT OF AGRONOMY**

**EFFECTS OF COBALT-60 GAMMA IRRADIATION ON GROWTH  
AND YIELD OF PEARL MILLET (*Pennisetum glaucum* L.) IN THE  
GUINEA SAVANNAH AGROECOLOGICAL ZONE OF GHANA**

**SALIFU BABA**

**2015**



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AGROECOLOGICAL ZONE OF GHANA**

**BY**

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**(UDS/MCS/0010/13) THESIS SUBMITTED TO THE DEPARTMENT  
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OF MASTERS OF PHILOSOPHY DEGREE IN CROP SCIENCE**



**2015**

### DECLARATION

I hereby declare that this is the result of my own work and that no previous submission has been made in this university or elsewhere for a degree. References made therein are duly acknowledged.

SALIFU BABA (STUDENT)



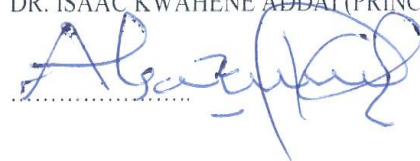
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I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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## ABSTRACT

Millet remains a neglected and under-utilized crop with low yields under the local environmental conditions. Four experiments namely Experiment I ( $M_1$  generation/dosage response study), Experiment II ( $M_1$  generation/field study), Experiment III ( $M_2$  generation) and Experiment IV ( $M_3$  generation) were carried out at the University for Development Studies, Tamale, Ghana, from June to July, 2014; August to November 2014; December 2014 to March 2015 and May to July 2015, respectively. Treatments used in Experiment I were made up of 7 gamma ray doses; 100, 200, 300, 400, 500, 600, 700 Gy and a control (0 Gy). Experiment

(dosage response study) was conducted to assess the sensitivity of pearl millet variety Naara to gamma irradiation. Parameters measured were seed germination percentage, seedling survival and seedling height. Results of the study indicated that gamma ray doses 400, 500, 600 and 700 Gy reduced seed germination and were lethal as they resulted in more than 50% reduction in seedling survival. Hence these doses were eliminated from subsequent experiments. The  $LD_{50}$  was predicted to be 309 Gy. In the  $M_1$ ,  $M_2$  and  $M_3$  studies, the effect of gamma irradiation on growth and yield of pearl millet variety Naara were investigated. Parameters measured were seed germination, plant survival, plant height, number of tillers and productive tillers, earliness to flowering, head length, width and weight, 100 seed weight and grain yield. The 100 Gy predominantly produced plants with higher values in almost all parameters measured in all the generations. Though, 200 and 300 Gy gamma ray doses performed less than the control in some parameters measured in the  $M_1$  generation, their performance subsequently



increased progressively in the M2 and M3 generations. In the M3 generation, all gamma ray doses improved all parameters measured, however, plant height and number of tillers were decreased by 100 Gy gamma ray dose. The study therefore recommends that promising lines selected from the M3 generation be advanced to further generations. Studies in the nearest future generations should be broadened to include nutritional and molecular analysis of selected promising mutant lines.



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To all I say God bless you.



## **DEDICATION**

This work is dedicated to my parents, Mr. Yahaya Salifu and Mrs.Salamatu Yahaya



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$w = \frac{c}{7f}$  (Equation 3) ..... 47



## LIST OF ABBREVIATIONS

BSTID	Board on Science and Technology for International Development
NRC	National Research Council
ECARSA	Eastern and Central Africa Regional Sorghum and Millet
M FAO	Food and Agriculture Organization of the United Nations
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
ILO MoFA	International Labour Office
	Ministry of Food and Agriculture
N A S	National Academy of Science
N P C	National Population Council
SADC	Southern Africa Development Community
SAFGRAD	Semi-Arid Food Grains Research and Development
SARI	Savanna Agricultural Research Institute
SRID	Statistics Research and Information Directorate
	UN United Nations Secretariat
WAP	Weeks After Planting
WFP	World Food Program



## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

Millet represents a highly valuable and diverse group of cereal and forage crops that typically produce small seeds. They are widely grown around the world as cereal crops or grains for both human food and fodder (Kannan *et al.*, 2014; Kholova and Vadez, 2013; Sumathi *et al.*, 2010). Millet is an important cereal crop in the semi-arid tropics of Asia and Africa, with 97% of its production occurring in developing countries (McDonough *et al.*, 2000). Millets are distinctive in their adaptability to adverse agro ecological conditions and requires minimal agronomic inputs, with good nutritional properties (Bashir *et al.*, 2011; Subi and Idris, 2013). Millets represent critical plant genetic resources for the agricultural and food security of poor farmers that inhabit arid, infertile and marginal lands. Similar to maize and sorghum, millets follow the C4 photosynthetic pathway of carbon assimilation (Brutnell *et al.*, 2010) hence they prevent photorespiration and as a consequence efficiently utilize scarce moisture in the semi-arid regions. Six millet species: kodo [*Paspalum scrobiculatum* (L.)], finger [*Eleusine coracana* (L.) Gaertn], proso [*Panicum miliaceum* (L.)], foxtail [*Setaria italica* (L.) P. Beauvois], little (*Panicum sumatrense*, syn.) and pearl [*Pennisetum glaucum* (L.) R. Br.] were recently shown to have an anti-proliferative property and might have a potential in the prevention of cancer initiation (Chandrasekara



and Shahidi, 2011), due to the presence of large amounts of phenolic compounds (Rao *et al.*, 2011).

Pearl millet constitutes the sixth most important cereal crop cultivated annually under rain fed condition in arid and semi-arid areas of Africa and the Indian subcontinent (Khairawal *et al.*, 1999; FAO, 2007). Millets are rich sources of human and livestock nutrition in developing countries. They contain high amount of

vitamins E, K and BI (100, 1.8 and 842 mg100g) respectively, calcium (37

mg100g), iron (114 mg100g), potassium (250 mg100g), magnesium (0.8

mg100g), zinc (2 mg100g) and protein (9.5 g100g) (Obilana and Manyasa, 2003). The grains of most millets do not contain gluten, a substance that causes celiac disease or other forms of allergies in wheat and related grains including barley and rye (Leder, 2004).

As a result, the crop represents an important staple crop among millions of small-scale farmers (FAO, 2007; Kharkwal *et al.*, 1999; McDonough *et al.*, 2000).

Nuclear techniques have a lot of applications in modern agriculture (Abdul Majeed *et al.*, 2010).

Its usage in genetic improvement of seeds and vegetative propagated crops are widespread (Jain, 2005; Ahloowalia *et al.*, 2004). Induced mutation is one of the best alternatives for improvement of crops as it can help to create and regenerate the variability, which is generally lost in the process of natural selection and adaptation of crops to various stresses (Khan and Goyal, 2009).

Genetic variability is the bedrock to a successful crop improvement program as it provides spectrum of variants for the effective selection by plant breeders (Jain, 2010). Mutation breeding is a reliable tool in crop improvement. It



supplements the existing germplasm and has been adopted to improve certain desirable characteristics in plants (Wilde *et al.*, 2012). Gamma sources are useful for irradiating a wide range of plant materials: seeds, whole plants, flowers, anthers, pollen grains and single cell cultures or protoplasts (Muthusamy *et al.*, 2003). Radiations have been used successfully to induce useful mutations for plant breeding and in fact great success in crop plants has been achieved in developing countries through induced mutagenesis since the 1930s (Ahloowalia *et al.*, 2004; Mohammed and Abdallah, 2011; Avinash, 2013). The lower doses/concentrations of the mutagenic treatments could enhance the biochemical components, which are used for improved economic characters (Muthusamy *et al.*, 2003). Gamma radiation can induce useful as well as harmful effects on crops. The need arises therefore to predict the most beneficial dose for improvement of specific traits of crop plants (Jamil and Khan, 2002).

## 1.2 Problem Statement

A food-secure world; where all people have access to nutritious and affordable food that provides the foundation for active and healthy lives is a pressing global issue (Jain, 2010; FAO, 1996b). Ghanaian agriculture is important for its food security in that it produces the food people eat and provides the

primary source of livelihood for more than 60% of its workforce (MoFA/SRID, 2010). Food security and nutrition are essential dimensions of sustainable development. This implies that inadequate food security and nutrition take an enormous toll on economies and may have negative consequences for the livelihoods and economic capabilities



of vulnerable populations (von Grebmer *et al.*, 2011; FAO, 2010). Strong interdependencies therefore exist between food security and nutrition. The United Nations estimates that in about 842 million people, approximately one in eight are currently undernourished; and approximately two billion suffer from micronutrient deficiencies (FAO/WFP, 2013). Majority of these people live in developing countries, where more than 14% of people are unable to meet their dietary energy requirements (FAO, 2007). The highest prevalence of undernourishment is in Sub-Saharan Africa and Western Asia (Rosegrant, 2011).

Projected increase in Ghana's population further presents challenges to achieving its food security goal. According to United Nations population estimates and projections, Ghana's population has increased rapidly over the years from 6.7 million in 1960, 18.9 million in 2000 to 24.2 million in 2010. With a current population growth rate of 2.4%, the population is expected to double in 29 years (NPC, 2011). Feeding this growing population in the years to come will require the production of more food and distributing it in a manner that reaches more people (FAO, 2009).

A significant boost in local food production will be necessary to meet growing demand. The increase in food production will need to come from improvements in yields and productivity of existing farmlands as well as bringing limited and currently unproductive lands into production.

Currently, some 57.1 % (13,628,179 ha) of Ghana's land surface (23,853,900 ha) represents the agricultural land area (area under rain fed cultivation, total area





under irrigation and area not under cultivation (FAO, 2009). Developing countries have around 2.8 billion ha of land of varying quality that could potentially be used to grow rain fed crops for yields above an acceptable minimum level. Nearly 970 million ha of these lands are already under cultivation (FAO 2003). Achieving an

increase in production through the expansion of cultivated area will be more **difficult** than in the past. Land and water resources are increasingly stressed and are becoming more scarce and diminished in quality due to resource degradation and competition from uses other than for food production (Bruinsma, 2009; FAO/IIASA, 2000). Scarcity of these resources would be compounded by competing demands from urbanization, industrial uses and their use in biofuel production (Bruinsma, 2009). At the same time, forces such as climate change and the need to preserve resources for future generations could change land availability (Parry, 2007).

Increases in yield per unit cultivated area has been the mainstay of historic production increases and will continue to play this role into the future, as production increase through area expansion is hindered by availability and access to land resources (FAO, 2009).

Average yield of cereals have been increasing at a nearly linear fashion for the past five decades (FAO, 2012), implying a falling growth rate. Local constraints to increasing yields remain a significant concern in many countries, threatening improvements in local food supplies in countries where they are most needed. After accounting for differences among countries, global yield of cereals is projected to increase from 3.3 tones/ha in the base year to 4.30 tones/ha in 2050 (FAO, 2012). Therefore, crop varieties with high



yielding potential and stability, together with better management are needed to meet the twin goal of increasing crop productivity and sustainability.

Though considerable progress has been made through conventional breeding in development of crop plants with improved grain yield and quality, this progress is not satisfactory in view of current demand to rapidly increase crop productivity to meet the food needs of the ever increasing human population (Huang *et al.*, 2004). The main limitation of conventional breeding is that the required characteristics may not be present in the breeding population, nor in plants that can be used for conventional plant breeding crosses (Roychowdhury and Tah, 2011).

### 1.3 Justification

Despite its major contribution to the livelihood and daily calorie intake of the people of Northern Ghana, millet still remains a neglected and under-utilized crop whose yields are low under the local environmental conditions. The area planted to millet in Ghana for the past decade has increased by 0.8% but yields, averaging 1300 kg/ha have not shown any significant change (MoFA/SRID, 2010). This is attributed to yield reducing factors such as genetically low yielding landraces, Strega infestation and pests and diseases.

Genetic enhancement strategies should focus on improving the yield and nutritional potential of crops through improvement programs. Crop improvement through conventional breeding dates to the remote past, but with the passage of time this method is presumed to be time consuming, laborious as well as showing limited genetic variability among existing varieties of crop plants



(Huang *et al.*, 2004; Roychowdhury and Tah, 2011). Trans genesis has also shown to offer a promising future to crop genetic improvement. However, issues of health, religion, social, and ethical interest concerning the release of transgenic plants to the environment still remain a course for discussion (Roychowdhury and Tah, 2011). Contrary to conventional and transgenic breeding, nuclear techniques are widely applied in agriculture to improve genetic diversity (Jain, 2010). Unlike conventional breeding procedures which involve the production of new genetic combinations from already existing parental genes, nuclear technology causes exclusively new mutations that may lead to new phenotypes (Abdul Majeed *et al.*, 2010; Micke, 1996). Experimental mutagenesis has already made significant contribution to crop improvement in numerous countries all over the world. This is evident from the fact that more than 2250 varieties of different crops had been released that were derived as direct mutants or from hybridization involving desirable mutants (Ahloowalla *et al.*, 2004). Example is the Tek bankye, a variety released in Ghana through gamma irradiation (Safo-. Kantanka, 1993).

It is obvious, therefore, that a significant portion of the required increases in food production cannot be attained by the further deployment of additional land and water resources. The increased use of agrochemicals and inorganic fertilizers for yield enhancement is also not a sustainable option on account of its deleterious impacts on health and the environment. Simply, more food must be produced with fewer inputs. The admixture of complementary solutions being adduced for feeding the world's teeming population with fewer agricultural inputs and with minimal ecological footprints constitute the ecosystem-based and knowledge-

intensive paradigm that is commonly referred to as sustainable crop production intensification (Chatham House, 2009; FAO, 2011).

According to FAO (2011), to realize the possibility of achieving low-input agriculture, which is preferred for the 21<sup>st</sup> Century, farmers require a suite of improved crop varieties that are genetically diverse, climate change resilient, input use-efficient, high yielding, have enhanced nutritional and other quality attributes and have been bred for adaptation to a range of agro-ecosystems and farming practices. But, the envisaged genetically diverse portfolio of suitable millet varieties is neither available to farmers (Tester and Langridge, 2010; McCouch, 2004) nor do conventional breeding strategies hold promise for delivering such genetic diversities (Mba *et al.*, 2012a). The extremely narrow genetic base of the available varieties, especially neglected crops like millet and the parental lines for breeding new ones nullify efforts to enhance productivities in farmers' fields, increase vulnerabilities and thereby imperil food security (Mba, 2013). Mutation breeding is thus a supplementary approach for crop improvement, and has played a productive role in sustainable agriculture (Larik and Jamro, 1993; Larik *et al.*, 2009). Due to lack of sufficient natural variability, mutation breeding in crop species can significantly accelerate many breeding endeavors, by generating variability which have proven difficult with classical breeding procedures (Roychowdhury, 2011; Roychowdhury and Tah, 2011).



## Objectives

### The study seeks to:

- Determine the optimum gamma radiation doses that will induce mutation in millet and their effect on growth and grain yield of grain millet.
- Create genetic variation in millet through experimental mutagenesis and select mutants with improved grain yield.



## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Origin and distribution

Millet is a highly valuable small-seeded grass, widely grown around the world as cereal crops or grains for both human food and fodder (Kannan *et al.*, 2014; Kholova and Vadez 2013). The crop is favored in semi-arid regions due to its productivity and short growing season under dry and high temperature conditions (Subi and Idris, 2013). It originated from central tropical Africa and is widely distributed in the drier tropics and India. It was later introduced into the western state in the 1850s and became established as minor forage in the Southeast and Gulf Coast states (BSTID-NRC, 1996). Millets are important crops in the semiarid tropics of Asia and Africa, with 97% of its production occurring in developing countries (McDonough *et al.*, 2000).

The two major millets, produced at the worldwide level are pearl millet (*Pennisetum glaucoma* (L.) R. Br.) and finger millet (*Eleusine coracana* (L.) Gaertn). Their center of origin is Sub-Saharan Africa: The West African dry-lands for pearl millet and the East African sub-humid uplands for finger millet. They represent global millets. Because they are cultivated in different geographical areas, they account for most world-wide millet production and trade, and have received most of the research and agricultural programs devoted to millets. Domestication of pearl millet took place in Africa (Marchais and Tostain, 1993), but different geographical origins for this crop have been proposed along the



Sahelian zone from Mauritania to Sudan (Brunken, 1977). While it is believed to be a product of multiple domestications (Parterres 1976), others proposed a single distributed across the semi-arid tropics of Africa and Asia.

Acreage under pearl millet cultivation in Africa is highest in Sudan (Ryan and Spencer, 2001). Although several species of millets are cultivated worldwide, data regarding the production of millets are often merged together. Millet production in Africa has been stagnant in recent times. Africa and Asia account for about 94% of global millet output, estimated at about 28 million tons in 1997 (McDonough *et al.*, 2000). The global area under millets cultivation has, however, shown a slight drop from 38.1 million ha in 1981 to 37.6 million ha in mid 1990s (FAO, 2004). Production figures obtained for millet in Africa between 1970s and 2000 indicated an increment of 22%, while other regions registered substantial decline (World Bank, 2004).

Pearl millet is planted on 18.5 million ha in Africa and 16.99 million ha in Asia (Ryan and Spencer, 2001). Global production of its grain is estimated at 23.38 million tons a year, to which India contributes nearly half. Approximately one-third of the world's millet is grown in Africa and Asia, about 70% of it in West Africa (Ryan and Spencer, 2001). Major producing countries in Africa include Nigeria, Niger, Burkina Faso, Chad, Mali, Mauritius and Senegal in the west, and Sudan and Uganda in the east (Ryan and Spencer, 2001). Six countries (China, Ethiopia, India, the Niger, Nigeria and the former Soviet Union) are estimated to account for about 80% of global millet utilization (Obilana and Manyasa, 2003).



Of the 30 million tons of produced in the world ,about 90% is utilized in developing countries, and only a tiny volume is used in the developed countries (Obilana and Manyasa,2003).the exact statistical data are unavailable for most countries, but it is estimated that a total of 20 million tones are consumed as food, the rest being equally divided between feed and other uses such as seed, the preparation of alcoholic beverages and waste (BSTID-NRC,1996).World consumption of millet as food has only grown marginally during the recent past in contrast to the significate increase in consumption of other cereals (BSTID-NRC,1996).

## 2.2 Climatic and edaphic requirements

Pearl millet can grow in a wide range of ecological conditions and can still yield well even under unfavorable conditions of drought stress and high temperatures. It is generally grown between 35-37° C north or south latitude and can be grown up to altitudes of 1400m, in warm and hot countries characteristic of the semi-arid environment (Prasad and Staggenborg, 2011).

Pearl millet is a warm weather crop and grows best at 20-28°C (singh et al.,1998). the crop is more tolerant to higher temperatures than probably any other cultivated cereal. These useful characteristics mean that it is finding a new niche in some unexpected/ unproductive /unproductive geographic area. The best temperature for seed germination of pearl millet is 23 - 32 °C. The seed of pearl millet does not germinate, and would not grow well under cold soil conditions. Poor emergence and seedling growth may result if planted before soil temperatures reach 23°C (Singh *et al.*, 1998).







The optimum rainfall requirement of pearl millet ranges from 350 - 500 mm. Pearl millet can, however, be grown in areas, which receive less than 35 cm of annual rainfall. Prolonged spells of warm, dry weather may be detrimental and may lead to reduced crop yields (Kanan *et al.*, 2014). Early maturing varieties are planted in the lowest rainfall areas. However, pearl millet requires evenly distributed rainfall during the growing season. Conversely, excess of rain at flowering stage can cause crop failure. At harvest time, dry warm weather is most suitable. Although pearl millet can respond to good moisture supplies during its growth, it is nevertheless one of the toughest, drought tolerant crops available (Gowda *et al.*, 2009). Pearl millet maintains its popularity in the regions where the weather is very unpredictable. The ability of the crop to grow in drier environments is due to a number of physiological and morphological characteristics such as rapid and deep root penetration (root depths of 3.6m have been recorded), root system well-developed and having specialized cell walls that prevent desiccation and tillering capacity of the crop to compensates for any reduction in yield contributing components such as number of heads, length of the head and grain weight (Payne *et al.*, 1990).

The crop tolerates poor, infertile soil better than the other crops. It performs poorly in clayly soils and cannot tolerate water logging (Izge, 2006). It is tolerant to subsoils that are acidic (even those as low as pH 4-5) and high in aluminum content. Pearl millet responds well to management inputs, therefore it has high potential of becoming an important component of intensive agriculture especially in arid and semi-arid regions (Izge, 2006).



### **2.3 Economic importance**

Millet is an important crop to the livelihood of the rural poor population, especially in the semi-arid tropics of Africa and Asia. Despite their potential to contribute to food security, health and nutrition, income generation and environmental services, they are not commercialized on a national scale (Prasad and Staggenborg, 2011). Millet plays an important role in the nutritional security of people in the dry lands of Sub-Saharan Africa, including the Sahel region, where they have a comparative advantage over other major staple crops in terms of resilience to traditional cultivations and food systems (Prasad and Staggenborg, 2011). The conservation, use, and availability of millet genetic diversity is increasingly important in the view of the evolving needs and manifold challenges of small-scale farmers in arid and semi-arid lands throughout Sub-Saharan Africa.

Taylor *et al* (2006) explained that commercial processing of millet into value added products in developing countries has the potential to stimulate economic development in those countries. Therefore, policies that support increased production of millet should be viewed in a holistic approach regarding the contributions they can make to the macro economy and not only as a means of increasing food security to those in semi-arid areas. According to Taylor (2003), millet and sorghum are very significant towards the achievement and maintenance of food security in Africa. Similar report was made by FAO (2008) that small grains are the best resort to avoiding chronic food shortages in rural communities within the semi-arid regions especially of the sub Saharan region. This is attributed to their high levels of adaptability to conditions

within the African terrain (Taylor, 2003). According to Alumira and Rusike (2005) improved millet varieties can reduce the probability of zero yields. Thus, they can make a significant contribution to household food security in drought years.

Millet is a high energy, nutritious food recommended for the health and wellbeing of infants, lactating mothers, the elderly and convalescents. Millets and sorghum provide 75% of total caloric intake for the poor people living in the semiarid tropics and sub-humid drought-prone areas. Pearl millet provides over 1,000 calories per person per day for over 38 million people in four countries, Sudan, Niger, Mali and Burkina Faso, in Sahel Africa (Dendy, 1995), and provides 13.40 kg/yr per capita food use. Malt and flour from millet are used throughout the African continent to prepare indigenous food and drinks. Millets are relatively superior in nutritional composition to other cereals. They contain high amount of -1 vitamins E, K and B1 (100, 1.8 and 842 mg/100g) respectively, calcium (37mg/100g), iron (114 mg/100g), potassium magnesium (0.8 mg/100g) and zinc (2 mg/100g) (Leder, 2004; Obilana and Manyasa, 2003). They generally contain high protein (up to 9.5 g/100g), phosphorus and potassium (up to 250 mg/100 g and 314 mg/100 g respectively) (Obilana and Manyasa, 2003). The grains of most millet varieties do not contain gluten (Leder, 2004). Thus, people with celiac diseases or other forms of allergies can replace certain gluten containing cereal in their diets with pearl millet. It is fitted for flat bread especially because it lacks gluten.





About 80% of the world's millet is used as food, with the remaining being used for stock feed (2%), beers (local and industrial) (3%), other uses (15%) and bird seed (ICRISAT/FAO, 1996). Millets are utilized in the preparation of several food and they differ from country to country and occasionally from region to region. The stiff or thick porridges (Tuwo) are the most popular foods, commonly consumed in all the Sahelian countries and Northern Ghana. The steam-cooked product 'Couscous' is more commonly consumed in the Francophone countries including Senegal, Mali, Guinea, Burkina Faso, Niger and Chad. The thin porridge 'bouillie' is also popular in these countries. Three countries among others have unique foods from pearl millet specific to them. Malting and brewing local beers using millets is significant in Uganda, Zimbabwe, Zambia, Namibia and Ghana. Non-alcoholic local beverages are also commonly made from millets in most regions of West Africa.

The green fodder is rich in protein, calcium, phosphorus and other minerals with oxalic acids in safe limits. It is more digestible when fed green to animals rather than chaffed straw (Chopra, 2001). Nevertheless, its usage for animal feed either as forage, grain or residue is still insignificant, with about 7% (< 2 million tons) of total production going into stock feed (ICRISAT/FAO, 1996).

Pearl millet reduce production cost of broilers as it is equal to or better than typical maize-soybean poultry diets and can be fed up to 10% of the ration without grinding (Davis *et al.*, 2003; Hidalgo *et al.*, 2004). It is well-adapted to regions where many numbers of broilers are produced around the world (Radcliffe *et al.*, 2001). Pearl millet grain is at par or even better than maize in poultry diets (Singh

and Barsaul, 1976; Sharma *et al.*, 1979). The relatively high energy density of

pearl millet is in relation to its higher oil content (4.8 g/100g) relative to other grains (Hill and Hanna, 1990). The grain of pearl millet appears to be generally free of any major anti-nutritional factors, such as the condensed tannins in sorghum grain that have a pigmented taste, which reduces protein availability.

## **2.4 Constraints to millet production**

### **2.4.1 Lack of suitable varieties**

Most available millet varieties are characteristically late maturing (taking about 4 months to mature) and lack Striga resistance. Varieties also lack drought resistance, insect resistance, disease resistance, bird and cold tolerance (Govindaraj *et al.*, 2010). There are breeding efforts to develop cold tolerant varieties for the highlands. In the dry lowland areas, priority research is to develop superior varieties and hybrids for the lower elevations, which mature early and are tolerant to drought. In the dry and sub-humid medium-altitude areas, breeding efforts are aimed at developing superior medium maturing varieties. Using improved varieties in specific region has increased yields. Sudan has recorded increments of up to 3.5 t/ha in Tabat and Rabih varieties, while in Kenya - KAK7780 and 1S21055 varieties yielded 3.5 tons/ha. There are five released varieties, which are resistant to Striga but none are resistant to drought. Over 15 varieties which have been evaluated for food quality, while only one dual purpose variety (E1291) has been released in Kenya (ECARSAM, 2004). The yields realized by the resource-poor farmers have remained considerably low: less than one t/ha compared to 2-3 t/ha in the research institutes, partly as a result





of inadequate dissemination of the released varieties, variation in genotype performance on environments, limited adaptation, and changing environments (ECARSAM, 2004). Yield losses from insect pests and diseases are quite significant and a resistant variety has not yet been developed. Marketing of millet products is essential if the crops have to become competitive. It is, therefore, important to develop varieties with different end-uses. Moreover, there is an increasing trend in changing weather pattern and variable agro-ecologies and pockets of millet production environment, which are not adequately addressed. More genotypes should be screened for yield improvement and stability in a wide range of environments to serve current and future needs.

#### **2.4.2 Poor agronomic practices**

Declining soil fertility, poor water resource management, poor cultural Striga control, poor chemical Striga control and limited use of inputs such as fertilizer, seed, chemicals, farm implements (Rohrbach, 2004). Traditionally, millet is mostly produced under rain-fed conditions. In the traditional culture, millet is usually intercropped with maize or legumes, or grown continuously without rotation. Broadcasting is a standard practice. Very little fertilizer or manure is used, and weed control is often done late after planting (SAFGRAD/ICRISAT, 1986).

For many years, the agronomy of millet production has been studied and recommendations set for preparing the seedbed, sowing dates, plant spacing, crop rotation, fertilizer rates and weeding regimes. In some parts of Africa where millet is grown under irrigation, heavy disc ploughing, harrowing, levelling and ridging

are used to prepare the seedbed (ECARSAM, 2004). The crop rotation program includes cotton, wheat, groundnuts/vegetables and a fallow period (ECARSAM, 2004). The modern farming environment is dynamic and new challenges arise. Suitable agronomic recommendations should be continued whether farming conditions change or new technologies and inputs are introduced.

#### **2.4.3 Poor control of insect pests and diseases**

Crop damage by insect pests, especially stem borers is a serious problem throughout Africa with East and Central Africa being the most affected. The stem borer situation is complicated by the presence of three genera of the pest, namely *Chilo*, *Busseola* and *Sesamia* (ECARSAM, 2004). *Chilo* is important in the low elevation areas of eastern Africa, while *Busseola* is prevalent in the higher elevation. *Sesamia* is less destructive than the other two stem borers. Breeding for resistance to these pests has proved difficult since only low levels of resistance have been identified in germplasm accessions (ECARSAM, 2004). Improved variety 76T1 #23 in Ethiopia and variety KARI Mtama1 in Kenya has been found to be resistant to the stem borer. Current research results show that chemical and cultural methods have been largely used to control the stem borer. In Tanzania, chemicals like Thiodan and Endosulfan have given good results in controlling the pest (Rohrbach, 2004). Field sanitation, crop residue management, early planting and intercropping are some of the cultural methods used to control the stem borer. Spraying with botanicals and biological control using parasitoids and fungus has been tried with some success (Rohrbach, 2004).





Disease problems in the region are considered to have lower priority compared to breeding, agronomic and entomological problems. Smuts and millet blight have been reported in all of Africa (Esele, 1995; Mansuetus, 1995). Smuts infestation can be managed through cultural control and proper seed treatments. Leaf blight is a common disease in the highlands. The disease could be subdued through cultural control or use of resistant varieties. Blast is the major disease affecting finger millet (Esele, 1995). The disease could be controlled through cultural methods or use of resistant varieties. Current research results indicate that there are a few disease resistant varieties (Mansuetus, 1995). The International Crop Research Institute for the Semi-Arid Tropics has identified nine varieties resistant to anthracnose in the region (ICRISAT, 1989). The pearl millet variety, Ashana, grown in Sudan, is resistant to downy mildew.

#### **2.4.4 Poor seed production and distribution**

This is evident through lack of seed distribution policy, poor seed certification and weak seed production schemes (Govindaraj *et al.*, 2010). Quality seed production is crucial to agriculture and strong linkages should be forged between research and seed production. A seed unit should produce adequate and good quality seeds of improved or hybrid varieties to meet farmers' demand. Lack of effective regional seed industry policy is one of the bottlenecks to rapid movement of improved varieties and hybrids. It is, therefore, essential to harmonise germplasm and seed movement regulations (SA FGRAD/ICRISAT, 1986; SADC/ICRISAT, 1987).

Commercial seed companies in the Africa countries have placed low priority on sorghum and millets' seeds (ECARSAM, 2004). Consequently, farmers lack





adequate quality seed. To increase sorghum and millet production in the African sub-region, the seed production and distribution system should be strengthened and farmers encouraged to use high quality seeds for better performance. Linkages between breeders, extension service, seed traders and farmer's organizations should be established and supported to develop an effective seed production system (ICRISAT, 1989).

## **2.5 Conventional breeding techniques**

The genetic modification of crop plants has been practiced for thousands of years, and in its various forms, has provided all the crops that humanity depends on for food, feed and fiber (Lemaux, 2009). The repeated selection of seeds from plants with good crop characteristics over time is the backbone of traditional breeding methods, the main objectives of which are to improve yield, quality, agronomic suitability and resistance to pests and diseases.

Traditional plant breeding relies on two basic processes: recognition of natural variation within a crop and selection of the desirable or required characteristic (Roychowdhury and Tah, 2011). It is effective, and also relatively cheap, and has provided the vast of our major food crops and continues to do so. The main limitation of conventional breeding is that the required characteristics may not be present in the breeding population, nor in plants that can be used for conventional plant breeding crosses (Roychowdhury and Tah, 2011).

Food distribution and equity issues have become a problem in Africa and Asia (FAO, 2008). Even today, when humankind is living in a world of relative



abundance, an estimated number of 850 million people are undernourished or chronically hungry (FAO 2008). Development of crop plants with improved grain yield and quality is very important to meet the growing food demand. Exploitation of naturally occurring inter and intra-specific genetic variability by hybridization of selected plants has been proposed (Munns *et al.*, 2006). Though considerable progress has been made through conventional breeding in achieving this goal, this progress is not enough in view of current demand to rapidly increase crop productivity to meet the food needs of the ever increasing human population (Munns *et al.*, 2006).

Producing more food in a sustainable manner will require yield increases, but also more land (FAO, 2010). However, with part of the current arable land and food crops now being diverted to biofuel production, the projected expansion of food production by 13% by 2030 in developing countries (120 million ha) will probably account for significant deforestation (FAO 2003). To avoid this, good agronomic practices, crop intensification and crop varieties with improved yields and other desirable traits are to be adopted to increase productivity per unit land area.

## **2.6 Mutation breeding**

The development of crops with relatively high yielding ability and nutritional quality through selection and breeding is of considerable economic value for increasing crop production. Plant breeding requires genetic variation of useful traits for crop improvement. However, genetic variability at the specific and varietal level could be lost in the process of adaptation to various stresses and through natural selection (Mba *et al.*, 2012b).



Conventional breeding is the oldest breeding technique relative to transgenesis and mutation breeding (Roychowdhury *et al.*, 2011). Limited genetic variability exists among naturally occurring varieties of crop plants, from which selection can be made. Contrary to conventional breeding, nuclear techniques are widely applied in agriculture for improving genetic diversity. Unlike conventional breeding procedures which utilize genetic diversities already existing in the parental genes, nuclear technology causes exclusively new genotypes arising from high mutation frequencies (Roychowdhury *et al.*, 2011). Mutation breeding is a supplementary approach for any crop improvement program and has played a productive role in sustainable agriculture.

Diversifying the limited genetic variability for agronomic traits of interest, especially grain yield and quality, together with their associated attributes, and the development of new crop cultivars are much demanding in this modern era (Roychowdhury *et al.*, 2012). Due to lack of sufficient natural variability, mutation breeding in crop species can significantly accelerate many breeding endeavors by generating variability, which have proven difficult with classical breeding procedures (Roychowdhury *et al.*, 2011). Plant breeders have, therefore, resorted to mutation breeding as a technique for crop improvement (Elliot, 1958). Mutation breeding has

played a productive role in sustainable agriculture (Larik and Jamro, 1993; Larik and Hafiz, 1981) as a supplementary approach for crop improvement which increases unselected genetic variability for practical breeding application. Mutation breeding has established itself as a reliable tool in crop improvement to supplement existing germplasm and has been adopted to improve

certain desirable characteristics in plants. However, Kharkwal *et al.* (1988) made a survey of mutation breeding and considered related literature. They suggested that desirable results will be obtained from the induced variability when it is fully integrated with conventional crop breeding programs.

Basic tool of nuclear technology for crop improvement involves the use of ionizing radiation which causes induced mutations in plants. These mutations might be beneficial and have higher economical values (Abdul Majeed *et al.*, 2010). The mutation frequency rate of spontaneous mutations is very low and relatively difficult for plant breeders to exploit and utilize for improvement in desirable traits in crop plants. Mutation induction has become a proven way of creating variation within crop varieties (Maluszynski, 1990). Therefore, the purpose of induced mutations is to enhance the mutation frequency rate in order to select desirable mutants for crop improvement. It has been found that irradiation of seeds increases mutation frequency and widen the mutation spectrum (Micke, 1996). Experimental mutagenesis has been investigated and applied globally in crop breeding in various agricultural and research institutes during the last half century (Siddiqui and Khan 1999; Anitha *et al.*, 2005). Induced mutation has been perceived as an important tool to create additional variability for quantitative and qualitative traits in a number of crop plants (Song and Kang, 2003)

Experimental mutagenesis has already made significant contribution to crop improvement all over the world. This is evident from the fact that more than 2250 varieties of different crops had been released that were derived as direct mutants or from hybridization involving desirable mutants (Ahloowalla *et al.*, 2004). Both





physical mutagens (mainly gamma rays) and chemical mutagens (mainly ethyl methane sulphonate), have been used and their proper doses established for different crops (Guenet, 2004). The use of induced mutation has over the past 50 years, played a major role in the development of superior crop varieties translating into a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares (Jain, 2010). Exploitation of natural and induced genetic diversity is the basic requirement of plant breeding in developing plant varieties for sustainable food production (Fasoula and Fasoula, 2002). Plant breeders are however handicapped due to lack of availability or non-existence of desired genotypes. Induced mutation has been extensively applied in creating genetic variability (Ashraf *et al.*, 2003) and according to Maluszynski and his associates (2000), 2,200 mutant varieties of different crops with enhanced agronomic characteristics have been developed and released the farmers for cultivation all over the world. Great success in crop plants has been achieved in developing countries through induced mutagenesis since the 1930s (Ahloowalia *et al.*, 2004). Mutagenic agents, such as radiation and certain chemicals are useful in inducing mutations and generating genetic variations from which desired mutants may be selected. Mutations are induced in both seed and vegetative propagated crops by physical and chemical mutagen treatments and these treatment cause breakages in the nuclear DNA. During the process of DNA repair mechanism, new mutations are induced randomly, which are heritable (Jain and Maluszynski, 2004). The changes can occur also in cytoplasmic organelles, or these may result in chromosomal or



genomic mutations that enable plant breeders to select useful mutants such as flower color, flower shape, disease resistance and early flowering types (Crino *et al.*, 1994; Donini and Sonnino, 1998; Jain and Maluszynski, 2004). Nuclear techniques have a lot of applications in modern agriculture. Its usage in genetic improvement of seeds and vegetative propagated crops are widespread (Jain, 2005; Ahloowalia *et al.*, 2004). Crops are induced to mutate through the exposure of their propagules to physical and chemical mutagenic agents. Among them, gamma rays and ethyl-methane sulphonate (EMS) are widely used for mutation induction (Mba *et al.*, 2012b). Mutation induction started with the discovery of radiations of X-rays by Roentgen in 1895, radioactivity by Becquerel in 1896 and radioactive elements by Marie and Pierre Curie in 1898. Radiation induced mutation has been the most common, accounting for 90% of induced mutations (Jain, 2005). Ionizing radiations (X-rays, gamma-rays, alpha and beta particles, protons and neutrons) constitute the most commonly used physical mutagens (Mba *et al.*, 2012b; Mba and Shu, 2012). Widespread usage of ionizing radiations relative to ultraviolet radiation (UV) is due to its ability to penetrate deeper into tissues and can induce a great number of different types of chemical changes. Physical mutagens allow for a sufficient reproducibility and, particularly for gamma rays, a high and uniform penetration in plant tissues (Jain, 2005), resulting in its preference to chemical mutagens. Gamma irradiation has been used successfully to induce useful mutations for plant breeding (Jamil and Khan, 2002).



Chemical agents known to induce mutations include base analogues, alkylating and intercalating agents and chemicals that modify DNA structure (Roychowdhury and Tah, 2011). These agents are very useful in crop improvement, providing high mutation rates and mostly point mutation (Jain, 2005). The most commonly used among these category is those belonging to the alkylating agents; ethyl methane sulphonate (EMS), diethyl sulphate (DES), ethylene imine (EI), ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH), methyl nitroso urea and (. MNH) together with azides (Mba, 2013).

### **2.6.2 Mutation induction for improvement in nutritional quality**

Issues of enhanced food security depend primarily on increasing agricultural production. Food crops make significant contributions to global food security by providing a vast array of foods that supply essential nutrients (Omonona and Agoi, 2007). Ensuring that people have access to adequate nutrient-rich food is essential for safe guarding their safety, health and well-being. About 97% of all millet breeding efforts have been geared towards improving the grain yields with very little attention to the nutritional quality (Andrews and Kumar, 1992). Research on grain quality has been on the evaluation of the physical and functional properties of the grains with very little effort geared towards improving their nutritional values though millet is mainly used as food (Al-Salhi *et al.*, 2004).

Among the different techniques of crop improvement, mutagenesis and the isolation of improved or novel phenotypes can result in mutant varieties endowed with desirable nutritional traits (Schum, 2003). Induced mutations have been used through the Joint effort of FAO/IAEA with more than 1800 cultivars obtained



either as direct mutants or derived from their crosses, released worldwide in 50 countries (Ahloowalia and Maluszynski, 2001). Crop varieties with improved characteristics, such as early maturity, resistance to pests and diseases and tolerance to environmental stresses have been achieved through experimental mutagenesis (Ahloowalia and Maluszynski, 2001; Maluszynski and Kasha, 2002). Experimental mutagenesis has been investigated and applied in crop breeding in various countries throughout the world during the last half century. Induced mutation has been useful in the evolution of new varieties of crops (Micke *et al.*, 1985) and has played an important role in enhancing their nutritional quality through the introduction of mutant genes. Seed irradiation during the pre-sowing is one of the most effective methods to induce mutation, which could lead to improved yield components and chemical composition (Khan, 1970; Selenia and Stepanenko, 1979).

### **2.6.3 Induced mutation using gamma irradiation**

Gamma rays are the most important physical mutagen and have proven to be very useful in improvement of characters and productivity in many plants (Jawardena and Peiris, 1988; Sharma and Rana, 2007). The ability of gamma rays to induce mutation in crop plants depends on the species and the dosage of irradiation (Artk and Peksen, 2006) and according to Kiong *et al* (2008) the morphological, structural and the functional changes are indeed dependent on the strength and duration of the gamma- irradiation stress. Gamma irradiation has achieved much success in mutation breeding of most cultivated crops and ornamentals (Song and Kang, 2003). It has proven to be a very reliable means of producing new genetic





variation from which selections can be made by breeders through enhancement of the expression of recessive genes whose effects under normal conditions are Masked by dominant genes (Yoon *et al.*, 1990; Schum, 2003; Song and Kang, 2003).

Ionizing radiations are parts of the electromagnetic spectrum that, on account of their relatively high energy levels (10 keV to several hundred keV), are capable of dislodging electrons from the nuclear orbits of the atoms that they impact upon (Mba, 2013). The impacted atoms therefore become ionized hence the term ionizing radiation. Gamma rays are known to influence plant growth and development by inducing cytological, genetically, biochemical, physiological and morphogenetic changes in cells and tissues (Gunckel and Sparrow, 1961).

The mutagenicity of these agents is derived from a combination of their ability to produce dimmers and reactive ions which in turn cause damage to living organisms. Gamma rays may act directly on the cellular component (Kovacs and Keresztes, 2002) or indirectly on water molecules, causing water-derived radicals. Radicals react with each other or nearby unchanged molecules in a very short time, resulting in breakage of chemical bonds or oxidation of the affected molecules. These radicals have the potential to damage or modify important components of plant cells differentially based on the dosage of irradiation (Ashraf *et al.*, 2003). The major effect of gamma rays in cells is DNA breaks. Since DNA consists of a pair of complementary double strands, breaks of either a single strand or both strands can occur (Kim *et al.*, 2004). However, the latter is believed to be much more important biologically. Because of the double-stranded structure of the

DNA, most single-strand breaks can be repaired normally; with the intact strand serving as a template for repair of its' damaged, opposite strand. The repairing process is more tedious and erroneous in double-strand breaks (Wi *et al.*, 2005). These erroneous repairs induce mutations with consequent effects including changes in cellular plant structure and metabolisms such as dilation of thylakoid membranes, changes in photosynthetic processes, modulation of the ant oxidative system and accumulation of phenolic compounds (Kim *et al.*, 2004; Wi *et al.*, 2005).

Gamma rays have been used to irradiate a wide range of plant materials; seeds, whole plants, plant parts, flowers, anthers, pollen grains and single cell cultures or protoplasts (Sharma and Rana, 2007). Gamma irradiation has an intensive effect on growth and development of plants by inducing genetic, cytological, biochemical, physiological and morphogenetic modifications in cells and tissues depending on the irradiation dosage (Gunckel and Sparrow, 1961). They can be useful for the alteration of physiological characters (Kiong *et al.*, 2008). The uniqueness of these rays is due to their high penetration power and accounts for their wider application for the improvement of various plant species relative to other ionizing radiations (Moussa, 2011), having the energy level from around 10 keV to several hundred keV. The lower doses of the mutagenic treatments could enhance the biochemical components, which are useful to the improvement of economic characters such as yield and nutritional quality (Muthusamy *et al.*, 2003).





The effectiveness of radiation to induce mutations is assessed based on a number of radiobiological parameters. Mutagenic radiations are a useful tool to all breeding programs associated with flowering crops (Krasaechai *et al.*, 2009). The effect of gamma rays on the trait of crop plants is dependent on such factors as crop species and the irradiation dosage (Artk and Peksen, 2006). Changes in crops resulting from radiation exposure include; metabolism and cellular changes in plants, modulation of the anti-oxidative system, photosynthetic alterations and phenolic compounds accumulation (Kim *et al.*, 2004; Wi *et al.*, 2005). Seed irradiation during the pre-sowing is one of the most effective methods to improve plant production, yield components and chemical composition (Kovacs and Keresztes, 2002). Induced mutations have been used for the improvement of major crops such as wheat (*Triticum* spp.), rice (*Oryza saliva* L.), barley (*Hordeum vulgare*), cotton (*Gossypium hirsutum* L.), and chickpea (*Cicer arietinum* L.) all propagated by seed (Ahloowalia and Maluszynski, 2001). Success has been achieved in numerous crops by several scientists through induction of mutation. Improved barley variety with early maturity, high protein contents and stiff straw has been developed by mutation breeding techniques (Javed *et al.*, 2000). Khan and Goyal (2009) developed three high grain yielding and early maturing mutants by treating seeds of *Brassica juncea* L. Cv. S-9 with gamma rays (0.75-1 Gy) and EMS. Rao *et al* (1975) discussed the findings related to the different researches with gamma irradiation in wheat and they reported that the doses above 50 Gy created a bad influence on bread quality. Ghafoor and Siddiqui (1976) studied the effects of gamma rays on tiller number and plant height in six cultivars of wheat.

The results showed that these cultivars differed significantly for both characters under different exposures. Hassan (1986) observed that 40 Krad dose caused maximum reduction in various genetic parameters of wheat and triticales.

#### **2.6.4 Induced mutation using chemical mutagens**

It was about two decades after the demonstrations of the mutagenicity of physical agents that nitrogen mustard (component of poisonous mustard gas used in World Wars I and II) were shown to cause mutations in cells (Mba *et al.*, 2010). This paved way for the identification of several other chemical mutagens that modify DNA structure. Their effects on DNA molecules manifest in deamination, the induction of transitions and insertions, the stoppage of transcription and replication and even strand breaks. These chemicals include base analogues (5-bromouracil, 5-bromodeoxyuridine and 2-aminopurine, alkylating agents (ethyl methane sulfonate, diethyl sulfonate, 2-chloroethyl-dimethyl amine and ethylene oxide) and intercalating agents (acridine orange, proflavin and ethidium bromide).

#### **2.7 Induced mutation in millet**

Many have expressed concerns that genetic variability in millet is limited and that breeding efforts would be enhanced if the range of variability could be broadened (Abuali *et al.*, 2012; Subi and Idris, 2013). These concerns have brought about the direction of mutation research geared towards the finding of mutagenic agents that are efficient on millet and are capable of producing variant mutant forms that may exhibit some usefulness in crop improvement programs (Abuali *et al.*, 2012).





Despite the potential contribution of millet to food security, health and nutrition, and income generation, it still falls in the category of neglected and underutilized crops. During the past two decades, several attempts have been made to boost the yield of millets. Experimental mutagenesis has proven to be one of the most important and viable techniques that can be exploited to develop and release new genotypes and high yielding cultivars of crops (Siddiqui and Khan, 1999). This can be achieved by exposure of seeds to mutagenic agents such as ionizing radiations or chemical mutagens (Vasline *et al.*, 2005).

Burton and Powell (1966) exposed dried seeds of 10 pearl millet inbreds to mutagenic treatments;  $5.67 \times 10$ ,  $1.14 \times 10$  and  $1.70 \times 10$  (total doses of flux x time) thermal-neutrons (TN) or for four hours with 0.2, 0.4, and 0.6% ethyl methane sulfonate (EMS) in unbuffered water solution. Among the 13 characteristics studied, 5 exhibited significant difference in inbred x treatment interactions. Reduction in seedling height, plant height, number of leaves as well as self and sibbed seed set and inhibition of seedling emergence and also delayed seed maturity were observed in all treatments. Reduction in seed emergence and survival was achieved with EMS. However, the mutagenic treatments;  $1.70 \times 10$  TN and 0.4% EMS increased the average chlorophyll-deficient seedling mutation rate by 5-folds. More chromosomal interchanges were induced with the low TN treatment than with the high EMS treatment. Mohan (1973), soaked seeds of two inbreds of millet in 0.005, 0.10, and 0.20% aqueous solutions of N-nitroso-N-methyl urea (NMH) for 4 hours after a 9-hour pre-soak in water. The 0.20% dose was almost lethal. Two-thirds and one-third of the  $M_I$  plants produced  $M_2$

chlorophyll-deficient mutant segregating progenies at the 0.005 and 0.10% doses, respectively.

Vijendra Das (1978), irradiated dry seeds of two genotypes, HB3 (an F hybrid) and MS 7625, with 40, 50, 60, 70, 80 and 90 kR of X-rays (50 kVp) and 10, 20, 30, and 40 kR of  $^{60}\text{Co}$  gamma rays. The approximate  $\text{Mi}$  Lethal dosage fifty

( $\text{LD}_{50}$ ) was 60 kR for X-rays and 20 kR for gamma rays. Gamma rays produced more  $\text{Mi}$  lethality, growth reduction, pollen sterility and a higher  $\text{M}_2$  mutant frequency than X-rays. Gamma rays also showed the higher mutagenic efficiency for the genotypes that were studied.

Burton and Hanna (1982), soaked inbred 'Tift 23DBJ\*' (fertile maintainer for  $\text{A}_1$  sterile cytoplasm) seeds in water solutions of 200 and 500 ppm streptomycin (STY), 50 ppm mitomycin (MIT) and 250 and 1000 ppm ethidium bromide (EB) at  $5^\circ\text{C}$  for 40 hours for the purpose of inducing cytoplasmic male sterile mutants.

The 250 and 1000 ppm EB doses increased male sterile mutant frequencies by over 50- and 100-fold, respectively, over the control (untreated inbreds). However,

by the  $\text{M}_3$  generation, most of the male sterile mutants had reverted to fertile pollen shedders. One percent of 402  $\text{M}_2$  progenies of  $\text{M}_1$  self-feed plants segregated

for chlorophyll deficient plants indicating that EB may also be considered a nuclear mutagenic agent. The 200 and 500 ppm STY and 50 ppm MIT treatments increased the frequencies of stable cytoplasmic male sterile mutants by 2.9, 3.6, and 6.2 times, respectively, over the control. Appropriate crosses with maintainer and restorer inbreds indicated that the induced mutants had similar sterility

maintainer and fertility restorer requirements as did the  $\text{A}^\wedge$  cytoplasm.





Muduli and Misra (2007), induced mutation in two varieties of finger millet: VR 708 (short height, early maturing with brown seeds) and GPU 26 (tall, late maturing with light brown seeds). Dried seeds of these varieties treated with three doses each of gamma rays, ethyl methane sulphonate (EMS) and nitrous guanidine (NG) employed separately and in combinations. The nine single mutagenic treatments were 150, 300 and 450 Gy of gamma rays, 0.15, 0.30 and 0.45% of EMS and 0.015, 0.030 and 0.045% of NG coded as G1, G2, G3, E1, E2, E3, N1, N2 and N3, respectively. The two combination treatments were 300 Gy gamma rays + 0.30% EMS and 300 Gy gamma rays + 0.030% nitroso guanidine (NG), coded as GE2 and GN2, respectively. The results of the experiment indicated significantly superiority in yield of nine of the eleven treatments in the M3 generation for both varieties with yield/plant ranging from 6.380g (N1) to 6.993g (E2) in VR 708 and 8.468g (GN2) to 9.225g (E2) in GPU 26 in different treatments.

Sani *et al* (2013) determined the radio-sensitivity of pearl millet (HKP) variety to gamma induced mutation to estimate the LD<sub>50</sub> capable of producing desirable mutants. The results obtained showed no significant difference in percentage germination among the treatments, except 700 Gy which was inhibitory. Seedling height also decreased progressively, with radiation dose, from 100% in the control, to 40% at the highest dose (700Gy). The LD<sub>50</sub> was successfully determined using seedling height as the determinant and was found to be 669.3 Gy.



Ambli and Mullainathan (2014) exposed dried seeds of pearl millet (*Pennisetum typhoides* (Bum.) Stapf.) variety Co (Cu) -9 to gamma ray doses from  $^{60}\text{Co}$  and different concentrations of EMS to ascertain their effect on seed germination and other characteristics. Results obtained showed a gradual decrease in germination

same for the other characters (survival, seedling height, root and shoot length) measured in the M1 generation. In addition, The LD50 value using seedling survival as an indicator, was found to be 20krad in gamma rays and 30mM in EMS.

## 2.8 Mutation breeding in other major crops

Several positive mutants of agricultural crops have been created by using gamma irradiations (Javed *et al.*, 2000; Rehman *et al.*, 1987; Gustaffson *et al.*, 1971). Successful improvements in crop characteristics have been achieved through mutagenic inductions. Khizar *et al* (1990), studied the effect of gamma rays' doses on two varieties of sorghum (DS- 25 and Pak-SS-II). They reported an induced variability due to gamma irradiations for percentage emergence, days to 50% flowering, plant height and yield/plant. Data pertaining to yield/plant revealed significant difference in mean value due to variety, various doses as well as their control. There was a significant reduction in yield in all treatments due to radiations. The reduction in yield was inversely correlated to the intensity of irradiation. Soeranto *et al* (2001) experimented on the radio-sensitivity of sorghum variety Keris through gamma induced mutagenesis from  $^{60}\text{Co}$  source to assess the





potential of the rays to create genetic variability within the variety. Results obtained in the  $M_1$  revealed a significant physiological effect due to irradiation treatment. Plant survival decreased with an increase in the dosage of radiation exposure. Results from the analysis of variance indicated that the irradiation treatments gave a significant effect on the phenotypic performance of plant height and harvest index in the  $M_2$ . Further in the  $M_4$  generation, a number of promising mutant lines had been registered. Some of these mutants had desirable agronomical characteristics such as semi dwarf, early maturing, big and condense head, high yielding, white and clean color of seeds. One of the promising lines, ET 20-B, was used as a model in post-harvest processing of sorghum grain. Nutritional content was analyzed and compared to that of the control variety and rice (Cilosari variety), specially for the milled grain. It was found that milled grain of ET 20-B line had nutrient contents, especially for fat and protein, higher than mutant lines obtained.

Larik *et al* (2009), exposed two sorghum varieties viz., DS-75 and Giza-3 to Co 60 gamma source with doses of 10, 20, 30 and 40 krad. Results obtained for mean varietal performance revealed a consistent decrease in seedling emergence percentage, ear head length and 1000-seed weight in both cultivars except in the 10 kR treatment and an increase in percentage plant with abnormal leaves and stem in  $M_1$  generation with increasing radiation dosage in both varieties. Mean yield per plant in  $M_1$  generation revealed a shift towards the negative direction except in the 10 kR dose, which suggests that lower dose (10 kR dose) of gamma



radiation can be useful for breeding point of view for selecting higher yielding plants in the early generation. Since the 1970s, gamma rays, sodium azide and EMS, used in combination or alone, has been used for mutation induction and breeding in wheat (Rachovska, 1996). Rao *et al* (1975) discussed the findings related to the different researches with gamma irradiation in wheat and they reported that the doses above 0.05 Gy created a bad influence on bread quality. Ghafoor and Siddiqui (1976) studied the effects of gamma rays on tillers number and plant height in six cultivars of wheat. The results showed that the cultivars differed significantly for both the characters. Hassan (1986) observed that 40 Krad dose caused maximum reduction in various genetic parameters of wheat and triticale. Mohammed and Abdollah (2011) studied the effect of gamma irradiation on some physiological characteristics and protein content in wheat by exposing dried seeds of two cultivars (Alamot and Zagros) to gamma rays at 25, 50, 75, 100 and 125 Gy from Cobalt 60 source. The mutant lines obtained for both varieties in the M<sub>1</sub> from 25 and 50 Gy gamma irradiation were superior in terms of 1000 seed weight, grain yield, harvest index and protein content relative to the control though the differences observed were not significant. Gamma irradiation above 50 Gy, however, posed some inhibitory effects on these parameters. Avinash (2013) subjected seeds of two pea varieties of *Pisum sativum* L., *P. sativum* var. *Hortense* (garden pea) and var. *arvense* (field pea) to different doses of gamma irradiation in order to evaluate the effect on yield attributing characters. The effect of mutagen was studied with regard to grain yield parameters.

Data recorded from the population showed significant variability in different characters. Most of the physiological parameters (percent germination, maturation period and number of flowers) showed dose dependent decline in irradiated plants. Lower doses of gamma irradiation had stimulatory effect on yield attributing parameters such as number of pods per plant, number of seeds per plant, and pod size, in both varieties. *L'isum sativum* var. *arvense* (leafy) was found to be high yielding than var. *hortense* (leafless) and the genotype of the first variety was observed to be more sensitive to gamma irradiation than the latter.

Desai and Rao (2014) irradiated seeds of pigeon pea (Var. BSMR 736) with gamma rays (5, 10, 15, 20, 25Kr). The results showed that the germination frequency, shoot and root length decreased with increasing radiation doses. Germination frequency was high (95.89) in the control plants and low (66.09) in 25kr irradiated plantlets. Total protein content was high in plantlet irradiated with 5kr (12.60mg/g FW) whereas only 9.21mg/gFW was found in the control plants. Proline content was high in 25Kr plantlets (9.93μmoles/g FW) but less in 10Kr irradiated plantlets. Highest amount of chlorophyll was found in 25Kr irradiated plantlets (3.84mg/g FW) and least (2.18mg/g FW) was found in 15Kr irradiated plants. Additionally, the amount of chlorophyll a was higher than chlorophyll b in both irradiated and non-irradiated plantlets. Ariraman *et al.*, (2014) treated the seeds of pigeon pea (*Cajanus cajan* (L.) Millsp) with different doses of gamma radiation from 5-50 Kr and concentrate Ethyl Methane Sulphonate from 5- 50mM for studying emergence, growth and survival effects. The seed germination percentage decreased with increase in the



concentration/doses when compared to the control. The 50 percentage of seed germination and reduction was observed in 20 Kr of gamma rays and 25 mM of EMS and it is considered as LD<sub>50</sub> value for both the treatments (Ariraman *et al.*, 2014). The decrease in seed germination was more prominent with gamma rays than that of EMS treatments. The seedling parameters of gamma rays and EMS treated seedlings were progressively decreased with increase dose/concentration in all mutagenic treatments when compared to the control. The maximum seedling parameters were observed in 5 Kr of gamma rays and 5 mM of EMS. Minimum seedling parameters were observed in 50 mM of EMS and 50 Kr of gamma rays respectively.

Giri (2014) used two varieties of pigeon pea (*Cajanus cajan* (L.) Millsp) (ICPL-87 and BDN-708) to study mutagenic sensitivity. The seeds of both the varieties were treated with four different concentrations and dose of EMS (10, 20, 30, 40 mM) and Gamma Radiation (100, 200, 300, 400 Gy) respectively. There was decrease in percent germination and survival at maturity, while pollen sterility increases with the concentration of the mutagens.



## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Description of study area

Four experiments namely Experiment I ( $M_1$  generation and dosage response studies), Experiment II ( $M_1$  generation of whole seed lot planted on field), Experiment III ( $M_2$  generation planted for screening in the field obtained from  $M_1$  generation) and Experiment IV ( $M_3$  generation planted for screening in the field obtained from  $M_2$  generation) were conducted. All experiments were conducted at the University for Development Studies, Tamale, Ghana, during the dry and farming seasons of year 2014 and 2015. Studies started from June to July, 2014 for Experiment I and ended with Experiment IV from May to July 2015.

The experimental sites lie on an altitude of 183m and latitude  $09^{\circ} 25' N$  and longitude  $0^{\circ} 58' W$ . The area is within the Guinea Savannah agro ecological zone and is subjected to marked wet and dry season with a unimodal annual rainfall of approximately 1000 mm which is evenly distributed from May to October, reaching a peak in August and September. Temperature distribution is uniform with mean monthly minimum of  $23.4^{\circ}C$  and maximum of  $34.5^{\circ}C$ . The minimum relative humidity of the area is 46% and maximum relative humidity is 76.8% (SARI, 2008).



### 3.2 Experiment I (M<sub>1</sub> and dosage response studies)

#### 3.2.1 Seed irradiation and planting

Seeds of Naara variety of pearl millet were obtained from Savanna Seed Company in Tamale. They were used as the initial breeding material. Dried seeds were exposed to gamma irradiation from cobalt-60 source (because of the relative long half-life, cheapness and availability of cobalt-60) at the Radiation Technology Center of Ghana Atomic Energy Commission (GAEC), Accra on June 2014. Seeds were divided into 8 groups (10 g each, approximately 400 seeds), each representing a gamma irradiation treatment from the following doses: 100, 200, 300, 400, 500, 600, 700 Gy and a control (0 Gy), giving a total of eight treatments (Table 1). These doses were adopted in reference to a related study by (Sani *et al.*, 2013) in pearl millet and cowpea. The treatments were laid out in Completely Randomized Design (CRD) with three replications. Twenty-liter vegetable oil containers were split into two equal halves from the vertical and filled with 14 kg of sandy loam soil of Nyankpala soil series to constitute the experimental units as shown in Plate 1. Holes were perforated at the bottom of the containers to drain excess water during watering. Fifty seeds per treatment were sown per replicate. They were watered and made to stand overnight. Irradiated seeds and the control were sown the next day after irradiation.





**Plate 1: Effect of gamma irradiation on seed emergence and seedling survival**



$$\text{GP} = \frac{\text{Number of emerged seeds}}{\text{Number of sown seeds}} \times 100 \quad (\text{Equation 1})$$

Where GP= germination percentage

**Table 1: Gamma ray doses used in dosage response studies**

Gamma irradiation (Gy)	Treatment
0	T1
100	T2
200	T3
300	T4
400	T5
500	T6
600	T7
700	T8



### 3.2.2 Cultural practices

Two liters of water was applied to each experimental unit every morning. Hand picking of weeds were done at two weeks' interval and ten plants randomly tagged were observed for data collection.

### 3.2.3 Data collection

#### 3.2.3.1 Percentage germination

Number of seeds that had emerged 7 days after planting was counted. Averages were calculated and used to compute germination percentage as shown:



### 3.2.3.2 Percentage survival

Averages were calculated and used to compute survival percentage as shown:

$$SP = \frac{\text{Number of seedlings that had survived 3WAP}}{\text{Number of emerged seeds}} \times 100 \text{ (Equation 2)}$$

Where. SP= survival percentage, WAP= weeks after planting

### 3.2.3.3 Seedling height

Seedling heights were measured from the soil surface to the flag leaf with a ruler at 3 and 5 WAP. Averages were computed and recorded in centimeters (cm).

## 3.3 Experiment II (M<sub>1</sub> generation of whole seed lot planted on field)

### 3.3.1 Seed irradiation and planting

It was revealed from the Experiment I that gamma ray doses above 300 Gy (400, 500, 600 and 700 Gy), suppressed seedling survival (Figure 1). Seeds were irradiated according to results from Experiment I. Dried seeds were exposed to gamma irradiation from cobalt-60 source at the Radiation Technology Center of Ghana Atomic Energy Commission (GAEC), Accra on August 2014. Prior to irradiation, seeds were divided into 4 groups (20 g each), each representing a gamma irradiation treatment 100, 200, 300 Gy and a control (0 Gy). A total of 4 treatments (Table 2) were laid in Randomized Complete Block Design (RCBD), with four replications. Field was cleared, ploughed and harrowed. The field was demarcated into plots, each measuring 2.5 m x 2.5 m with 1 m and 2 m alleys between plots and replications, respectively. Treated seeds and the control were planted immediately at a spacing of 25 cm x 75 cm, and seeding rate of four per hill. After harvesting and drying, seeds from individual heads in respective



treatments were sampled and composited for advancement into the segregating **M2** generation.

**Table 2: Gamma ray doses used in M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> field studies**

Gamma irradiation (Gy)	Treatment
0	T1
100	T2
200	T3
300	T4

### 3.3.2 Cultural practices

Weeding was carried out at 4, 7 and 9 WAP. Bird scarring was carried out at the onset of seed filling till harvest.

### 3.3.3 Data collection

#### 3.3.3.1 Plant height

The height of each plant tagged per plot was measured from its base to the flag leaf. Means were computed and recorded in centimeters (cm).

#### 3.3.3.2 Number of tillers

Tillers were counted at the maximum tiller stage (8 WAP) in respective plots.

Numbers obtained for respective treatments were averaged and recorded.



#### ***C [3.3.3.3 Number of productive tillers***

Productive tillers (defined as number of tillers that produce heads and seeds) were counted in respective plots prior to flowering. Numbers obtained for respective treatments were averaged and recorded.

#### ***3.3.3.4 Days to 50% flowering***

Days taken to attain 50% flowering in respective plots were counted. Numbers obtained were averaged and recorded.

#### ***3.3.3.5 Head length***

Head length was measured using a ruler after harvesting and sun-drying to 13% moisture content. Values obtained for respective treatments were averaged and recorded in centimeters (cm).

#### ***3.3.3.6 Head width***

Circumference of head was measured using tape measure after harvesting and sun-drying to 13% moisture content, and recorded in centimeters (cm). Width of each head was then calculated as shown:

$$W = \frac{C}{\pi} \text{ (Equation 5)}$$

Where W= width and C= circumference

#### ***3.3.3.7 Head weight***

Weights of each head for individual treatments were taken after harvesting and sun-drying to 13% moisture content with an electronic scale (Sartorius TE 612). Averages were computed and recorded in grams (g).



#### **3.3.3.8 100 seed weight**

Weight of 100 seeds sampled at random from each head was taken with an electronic scale. Averages were computed and recorded in grams (g).

#### **3.3.3.9 Grain yield**

After harvesting and threshing, the seeds were sun-dried to 13% moisture content.

Weights of the dried seeds were recorded and converted to kg per ha.

### **3.4 Studies on the segregation of M2 and M3 generations**

#### **3.4.1 Land preparation and experimental design**

Land preparation was done manually. Field was laid out into individual plots measuring 1.95 m x 3.5 m, with 1 m and 2 m alley between plots and replications, respectively. Ridges were made, each measuring 3.5 m long, 0.25 m wide and 0.6 m apart on individual plots. The four treatments: seeds harvested from the control, 100, 200 and 300 Gy doses from M<sub>1</sub> were sowed in Randomized Complete Block Design (RCBD), with four replications. Seeds from the M<sub>1</sub> generation were planted 25 cm apart on each ridge.

#### **3.4.2 Cultural practices and screening**

Weed control was done manually using the hoe at 4 and 7 weeks after planting. Each plot received 48 liters of water twice a day, morning and evening. Scaring of birds was carried out at the onset of seed filling till harvest as stated in Experiment II (Page 38). Plants were monitored and screened for enhanced growth and yield characteristics. Single plant selection was done for promising progenies. Individual plant selection based on phenotypic variations in agronomic and yield

characters were carried out. Selected progenies from the M2 generation were processed for advancement to the M3 generation. Promising progenies from 100, 200 and 300 Gy treatments were selected.

### **3.4.3 Data collection**

#### **3.4.3.1 Plant height**

The height of each plant tagged per plot was measured from its base to the flag leaf. Means were computed and recorded in centimeters (cm).

#### **3.4.3.2 Number of tillers**

Tillers were counted at the maximum tiller stage (8 WAP) in respective plots. Numbers obtained for respective treatments were averaged and recorded.

#### **3.4.3.3 Number of productive tillers**

Productive tillers (defined as number of tillers that produce heads and seeds) were counted in respective plots prior to flowering. Numbers obtained for respective treatments were averaged and recorded.

#### **3.4.3.4 Days to 50% flowering**

Day taken to attain 50% flowering in respective plots were counted. Numbers obtained were averaged and recorded.

#### **3.4.4 Head length**

Head length was measured using a ruler after harvesting and sun-drying to 13% moisture content. Values obtained for respective treatments were averaged and recorded in centimeters (cm).



### **3.4 A. Head width**

Circumference of head was measured using tape measure after harvesting drying to 13% moisture content, and recorded in centimeters (cm). Width of each head was then calculated using equation (1).

#### **3.4.3.7 Head weight**

Weights of each head for individual treatments were taken after harvesting and sun-drying to 13% moisture content with an electronic scale (Sartorius TE 612). Averages were computed and recorded in grams (g).

#### **3.4.3.8 100 seed weight**

Weight of 100 seeds sampled at random from each head was taken with an electronic scale. Averages were computed and recorded in grams (g).

#### **3.4.3.9 Grain yield**

After harvesting and threshing, the seeds were sun-dried to 13% moisture content.

Weights of the dried seeds were recorded and converted to kg per ha.

### **3.5 Data analysis**

Count and percentage data were transformed using square root and arc-sine transformations before statistical analysis. Data obtained were subjected to ANOVA using Genstat statistical package, 12th edition. Means were separated using LSD at 5%.



## CHAPTER FOUR

### RESULTS

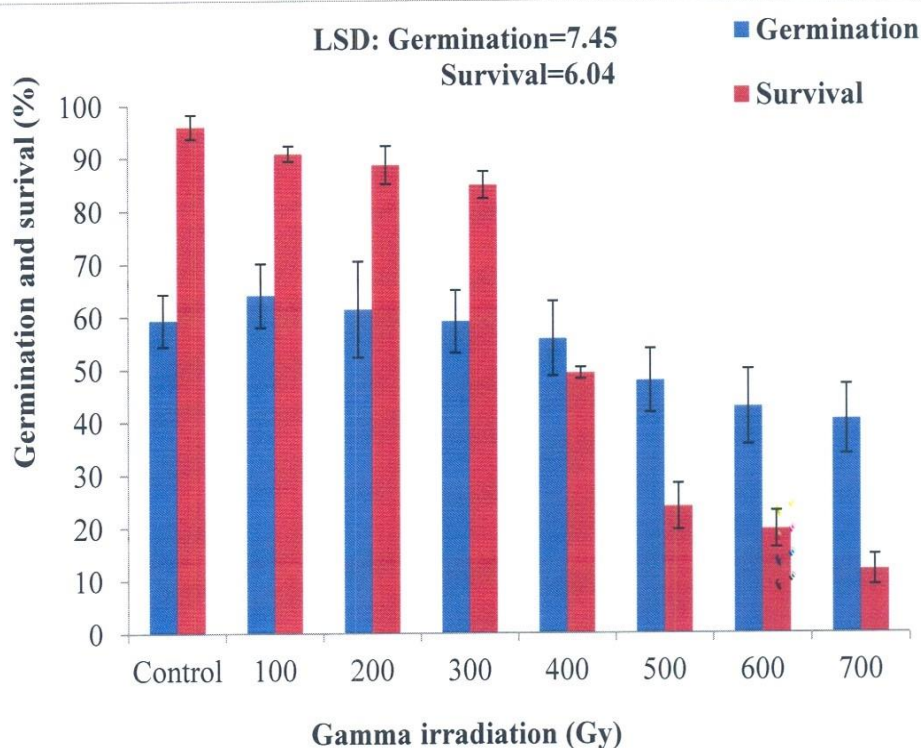
#### 4.1 Dosage response studies

##### 4.1.1 Seed germination and seedling survival

Seed germination and seedling survival were significantly ( $P < 0.05$ ) affected by gamma irradiation treatment. Lower gamma irradiation doses slightly enhanced seed germination over the control (Figure 1) but not statistically significant. The highest percentage in seed germination was recorded by plant irradiated with 100 Gy of Gamma rays. This was similar to percentages recorded for plants from 200, 300, 400 and 0 Gy plots (Figure 1). Contrary, 600 and 700 Gy gamma ray doses significantly reduced seed germination of millet.

The highest survival rate was recorded in the control plots, and this varied significantly from all the treatments (Figure 1). It was followed closely by plants from the 100 Gy plots, the performance of which was also significantly similar to plants from 200 Gy gamma irradiation treatments (Figure 1). Apparently, 400, 500, 600 and 700 Gy gamma irradiation treatments proved to be lethal, as plants in these plots recorded survival rates below 50% and were eliminated from subsequent field studies as shown in Table 2.





**Figure 1: Effect of gamma irradiation on seed germination and seedling survival in the M<sub>1</sub> generation at 3 WAP. Bars represent SEM.**

#### 4.1.2 Determination of LD<sub>50</sub>

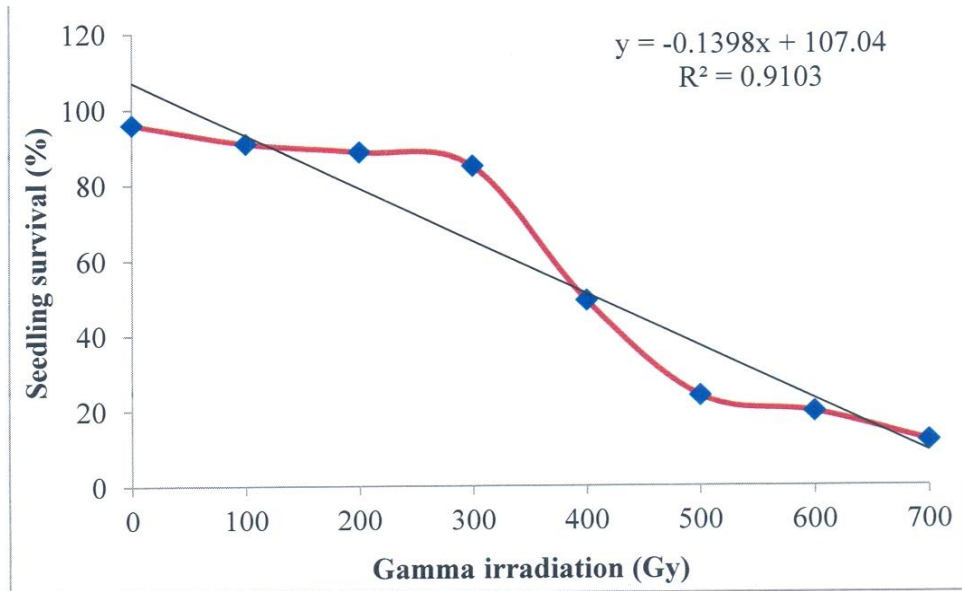
**LD<sub>50</sub>** is the gamma ray dose that will result in reductions of 50% in seedling survival of plants from the control plots. **From figures 2**

$$Y = -0.1398x + 107.04 \quad R^2 = 0.9103$$

LD<sub>50</sub> is the X value calculated with Y= 47.975 (50% of the control) LD<sub>50</sub>= 100.33 Gy







**Figure 2: Effect of gamma irradiation on seedling survival in the  $M_1$  generation at 3 WAP.**

## 4.2 Field studies during $M_1$ , $M_2$ and $M_3$ generations

### 4.2.1 Seed germination and plant survival

Results for seed germination and plant survival showed significant ( $P < 0.05$ ) effect due to gamma irradiation treatments in the  $M_2$  generation. In the  $M_3$  however, gamma ray irradiation did not significantly ( $P > 0.05$ ) affect this seed germination. In the  $M_2$  generation, the highest seed germination (92.1%) was recorded by plants from 100 Gy plots, and this varied significantly ( $P < 0.05$ ) from plants from the control, the 200 and 300 Gy plots (Table 3). Nonetheless, plants from the 0, 200 and 300 Gy plots produced relatively appreciable and significantly similar germination percentages (Table 3).

From table 4, survival rate was highest for plants from 100 Gy gamma irradiation treated plots, and was closely followed by plants from the control plots for the  $M_2$



generation. Survival rates recorded by plants from 200 and 300 Gy plots were significantly similar (Tab I [e 4]). The highest survival rate in the M3 was recorded by plant from the control plot, this was however significantly similar to plant from the remaining treatments.

**Table 3: Effect of gamma irradiation on seed germination of millet**

Gamma dose (Gy)	Germination (%)	
	M2 generation	M3 generation
0	85.03b	100.00
100	92.10a	99.50
200	87.50b	99.50
300	85.25b	99.25
<b>LSD (0.05)</b>	<b>4.99</b>	<b>1.01NS</b>

NS=Not significant; Means followed by the same letter (s) in each column are not significantly



**Table 4: Effect of gamma irradiation on survival of millet at 3 WAP**

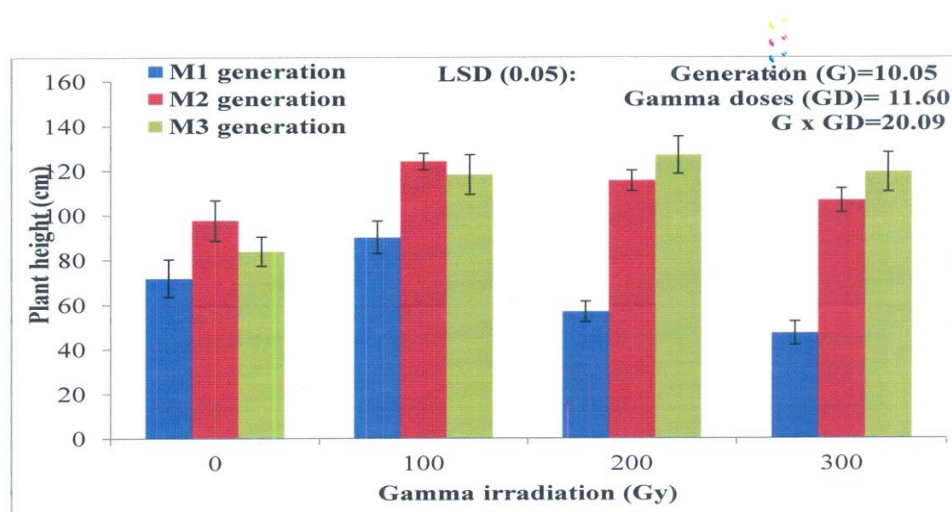
Gamma dose (Gy)	Survival (%)	
	M2 generation	M3 generation
0	99.58ac	98.00
100	99.58ac	97.50
200	98.18b	97.50
300	98.25bc	97.25
<b>LSD (0.05)</b>	<b>1.33</b>	<b>1.08NS</b>

NS=Not significant; Means followed by the same letter (s) in each column are not significantly different

#### 4.2.2 Plant height

Plant height was significantly ( $P < 0.05$ ) affected by gamma irradiation in all mutant generations. In the  $M_1$  generation, the highest mean height was recorded by plants from 100 Gy plot, and was significantly similar to height recorded by plants from the control plot (Figure 3). Plants from 200 and 300 Gy plots recorded significantly similar heights. The highest height in the  $M_2$  generation was obtained by plants from 100 Gy plots, it was however significantly similar to height reached by plants from 200 Gy plots. Plants from 300 Gy and the control plots also performed significantly similar in terms of height reached (Figure 3). In the  $M_3$  generation however, plants from 100, 200 and 300 Gy were not significantly different among themselves except with the control (Figure 3).





**Figure 3: Effect of gamma irradiation on plant height of millet at 10 WAP.**  
Bars represent SEM.

**Figure 3: Effect of gamma irradiation on plant height of millet at 10 WAP. Bars represent SEM.**

#### 4.2.3 Number of tillers

Gamma irradiation treatment had no significant ( $P>0.05$ ) effect on tillering in the M1 generation. However, in the M2 and M3 generations, tillering was significantly ( $P<0.05$ ) affected. In the M2 generation, plants from 100 Gy plots were outstanding and recorded the highest number of tiller, which varied significantly from all other treatments (Figure 4). It was followed by plants from 200 Gy plots, the performance of which was significantly similar to plants from the 300 Gy plots (Figure 4). In the M3 however, plants from 100, 200 and 300 Gy plots recorded significantly similar tiller numbers. In both generations, plants from the control plots recorded the least number of tillers, and varied significantly from all gamma irradiation treatments (Figure 4).

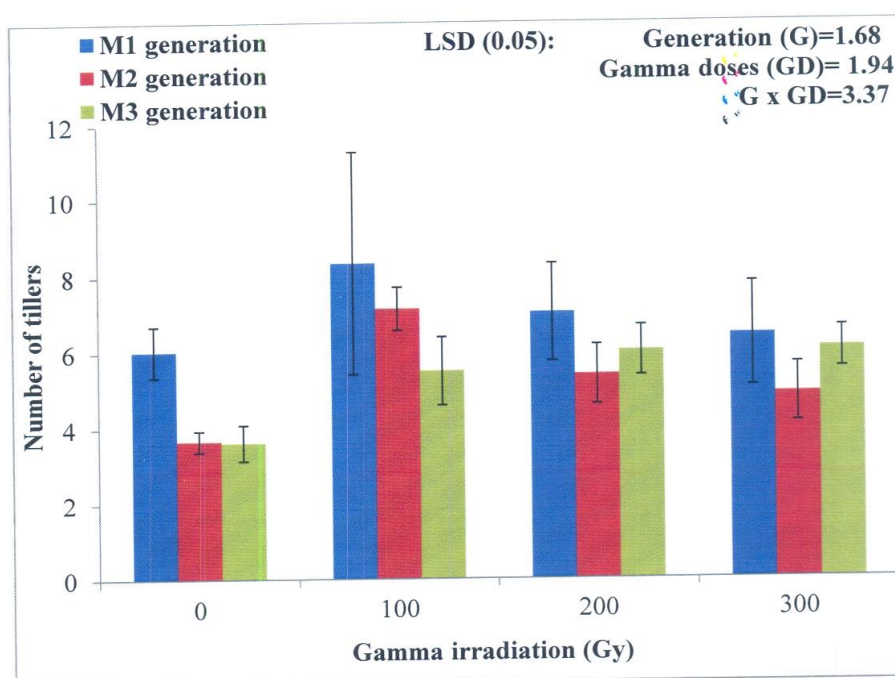
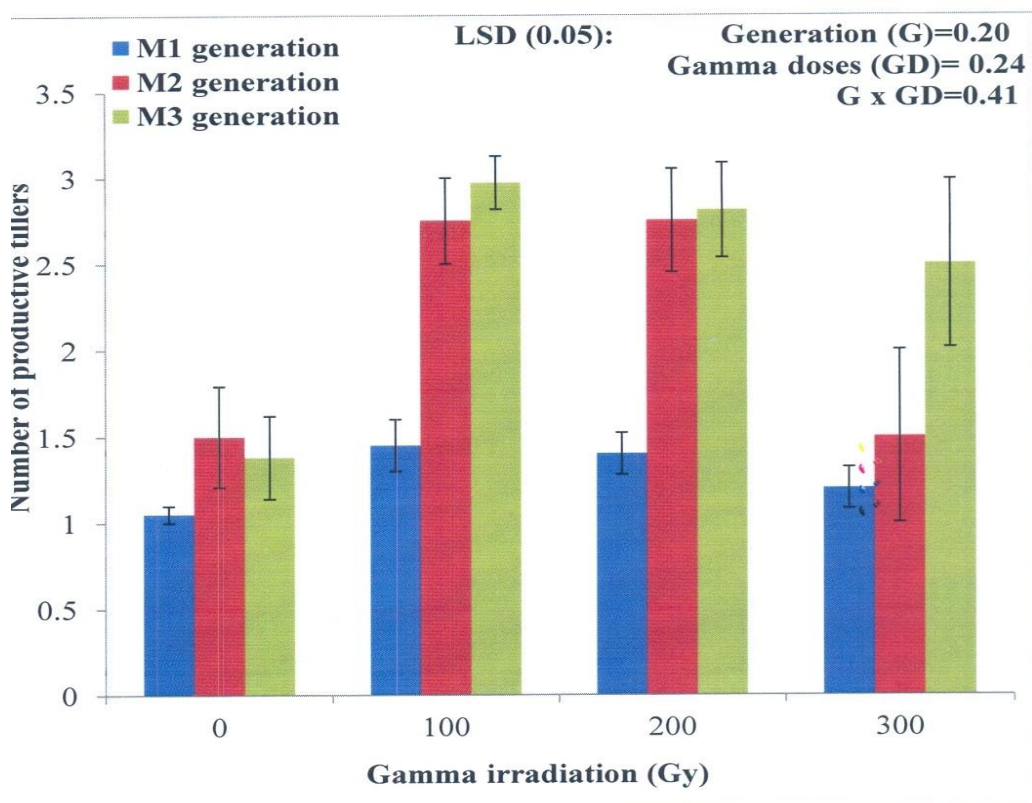


Figure 4: Effect of gamma irradiation on number of tillers. Bars represent SEM.

#### 4.2.4 Number of productive tillers

Gamma irradiation significantly ( $P < 0.05$ ) affected productive tillering in all mutant generations. Trend of productive tillering response to gamma ray treatment was similar in the  $M_1$  and  $M_2$  generations. The highest numbers of productive tillers in the  $M_1$  and  $M_2$  generations were recorded by plants from 100 and 200 Gy plots (Figure 5). This was followed by plants from 300 Gy plots. The control recorded least number of productive tillers and was significantly different from the remaining treatments.



**Figure 5: Effect of gamma irradiation on number of productive tillers. Bars represent SEM.**

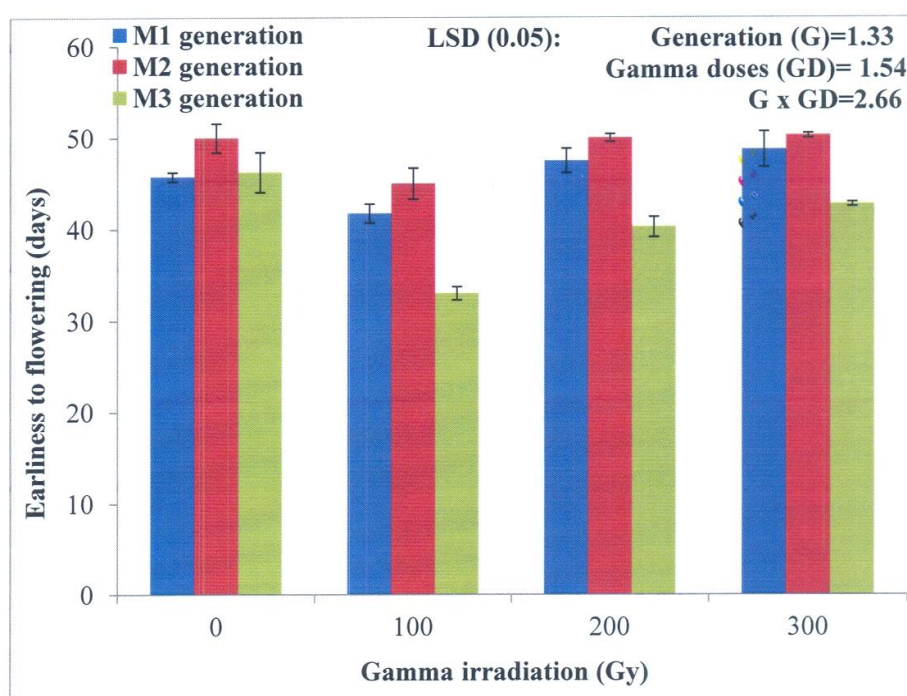


#### 4.2.5 Days to 50% flowering

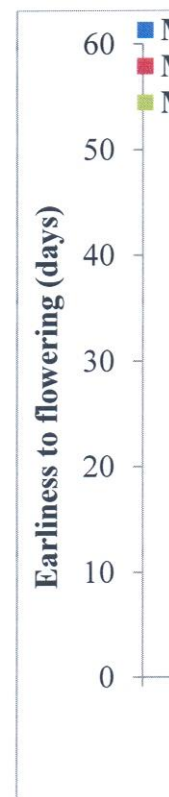
Gamma irradiation treatments generated significant ( $P < 0.05$ ) variability in flowering time (Figure 6). Trend of earliness in flowering was similar in both  $M_1$  and  $M_2$  generations. Evidently, plants from 100 Gy recorded the least mean number of days to attain 50% flowering, and varied significantly to number of days taken by plants from the control, 200 and 300 Gy plots. However, plants from the control, 200 and 300 Gy plots did not exhibit any significant variation in the mean number of days taken to attain 50% flowering; 45.75, 41.50 and 48.75



days respectively (Figure 6). Similarly, in the M3 generation, plants from 100 Gy plots earliest, and differed significantly from number of days taken by plants from control, 20 Gy plots to flower up to 50% (Figure 6). It was followed closely by plants from 200 an plots, the performance of which was significantly similar. Plants from the control plo highest number of days to achieve 50% flowering in all generations (figure6)



**Figure 6: Effect of gamma irradiation on earliness to flowering. Bars rent SEM.**



**Figure 6: E SEM.**

#### 4.2.6 Head length

Gamma irradiation did not significantly ( $P>0.05$ ) affect head length of millet in the  $M_1$  generation, it however affected it significantly in the  $M_2$  and  $M_3$  generations. Highest head length in the  $M_2$  generation was recorded by plants from 100 Gy plots, and differed significantly from plants from all other treatments (Figure 7). It was, respectively, followed by plants from 200 and 300 Gy plots of gamma irradiation, whose performances varied significantly from each other. Plants from the control plot recorded the least head length. Subsequently in the  $M_3$  generation, plants from all gamma ray treated plots, 100, 200 and Gy, similar head lengths, and except for 200 Gy different significantly from plants in(Figure 7)

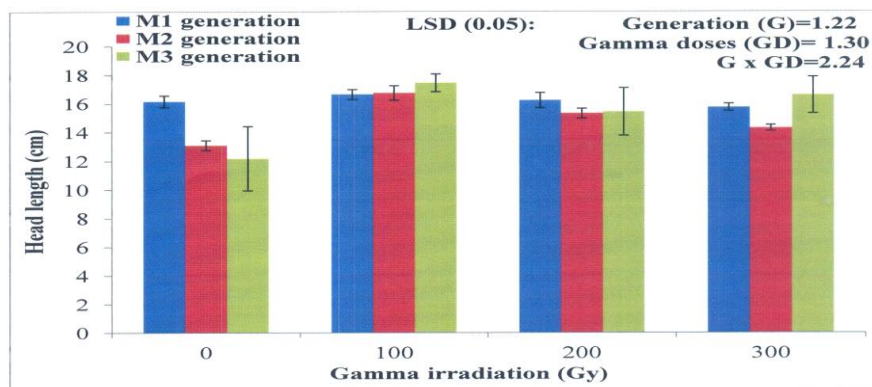


Figure 7: Effect of gamma irradiation on head length of millet. Bars represent SEM.



#### 4.2.7 Head Width

Gamma irradiation significantly ( $p < 0.05$ ) affected head width in all mutant generations. The widest width in the  $M_1$  generation was recorded by plants from 100 Gy plot (Figure 8). It was however significantly similar to head width of plants from the control plots, whose performance was in turn significantly similar to plants from 200 Gy plots. The shortest head width was recorded by plants from 300 Gy plots (Figure 8). Recording the highest head width in the  $M_2$  generation were plants from 100 Gy plots, and was significantly different from plants in 0 and 200 plots (Figure 8). In general, plants from the untreated control plots recorded the least values for head width. In the  $M_3$  generation, plants from 100, 200 and 300 Gy plots recorded statistically similar head widths and varied significantly from plants from the control plots (Figure 8).

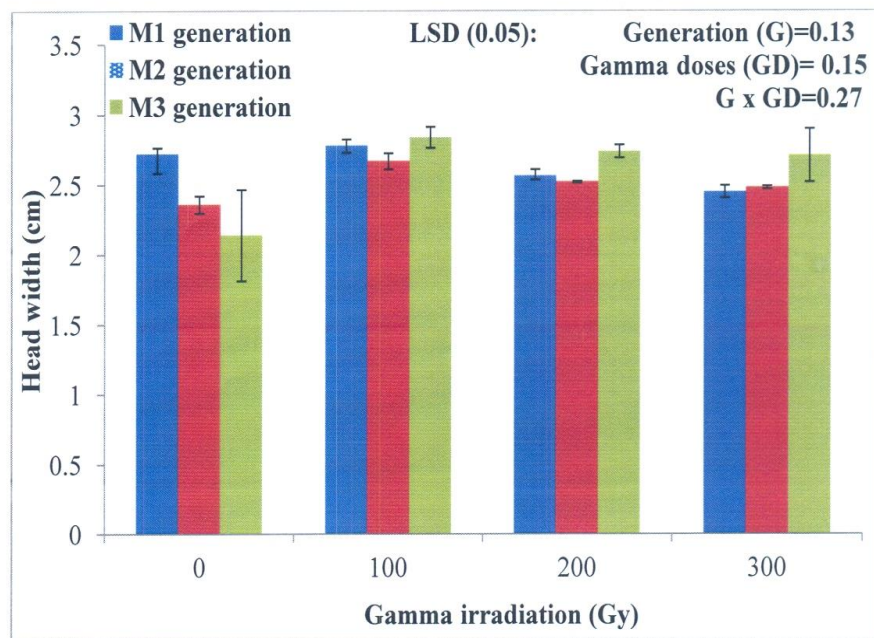


Figure 8: Effect of gamma irradiation on head width of millet. Bars represent SEM.



#### 4.2.8 Head weight

Gamma irradiation treatment significantly affected head weight in mutant generations.

Plants from 100 Gy plots recorded the highest head weight in the M<sub>1</sub> generation. This was, however, significantly similar to plants from 300 Gy plots (Table 5). It was followed by plants from the control and 200 Gy plots. Similar head weight was recorded by plants from the control and 100 Gy plots in the M<sub>2</sub> generation, while the least weight were recorded by plants from 300 Gy plots. In the M<sub>3</sub> generation, plants from the control, 100 and 200 Gy plots recorded significantly similar head weights (Table 5).

**Table 5: Effect of Gamma irradiation on head weight of millet**

**LSD (0.05): Gamma doses= 2.94 Generation=2.55**

Gamma

irradiation (Gy)

Head weight (g)

Generations	M1	M2	
0 20.86ab	12.29ab	25.10a	
100 22.81ab	16.16a	22.40ab	
200	12.62b	20.50bc	23.55a
300	15.09ab	16.30c	20.38b

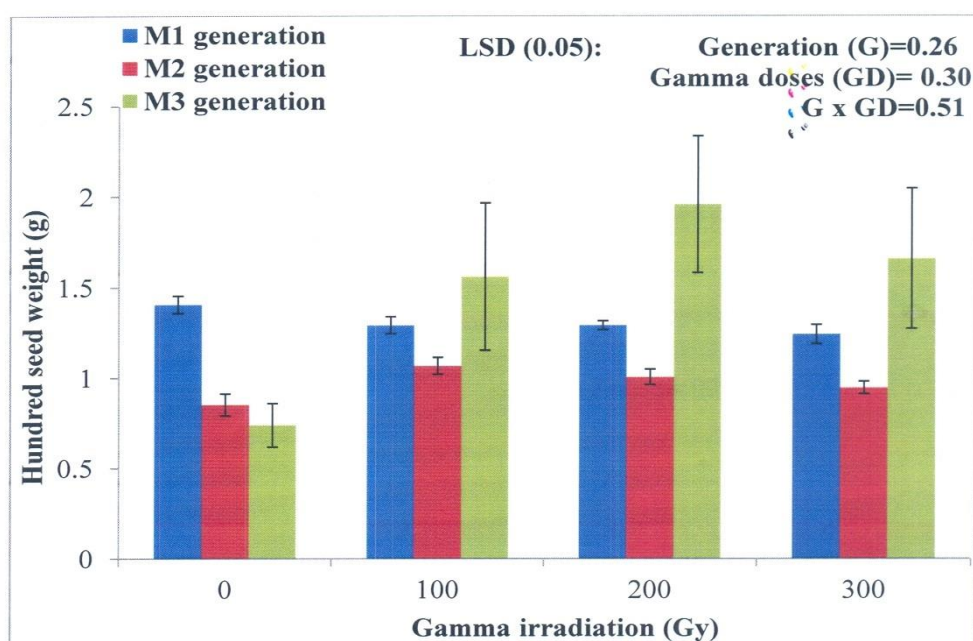
**Gamma doses x generation=5.01**

**CV (%)=18.60**

**NS= Not statistically different; Means followed by the same letter (s) in each column are not significantly different**

#### 4.2.9 100 seed weight

Gamma irradiation significantly ( $P < 0.05$ ) affected 100 seed weight of millet in the **M1**, **M2** and **M3** generations. Plants in control plots recorded the highest weight for this trait in the **M1** generation, and varied significantly from plants from 100, 200 and 300 Gy plots (Figure 9). The highest weight for 100 seeds count in the **M2** generation was recorded by plants from 100 Gy plot, this was, however, significantly similar to the weight recorded by plants from 200 and 300 Gy plots (Figure 9). Nevertheless, the weight recorded by plants from 300 Gy plot was significantly similar to plants from the control plots. In the **M3** generation, plants from all gamma irradiation treatments recorded significantly higher values than the control, but performed significantly similar among themselves (Figure 9).

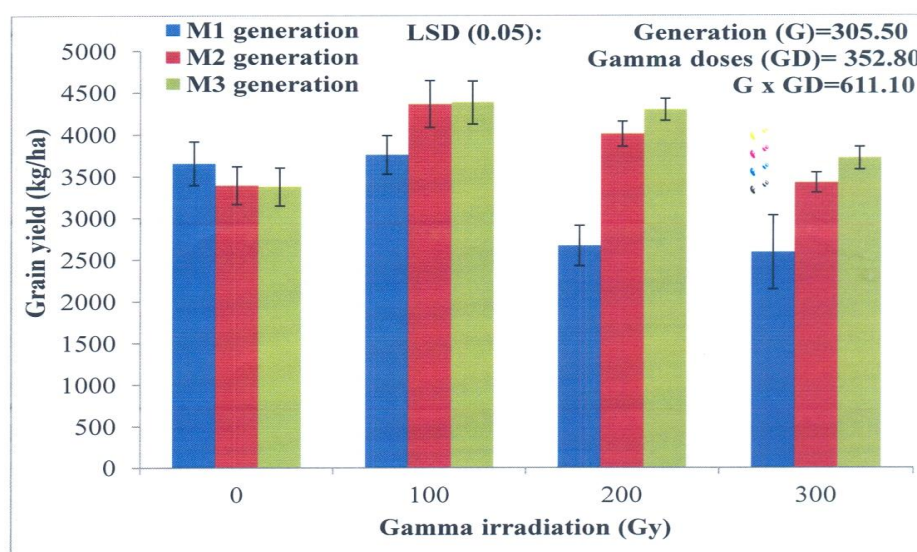


**Figure 9: Effect of gamma irradiation on 100 seed weight. Bars represent SEM**



#### 4.2.10 Grain yield

Grain yield was significantly ( $P < 0.05$ ) affected by gamma irradiation treatment in all the mutant generations. Grain yield was enhanced in plants treated at 100 Gy in M1 generation compared with the control plots, though the difference was not significant (Figure 10). Similarly, plants from 200 and 300 Gy plots recorded significantly similar grain yields. Pattern of grain yield response to gamma ray treatment was similar in the M2 and M3 generations. The highest grain yield was recorded by plants from 100 Gy treatment, it was, however, significantly similar to yield obtained by plants from 200 Gy plots (Figure 10). This was followed by plants from the control plot, yield of which was similar to plants from 300 Gy plot.



**Figure 10: Effect of gamma irradiation on grain yield of millet. Bars represent SEM.**



### 4.3 Genetic advance to selection

Observed genetic advancement in the M<sub>2</sub> and M<sub>3</sub> generations were highly variable. Some characters exhibited low percentage improvements, while others recorded higher percentages. Some characters recorded increments in the M<sub>2</sub> generation, but consequently decreased in the M<sub>3</sub> generation (Table 6). There was an increase in mean value of respective gamma irradiation treatments for characters such as plant height, number of productive tillers, head length, width and weight, and grain yield. Improvement in plant height, productive tillers and grain yield were recorded by all gamma ray doses in the M<sub>2</sub> generation. Consequently, 100, 200 and 300 Gy gamma ray doses improved plant height by 37.85, 102.81 and 125.64%, productive tillers by 89.66, 96.43 and 25.00%, and grain yield by 16.10, 50.19 and 32.21% over the M<sub>1</sub> generation (Table 6). Improvement in head length (0.60%) and width (1.22%) were recorded by plants from the 100 and 300 Gy gamma ray doses, respectively (Table 6). In the M<sub>3</sub> generation, all gamma ray doses improved all parameters measured, however, plant height and number of tillers were decreased by 100 Gy gamma ray dose (Table 6).



**Table 6: Genetic advancement in M2 and M3 populations**

Gamma ray doses (Gy)	Character	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>2</sub> -M <sub>1</sub>	M <sub>3</sub> -M <sub>1</sub>	M <sub>3</sub> -M <sub>2</sub>	Genetic advance in the M <sub>2</sub> over M <sub>1</sub> (%) [(M <sub>2</sub> -M <sub>1</sub> )/M <sub>1</sub> × 100]	Genetic advance in the M <sub>3</sub> over M <sub>1</sub> (%) [(M <sub>3</sub> -M <sub>1</sub> )/M <sub>1</sub> × 100]	Genetic advance in the M <sub>3</sub> over M <sub>2</sub> (%) [(M <sub>3</sub> -M <sub>2</sub> )/M <sub>2</sub> × 100]
100	Plant height	90.20	124.10	118.2	33.90	28	-4.75	37.85	31.04	-4.75
	Tiller number	8.35	7.15	5.50	-1.20	-2.85	-1.65	-14	-34.13	-23.08
	Productive tiller	1.45	2.75	2.97	1.30	1.52	0.22	89.66	104.83	8.00
	Earliness	41.75	45.00	33.00	3.25	-8.75	-(-12)	8.89	-0.21	-(-26.67)
	Head length	16.64	16.74	17.45	0.11	0.81	0.71	0.60	4.87	4.27
	Head width	2.78	2.67	2.84	-0.11	0.06	0.17	-3.96	2.16	1.02
	Head weight	16.16	22.40	22.81	6.24	6.68	0.41	38.61	41.34	1.83
	100 seed weight	1.29	1.07	1.56	6.24	0.27	0.49	-17.05	20.93	45.79
	Grain yield	3758	4363	4381	605.00	623.00	18.00	16.10	16.58	0.41
200	Plant height	56.90	115.40	126.90	58.50	70.00	11.50	102.81	123.02	9.97
	Tiller number	7.04	5.40	6.03	-1.64	-1.01	0.63	-23.30	14.35	11.67
	Productive tiller	1.40	2.75	2.81	1.35	1.41	0.06	96.43	100.71	2.18
	Earliness	47.50	50.00	40.25	2.50	-7.25	-(-9.75)	5.26	-15.26	-(-19)
	Head length	16.23	15.32	15.43	-0.91	-0.80	0.11	-5.61	-4.92	0.72
	Head width	2.57	2.52	2.74	-0.05	0.17	0.22	-1.95	6.61	8.73
	Head weight	12.62	20.50	23.55	7.88	10.93	3.05	65.56	86.61	14.88
	100 seed weight	1.29	1.01	1.96	-0.28	0.67	0.95	-21.71	51.94	94.06
	Grain yield	2668.00	4007.00	4296.00	1339.00	1628.00	289.00	50.19	61.02	7.21



300	Plant height	47.20	106.50	119.20	59.30	72.00	12.7	125.64	152.54	11.92
	Tiller number	6.45	4.90	6.09	-1.55	-0.36	1.19	-24.03	-5.58	24.29
	Productive tiller	1.20	1.50	2.59	0.30	1.39	1.09	25.00	115.83	72.67
	Earliness to flowering	48.75	50.25	42.75	1.50	-6.00	-(-7.5)	3.08	12.31	-(-14.93)
	Head length	15.74	14.30	16.60	-1.44	0.86	2.30	-9.15	5.46	16.08
	Head width	2.45	2.48	2.71	0.03	0.26	0.23	1.22	10.61	9.27
	Head weight	15.09	16.30	20.83	1.21	5.74	4.53	8.02	38.04	27.78
	100 seed weight	1.24	0.95	1.66	-0.29	0.42	0.71	-23.39	33.87	74.73
	Grain yield	2589.00	3423.00	3717.00	834.00	1128.00	294.00	32.21	43.68	8.59



## CHAPTER FIVE

### DISCUSSION

#### 5.1 Seed germination and seedling survival

The stimulating effect of lower doses of gamma ray on germination may be as a result of the activation of RNA or protein synthesis, which occurred during the early stages of germination after seed irradiation (Abdel-Hady *et al.*, 2008). Percentage reduction in seed germination at higher doses might have been due to the effect of gamma rays on meristematic tissues of the seed. It may also be attributed to disturbances at cellular level, caused either at physiological or physical level. Kumar and Mishra (2004) reported in okra (*Abelmoschus esculentus*) that germination percentage generally decreased with increasing doses of gamma irradiation. Reduced germination percentage with increasing doses of gamma irradiation has also been reported in *Pinus* (Thapa, 2004), Rye (Akgun and Tosum, 2004) and Chickpea (Khan *et al.*, 2005; Toker *et al.*, 2005). Gamma irradiation above 300 Gy resulted in less plant survival and accounted for their elimination from subsequent experiments. These results obtained in the present study were in accordance with the study by Moghaddam *et al.* (2011) on *Centella asiatica*, who noted that plantlet survival rate kept decreasing with increasing irradiation dosage for three weeks after irradiation. The study by Kiong *et al.* (2008) indicated that survival of plants to maturity after mutation depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose, among the factors, may be





responsible for less germ inability and reduction in plant growth and survival. These results are in agreement with those obtained by Park *et al* (2008) on *Hosta plantaginea*, Golubanova and Gecheff (2011) on Sudan grass and Cheema and Atta (2003) on Basmati rice.

According to Sani *et al* (2013) seedling height is a good indicator of the biological effect of gamma rays. The LD50 is the gamma ray dose that will result in 50% reduction in seedling survival in the control. This dosage is expected to produce enough genetic modification that will lead to new, desirable and viable mutant lines. It is also the gamma ray dose above which seedling survival is significantly reduced. The value obtained for LD<sub>50</sub> (309 Gy) in the present study, however, disagrees with the findings of Sani *et al* (2013) and Ambli and Mullainathan (2014) who predicted LD<sub>50</sub> in pearl millet to be 669.3 Gy and 20 Krd (200 Gy), respectively. Variations in LD50 values of the two studies may be due to differences in the genotypes used for the study and the environment in which they were evaluated.

## 5.2 Vegetative growth, tillering and flowering

The result indicated that gamma rays could cause a broad genetic variability in plant height. Stimulatory effect was observed in lower doses of gamma rays on plant height, and this may be due to changes in cell division rates as well as an activation of growth hormone, such as auxin (Zaka *et al.*, 2002; Gunckel and Sparrow, 1961). Wi *et al* (2007) noted that low doses of irradiation will induce the growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti-oxidative capacity of the cells to easily overcome stress





induced by the irradiation treatment. According to De-Vita *et al* (1993) water radiolysis, the predominant effect of ionizing radiation in organisms induces reactive oxygen species (ROS) formation. Therefore, in general, plant, bacterial and animal enzymes that are involved in cell protection against oxidative stress will display similar responses under ionizing radiation stress as under other stress factors (Zaka *et al.*, 2002). In contrast, high irradiation doses that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome (Preussa and Britta, 2003). Similar results were obtained by El-Ashry *et al.*, (1992) on *Lathyrus odoratus*, El-Mahrouk (2000) on *Gomphrena globose* and Abdel-Maksoud (1992) on *Solanum pseudocapsicum*.

Auxiliary bud development and floral differentiation, in general, occur at an early stage in plant development. Results reported by Khangyldin (1967) indicated that an increase in kinetin to auxin ratio increased buds, leaves and shoots growth. The same hormonal imbalance may be caused by gamma irradiation. The production of the growth hormone, kinetin is known to increase proliferation of tillers (Khangyldin, 1967). These results are in agreement with those obtained by El-Ashry *et al* (1992) on *Lathyrus odoratus* and Koli *et al* (2002) on Cumin. It is possible that gamma irradiation affects biochemical processes such as auxin levels that are known to regulate the rate and pattern of apical differentiation at these stages. This could have a direct effect on subsequent agronomic performance of a plant, such as production and proliferation of tillers. Low gamma ray doses are also believed to play a pivotal role in maintaining hormonal balance.

Reduction in tiller at higher doses could be attributed to reduced mitotic activities in meristematic tissues and probably reduced moisture content in seeds as reported by Muhammad and Afsari (2001). Similarly, Norfazrin *et al* (2001) noticed that higher gamma ray doses (600 and 800 Gy) had negative effect on the morphological characteristics of tomato and okra derived from irradiated seeds. A reduction in tillering in crops that are exposed to higher gamma ray doses had already been reported by Al-Salhi *et al* (2004), Token *et al.* (2005) and Kon *et al.* (2007).

The findings obtained in this study showed that gamma rays can change the number of days to flowering. There have been earlier reports by Karim *et al* (2008) on the potential of gamma ray to change flowering in either positive or negative direction. Mahala *et al.* (1990) found that mutagenesis could widen variability to either positive or negative direction and this will result in sufficient variability in the treated population and serve as basis for selecting early or late flowering in plants. The high efficiency of lower doses of gamma rays to stimulate early flowering is probably due to the fact that biological damage such as seedling injury, lethality and sterility increases with the increase in dose at faster rate during mutations (Konzak *et al.*, 1965). Suppressive effect by high doses of gamma rays may be related to auxin and DNA biogenesis in a relationship. Deoxyribonucleic acid is required for and is previously synthesized sequentially to auxin formation, the radiation block occurring in the formation of nucleic acid. The primary radiation block is in auxin synthesis, the auxin required for the





formation of DNA (Jan *et al.*, 2012). Generally, the effect of radiation is on an undefined entity in reaction and essential for both DNA and auxin synthesis (Jan *et al.*, 2012; Lage and Esquibel, 1995). Decreases in efficiency and negative effects on plant of gamma rays with increase in doses has also been observed in Foxtail millet by Gupta (2001) and in chickpea by Kharkwal (1998).

### 5.3 Yield components and yield

The beneficial effect of gamma rays on yield, especially at low doses has been reported in several investigations (Khizar *et al.*, 1990; Larik *et al.*, 2009). The results obtained in the can be compared with the studies of Veeresh *et al* (1995) who recorded an increase in seed and pod weight of winged bean at low doses of gamma irradiation, and a decrease at higher doses. Similarly, Kon *et al* (2007) reported a declining tendency in seed weight of long beans when exposed to higher gamma radiation doses. Similar results were reported by Kaul and Bradu (1972) in *Atropa belladonna*, Suhas *et al* (1976) in *Cassia angustifolia*, Selenia and Stepanenko (1979) in *Matricaria recutita* and Youssef *et al* (1998) in geranium. Reduction in seed weights at higher doses might be due to reduced plant stature or reduced moisture contents in shoot due to radiation effect (Abdul Majeed *et al.*, 2010).

According to Maman *et al* (2003) yield differences in agronomic crops are associated with yield components. van Oosterom *et al* (2002) reported that yield component studies with pearl millet have shown the number of productive tillers per plant correlated with yield. Correlation of seed and head weight has also been reported in related studies by Limon-Ortega *et al* (1998). The ability of low

gamma rays to enhance number of productive tillers, head and seed weight in this study therefore consequently resulted in yield enhancement in the present study.

Increased genetic advance in the treated populations in the present study are in conformity with results obtained for sesame and groundnut by Chavan and Chopde (1982) and Mathur *et al* (2000).



## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

Millet is relatively the most dependable in terms of productivity in the semi-arid regions of Africa, but is still a neglected and under-utilized crop. Current yield levels are low under the local environmental conditions. Therefore, in the present study, irradiation techniques were applied to investigate its effect on growth and yield in pearl millet in the Guinea savannah agro ecological zone of Ghana by irradiating with gamma ray's doses of 100, 200, 300 Gy and a control (0 Gy).

Sensitivity of the variety of pearl millet to gamma irradiation in the study area has been successfully predicted. The efficiency and success of plant breeding is in the existence of genetic variability and selection in the segregating populations. These were achieved in the present study. Superior strains were screened and selected based on variation in growth and yield traits in the M2 for advancement to M3 generation and also in the M3 for advancing into subsequent generations. The results of the study revealed significant variation in growth parameters, yield components and grain yield following the gamma ray treatments. Gamma irradiation has in fact, induced sufficient genetic variability in Millet. Grain yield and yield components were essentially used as selection criteria to screen desirable and superior lines for increased yield. High yielding mutant lines obtained could play a major role in breaking the yield constraints in pearl millet and improve the economic status of farmers in the study area.



Among the gamma ray treatments, 100 Gy predominantly enhanced almost all growth and yield parameters measured in all the generations as compared to the uneradicated control. Though, 200 and 300 Gy gamma ray treatments performed less than the control in most parameters measured in the M<sub>1</sub> generation, their performance subsequently increased significantly better than the control progressively in the M2 and M3 generations. All gamma ray doses enhanced earliness to flowering than the control in the segregating generations, among which 100 Gy was outstanding.

## 6.2 Recommendations

The study therefore recommends that;

- Promising lines selected from the M3 generation be advanced further.
- The scope of studies in future generations be broaden to include nutritional and molecular analysis of selected promising mutant lines and their reaction with biotic (insects and pests) and abiotic (drought, temperature, flood) stresses in the study area.



## REFERENCES

Abdel- Maksoud B. (1992). Gamma rays effect on *Solanum pseudocapsicum* L in the M1 generation. Alexandria journal of Agriculture Research, 37:227-247.

Abdul Majeed, Asif Ur Rahman K., Habib A. and Zahir M. (2010). Gamma irradiation effect on

some

Growth parameters of *Lepidium sativum* L. ARPN. Journal of Agriculture and Biological

Science, 5(1): 39-42.

Abdel-Hady M. S., Okasha E. M. Soliman S. S. A. and Talaat M. (2008). Effect of gamma radiation and

gibberellic acid on germination and alkaloid production in *Atropa belladonna* Aust. Journal of

Basic and Applied Science, 2(3): 401-405.

Abuali A. I., Abdelmulla A. A. and Idris A. E. (2012). Character association and path analysis in Pearl Millet

(*Pennisetum glaucum* L.). American Journal of Experimental Agriculture, 2 (3): 370-381.



Ahloowalia B. S. and Maluszynski M. (2001). Induced mutations - A new paradigm in plant.

Euphytica, 118 (2): 167-173.

Ahloowalla B. S., Maluszynski M. and Nichterlein K. (2004). Global impact of mutation

derived

varieties. Euphytica, 135: 187-204.

Akgun I. and Tosum M. (2004). Agricultural and cytological characteristics of M<sub>1</sub> rye

(*Secalmontanum*Guss.) as effected by the application of different doses of gamma

rays.Pakistan Journal of Biological Science, 7(5): 827- 833.



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

Alumira J. and Rusike J. (2005). The Green Revolution in Zimbabwe. *Journal of Agricultural and Development Economics*, 2 (1):50-66.

Ambli K. and Mullainathan L. (2014). Effect of gamma rays and EMS on seed germination and seed characters in Pearl millet (*Pennisetum typhoides*) (Burn.) Stapf. Var. CO(Cu)-9. *Journal of Chemical, Biological and Physical Science. Section B*; Vol.4, No.4; 3345-3349.

Andrews D. J. and Kumar K. A. (1992). Pearl millet for food, feed and forage. *Advances in Agronomy*. 48: 89-139.

Anitha Y. V., Vennila S. and Ganesan J. (2005). Mutation - an alternate source of variability. UGC national seminar on present scenario in plant science research, Annamalai University, Annamalainagar, pp 42.

Ariraman M., Gnanamurthy S., Dhanavel D., Bharathi T. and Murugan S. (2014). Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon pea (*Cajanus cajan* (L.) Millsp). *International Letters of Natural Sciences*, 16: 41-49.

Artk C. and Peksen E. (2006). The effects of gamma irradiation on seed yield and some plant characteristics of faba bean (*Vicia faba* L.) in M2 generation. *Ondokuz-Mays-Universitesi, -Ziraat- Fakultesi-Dergisi*, 21 (1): 95-104.





Ashraf M., Cheema A. A., Rashid M. and Qamar Z. (2003). Effect of gamma rays on Ma generation in Basmati rice. Pakistan Journal of Botany, 35(5):791-795.

Avinash A. (2013). Effect of gamma irradiation on yield attributing characters in two varieties of Pea (*Pisum sativum* L.). International Journal of Life Sciences, Vol. 1 (4):241-247.

Bashir E., Haussmann B., Ali A., Ismail M., Abdalla E., Bakhit O., Sabeel S., Muth P. and Panics H. (2011). Genetic analysis and bio fortification of Pearl Millet for Nutritional Values. Conference of Development on the margin, Tropentag 2011, October 5 - 7, Bonn, Germany.

Brock R. D. (1971). The role of induced mutations in plant improvement. Radiation Botany, 11: 181-196.

Brunken J. N. (1977). A systematic study of Pennisetum sect. pennisetum (Gramineae). American Journal of Botany, 64:161-176.

Brutnell T. P., Wang L., Swartwood K., Goldschmidt A., Jackson D., Zhu X. G., Kellogg E. and

Van Eck J. (2010). *Setaria viridis*: A Model for C4 Photosynthesis, Plant Cell, 22: 2537-2544.

Bruinsma J. (2009). The resource outlook to 2050: By how much do land, water use and crop yields need to increase by 2050? pp 33. Expert Meeting on How to Feed the World in 2050. Rome, FAO and ESDD.



**BSTID-NRC (Board on Science and Technology for International Development-National Research Council). (1996).** Lost crops of Africa. Academic Press, Washington D.C. pp. 127-213.

Burton G. W. and Powell J. B. (1966). Morphological and cytological response of pearl millet (*Pennisetum*

*typhoides*) to thermal neutrons and ethyl methane sulfonate seed treatments, Crop Science Journal, 6:180.

Burton G. W. and Hanna W. W. (1982). Stable cytoplasmic male sterile mutants induced in Tift 23DB, pearl millet with mitomycin and streptomycin, Crop Science Journal, 6: 194.

Chandrasekara A. and Shahidi F. (2011). Ant proliferative potential and DNA scission inhibitory activity of phenolic from whole millet grains, J. Funct. Foods, 3: 159-170.

Chatham House (2009). The Feeding of the Nine Billion: Global Food Security for the 21st Century; Chatham House: London, UK.

Chavan G. V. and Chopde P. R. (1982). Polygenic variability, heritability and genetic advance in irradiated sesame. Journal of M.arathwada Agricultural Universities, 7: 17-19.

Cheema A. A. and Atta B. M. (2003). Radio sensitivity studies in basmati rice. Pakistan Journal of Botany, 35: 197-207.

Chopra V. L. (2001). Breeding Field Crops. Oxford: IBH Publishing Co Pvt Ltd.



- Crino P., Bonito R. D. and Veronese P. (1994). Genetic variability in tomato plants regenerated from irradiated cotyledons. *Journal of Genetics and Breeding*, 48: 281-290.
- Davis A. J., Dale N. M. and Ferreira F. J. (2003). Pearl millet as an alternative feed ingredient in broiler diets. *Journal of Applied Poultry Research*, 12:137-144.
- Dendy D. A. (1995). Sorghum and millets: Production and importance. pp. 11-26. *In*: D.A.V. Denby (ed.), *Sorghum and millets: Chemistry and technology*. American Association of Cereal Chemists, Inc., St. Paul, M.N.
- Desai A. S. and Rao S. (2014). Effect of gamma radiation on germination and physiological aspects of pigeon pea (*Cajanus cajan* (L.) MILLSP) seedlings. *International Journal of Research in Applied, Natural and Social Sciences*, Vol. 2, Issue 6, 47-52.
- De-Vita J. R., Samuel V. T. and Rogenberg S. A. (1993). *Cancer, Principles and Practice of Oncology*. 4th edn. Philadelphia, Lippincott Co.
- Donini P. and Sonnino A. (1998). *Somaclonal Variation and Induced Mutations in Crop Improvement*, eds., Kluwer Academic Publishers, Dordrecht, pp 255-291.
- ECARSAM. (2004). Country reports.
- Ejeta G., Hansen M. M. and Mertz E. T. (1987). In vitro digestibility and amino acid composition of Pearl millet (*Pennisetum typhoides*) and other cereals. *Proceedings of National Academy of Sciences (USA)*, 84: 6016-6019.

El-Ashry A. I., Zagloul M. A. and Ghait E. A. A. (1992). Physiological studies on *Lathyrus odoratus*. 2. Effect of gamma radiation on growth and flowering of *Lathyrus odoratus* L., Bulletin Suez Canal University Applied Science, 1: 506521.

Elliot F. C. (1958). Plant breeding and cytogenetics. McGraw Hill Book Co. New York, U.S.A.

El-Mahrouk M. E. M. (2000). Induction of genetic variability in *Gomphrena globosa* L. plant by gamma-rays. Master of Science Thesis, Faculty of Agriculture, Tanta University Kafr El- Sheikh.

Esele J. P. (1995), Folia and head diseases of sorghum. African Crop Science Journal, 3:185-189.

Fasoula V and Fasoula A. (2002). Principles underlying genetic improvement for high and stable crop yield potential. Field Crops Research, 75:191-209.

Food and Agriculture Organization of the United Nations/ World Food Program (FAO/WFP). (2013). "High Level Consultation on Hunger, Food Security and Nutrition in the Post-2015 Development Framework". FAO/WFP led thematic consultation.

Food and Agriculture Organization (FAO). (2012). World Agriculture Towards 2030/2050: The 2012 revision ESA E Working Paper No. 12-03.

Food and Agriculture Organization of the United Nations (FAO). (2011). Save and Grow—A Policy Maker's Guide to the sustainable intensification of smallholder crop production. Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO). (2010). the State of Food Insecurity in the World 2010; World Bank. 2007; World Bank. 2008. Rising Food and Fuel Prices: Addressing the Risks to Future Generations. Washington, DC: World Bank.

Food and Agriculture Organization of the United Nations. (2009). How to Feed the World in 2050; Food and Agriculture Organization of the United Nations: Rome, Italy.

Food and Agriculture Organization (FAO). (2008). Emergency and Rehabilitation assistance: Consolidated appeals, Zimbabwe.

Food and Agriculture Organization of the United Nations (FAO). (2007). Annual Publication. Rome, Italy: Food and Agricultural Organization.

Food and Agriculture Organization of the United Nations (FAO). (2004). Food and Agriculture Organization of United Nations, 2004. FAOSTAT.

Food and Agriculture Organization of the United Nations (FAO). (2003). "World agriculture: towards 2015/2030 — An FAO perspective". Earth Scan. Rome, Italy.





Food and Agriculture Organization and International Institute for Applied Systems Analysis. (FAO/IIASA). (2000). Global agro-ecological zones-2000. Rome, Italy: FAO; and Laxenburg, Austria: IIASA.

Food and Agriculture Organization of the United Nations (FAO). (1996a). The Sixth world food survey. Rome.

Food and Agriculture Organization of the United Nations (FAO). (1996b). Rome Declaration and World Food Summit Plan of Action. Rome.

Fischer G., Van Velthuizen H., Shah M. and Nachtergaele F. O. (2002). Global agro-ecological assessment for agriculture in the twenty-first century: methodology and results. (Available at: HYPERLINK. "<http://www.iiasa.ac.at/Admin/PUB/Documents/RR-02-002.pdr> <http://www.iiasa.ac.at/Admin/PUB/Documents/RR-02-002.pdf>).

Ghafoor A. and Siddiqui K. A. (1976). Effect of gamma rays and fast neutrons treatments on tillers number and plant height in wheat. Pakistan Journal of Botany, 8:1-7.

Giri S. P. (2014). Studies of mutagenic sensitivity in Pigeon pea [*Cajanus Cajan* (L.) Mill sp.]. Bioscience Discovery, 5(2):227-229.

Golubanova I. and Gecheff K. (2011). M<sub>1</sub> cytogenetic and physiological effects of gamma-ray's in Sudan grass (*Sorghum sudanense* (Piper.) Stapf). Bulgarian Journal of Agriculture Science, 17(4): 417-423.

Govindaraj M., Shanmugasundaram P., Sumathi P. and Muthiah A. R. (2010). Simple, rapid and cost effective screening method for drought resistant breeding in Pearl millet.

ElectronicJournal of Plant Breeding, 1(4): 590- 599 (July 2010)

Gowda C. L. L., Serraj R., Srinivasan G., Chauhan Y. S., Redd B. V. S., Rai K. N., Nigam S. N., Gaur P. M., Reddy L. J., Dwivedi S. L., Upadhyaya H. D., Zaidi P. II., Rai H. K., Maniselvan P., Follkerstma R. and Nalini M. (2009). Opportunities for improving crop water productivity through genetic

enhancement of dryland crops. CAB International, 2009. Rain-fed A Unlocking the Potential.

Guenet J. L. (2004). Chemical mutagenesis of the mouse genome: an overview. Mutagenesis of the mouse genome, pp 9-24.

Gunckel J. E. and Sparrow A. H. (1961). Ionizing radiation: Biochemical, Physiological and Morphological aspects of their effects on plants. *In*: Encyclopedia of Plant Physiology. (ed.) W. Ruhland. XVI: pp. 555-611, Springer-verlag, Berlin.

Gupta P. K. (2001). Induced mutations in Foxtail millet (*Setaria italica Beauv*), chlorophyll mutations induced by gamma rays, EMS and DES. Theoretical and Applied Genetics, 45, pp. 242-249.

Gustafsson A., Hagberg A., Persson G. and Wikland K. (1971). Induced mutation and barley improvement. Theoretical and Applied Genetics. 41: 239-48.







Hanna W. W. and Young J. R. (1974). Gamma radiation effects on pearl millet pollen, *Crop Science Journal*, 14:600.

Hassan, S. (1986). Gamma irradiation induced variations in genetic parameters of wheat and tritical. *Sarhad Journal of Agriculture*, 2: 173-9.

Hidalgo M. A., Davis A. J., Dale N. M. and Dozier W. A. (2004). Use of whole pearl millet in broiler diets. *Journal of Applied Poultry Research*, 13:229-234.

Hill G. M. and Hanna W. W. (1990). Nutritive characteristics of pearl millet grain in beef cattle diets: Issues in New Crops and New Uses. *Journal of Animal Sciences*, 68:2061-2066.

Huang S., Adams W. R., Zhou Q., Malloy K. P., Voyles D. A., Anthony J., Kriz A. L. and Luethy M. H. (2004). Improving nutritional quality of maize proteins by expressing sense and antisense zein genes. *Journal of Agriculture and Food Chemistry*, 52: 1958-1964.

ICRISAT/FAO. (1996). The world sorghum and millet economies: facts, trends and outlook. ICRISAT, Patancheru, India and FAO, Rome, pp 68.

ICRISAT. (1989), International workshop on sorghum stem borers, 17 20th November (1997), ICRISAT, Patancheru, India.

ILO. (2007). Chapter 4. Employment by sector. *In* Key indicators of the labour market (KILM), 5<sup>th</sup> edition. Available at: [www.ilo.org/publidenglish/employment/strat/kilm/download/kilm04](http://www.ilo.org/publidenglish/employment/strat/kilm/download/kilm04.pdf). pdf.



IZge A. U. (2006). Combining ability and heterosis of grain yield components among pearl millet (*Pennisetum glaucum* L. R. Br) in breds. PhD Thesis, Federal University of Technology, Yola, Nigeria, pp. 148.

Jan S., Parween T., Siddiqi T. O. and Uzzafar M. (2012). Effect of gamma radiation on morphological, biochemical and physiological aspects of plants and plant products. Environment Review, 20: 17-39.

Jain S. M. (2010). Mutagenesis in crop improvement under the climate change. Romanian Biotechnological Letters. Vol. 15, No.2. 88-106.

Jain S. M. (2005). Major mutation-assisted plant breeding programs supported by FAO/ IAEA. Plant Cell Tissue and Organ Culture, 82: 113-123.

Jain S. M. and Maluszynski M. (2004). In vitro applications in crop improvement: Recent Progress. Mujib A., Cho M., Predieri S., Banerjee S., eds., IBH-Oxford, India. pp 169-202.

Jamil M. and Khan U. Q. (2002). Study of genetic variation in yield components of wheat cultivar Bukhtwar-92 as induced by gamma radiation. Asian Journal of Plant Science. 5 (1): 579-580.

Javed M. A., Khatri I. A., Khan A., Ahmad M., Siddiqui M. A. and Arain A. G. (2000). Utilization of gamma irradiation for the genetic improvement of oriental mustard *Brassica juncea* (L.) Coss. Pakistan Journal of Botany, 32: 77-83.

Jawardena S. D. L. and Perris R. (1988). Food crop breeding in Sri Lanka- Achievements and challenges. Biology News, 2:22-34.

Kannan B., Sen apathy S., Raj A. G. B., Chandra S., Muthiah A., Dhanapal A. P and Hash C. T. (2014).

Association analysis of SSR markers with phenology, grain, and Stover-yield related traits in Pearl

Millet (*Pennisetum glaucum* (L.) R. Br.). The Scientific World Journal, Volume 2014, Article ID

562327, pp 14.

Karim K. M.R., Islam A. K. M. R., Hossain M. M., Azad H. M. S. and Rahman W. M. (2008). Effect of

gamma rays on yield attributes of large seeded chickpea. Journal of Soil Nature, 2(2), pp.

1924.

Kaul B. L. and Bradu B.L. (1972). Studies on the induced mutation in medicinal plant. I. Spectrum of

adult plant mutations in *Atropa belladonna*. Planta Medica, 20: 205-10.

Khairawal I. S., Rai K. N., Andrew D. J. and Harnnarayana A. (1999). *Pearl millet breeding*. Oxford

and IBH Publishing Co. PVT Ltd.

Khan S. and Goyal S. (2009). Mutation genetics studies in mungbean IV. Selection of early maturing

mutant. Thai Journal of Agricultural Science, 42 (2): 109-113.

Khan M. R., Qureshi A. S., Syed A. H. and Ibrahim M. (2005). Genetic variability induced by gamma

irradiation and its modulation with gibberellic acid in M2 generation of Chickpea (*Cicer*

*arietinum* L.). Pakistan Journal of Botany, 37(2):285-292.



Khan B. M. (1970). Effect of gamma rays' irradiation on yield and the incidence of blight disease of gram. Agric. J. Pakistan., 21: 43-6.

Khangyldin V. V. (19617). Pea mutations induced by x and r rays. Plant Breeding Abstract, 37(2): 2057.

Kharkwal M. C. (1998). Induced Mutations for Improvement of Protein in Chickpea and Pigeon pea. Indian Journal of Genetics, 58(1): 61-68.

Kharkwal M. C., Jain H. K. and Sharma B. (1988). Induced mutations for improvement of chick pea, lentil, pea and cowpea. *In*: Improvement of Grain Legume Production Using Induced . Proc. workshop Pullman, IAEA, Vienna, pp. 89-109.

Khizar H., Akram Muhammed S., Fazal E. and Abdul-Shakoor. (1990). Gamma radiations induce variability in sorghum cultivars. Parkistani Journal of Agricultural Research, Vol.11,no.1.

Kholova J. and Vadez V. (2013). Water extraction under terminal drought explains the genotypic differences in yield, not the anti-oxidant changes in leaves of pearl millet (*PennisOtum glaucum*). Functional Plant Biology, 40, 44-53.

Kim J. H., Baek iSt H., Chung B. Y., Wi S. G. and Kim, J. S. (2004). Alterations in the photosynthetic pigments and antioxidant machineries of *Capsicum annuum* L. seedlings from gamma irradiated seeds. Journal of plant biology, 47: 314-321.





- Kiong A., Ling Pick A., Grace Lai S. H. and Harun A. R. (2008). "Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation". American Eurasian Journal of Sustainable Agriculture, 2(2): 135-149.
- Koli N. R., Sharma Y. and Sharma Y. (2002). Gamma rays induced variation in cumin (*Cuminum cyminum*, L.). Annals Agriculture Biology Research, 7(2): 161-164.
- Kon E, Ahmed OH, Saamin S, Majid N. M. (2007). Gamma radio sensitivity study on long bean (*Vigna sesquipedalis*). American Journal of Applied Sciences. 4(12), 1090-1093.
- Kovacs E. and Keresztes A. (2002). Effect of gamma and UV-B/C radiation on plant cells. Micron, 33: 199-210.
- Konzak C. F., Nilan R. A., Wagner J. and Foster R. J. (1965). Efficient chemical mutagenesis in the use of induced mutations in plant breeding. Radiation Botany, 5: 549-70.
- Krasaechai A., Yu L. D., Sirisawad T., Phornsawatchai T., Bundithya W. and Taya U. (2009). Anuntalabhochai, S. and Vilaithong, T., "Low-energy ion beam modification of horticultural plants for induction mutation". Surface and Coatings Technology, 203: 2525-2530.
- Kumar A. and Mishra M. N. (2004). Effect of gamma-rays, EMS and NMU on germination, seedling vigour, pollen viability and plant survival in M and M2

generation of Okra (*Abelmoschus esculentus* (L.) Moench). *Advances in Plant Science*, 17(1), pp. 295-297.

Lage C. L. S. and Esquibel M. A. (1995). Role of non-enzymatic synthesis of radiation. *Arquivos Biology Technology*, 38(4): 1173-1180.

Larik A. S., Memon S. and Soomro Z. A. (2009). Radiation induced polygenic mutations in Sorghum Bicolor L. *Journal of Agricultural Research*, 47(1).

Larik A. S. and Jamro G. H. (1993). Genotypic response to physical mutagens. *Proc. 2nd All Pak. Int. Sci. Conf.* December 20-30. pp. 161- 163.

Larik A. S. and Hafiz H. M. I. (1981). Wheat improvement by induced mutation. *Wheat Information Service*, 53:35-39.

Leder I. (2004). Sorghum and millets. In: Rileky G (ed.) *Cultivated plants, primarily as food sources*, in *Encyclopedia of Life Support Systems (EOLSS)*, Developed under the auspices of the UNESCO, Eolss Publishers, Oxford, UK.

Lemaux P. G. (2009). Genetically engineered plants and foods: A scientist's analysis of the issues (Part 11). *Annual Review of Plant Biology*, 60: 511-55.

Limon-Ortega A., Mason S. C. and Martin A. R. (1998). Production practices improve grain sorghum and pearl millet competitiveness.





Mahala S.V. S., Mor \$. R. and Yadav J. S. (1990). Induced genetic variability for oil content in mustard (*Brassic juncea* L. Czern and Coss). Oil crops Newsletter, IDRC, Ottawa, Canada, No.7, pp. 13-15.

Maluszynski M. (1990). Gene manipulation in plant improvement. II. (J.P. Gustafsson, ed), Plenum press, New York.

Maluszynski M., Nichterlein K., Van Zanten L. and Ahloowalia B. S. (2000). Officially released mutant varieties. The FAO/IAEA. Database. *Mut. Breed. Rev.*, No. 12, pp.1-84, IAEA, Vienna, Austria.

Maluszynski M. and Kasha K. J. (Eds.). (2002). Mutations, *In vitro* and Molecular Techniques for Environmentally Sustainable Crop Improvement. Kluwer Academic Publishers, Dordrecht/Boston/ London. ISBN 1-4020-0602-0.

Maman N., Mason S. C., Lyon D. J. and Dhungana P. (2003). Yield components of pearl millet and grain sorghum across environments in the central Great Plains. *Crop Science*, 44:1219-1222.

Mansuetus S. B. A. (1995). Sorghum diseases in Tanzania. *African Crop Science Journal* 3:203-207.

Marchais L. and Tostain S. (1993). Evaluation de la diversite' ge'ne'tique des Mils (*Pennisetum glaucum* (L.) R. BR.) au moyen de marqueurs enzymatiques et relation entre formes sauvages et cultive'es. In: Hamon S (eds) Le mil en Afrique diversite' ge'ne'tique et agrophysiologique: Potentialite's et contraintes pour

l'amélioration génétique et l'agriculture. Actes de la réunion thématique sur le mil  
(*Pennisetum glaucum*, L.), Montpellier du 24 au 26 novembre 1992.

Mathur R. K., Manivel P., Samder M. Y., Gor H. K. and Chikani B. M. (2000). Creation of genetic  
variability through mutation breeding in groundnut. *In: Proceedings of the DAE-BRNS*

Symposium on the Use of Nuclear and Molecular Techniques in Crop Improvement. December  
6-8, 2000. Bhabha Atomic Research Centre, Mumbai, India. pp. 203-213.

Mba C. (2013). Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and  
Agriculture. *Agronomy*, 3, 200-231.

Mba C. and Shu Q. Y. (2012). Gamma Irradiation. In *Plant Mutation Breeding and Biotechnology*;  
Shu, Q., Forster, B.P., Nakagawa, H., Eds.; CABI: Oxfordshire, UK. pp. 91-98.

Mba C., Guimaraes P. and Ghosh K. (2012a). Re-orienting crop improvement for the changing climatic  
conditions of the 21st century. *Agric. Food Secur.* 1,7.

Mba C., Afza R. and Shu Q. Y. (2012b). Mutagenic radiations: X-rays, ionizing

particles and ultraviolet. In *Plant Mutation Breeding and Biotechnology*; Shu,

Q., Forster, B.P., Nakagawa, H., Eds.; CABI: Oxfordshire, UK; pp. 83-90.

Mba C., Afza R., Bado S. and Jain S. M. (2010). Induced mutagenesis in plants using physical and  
chemical agents. In *Plant Cell Culture: Essential Methods*; Davey, M.R., Anthony, P., Eds.; John  
Wiley & Sons, Ltd.: Chichester, UK.







- McCouch S. (2004). Diversifying selection in plant breeding. *PLoS Biology*, 2: 1507-1512.
- McDonough Cassandra M., Rooney Lloyd W. and Serna-Sadivar Sergio O. (2000). "The millets". *Food science and Technology: Handbook of cereal science and technology* (CRC Press). 99<sup>th</sup> ed: 117-210.
- Micke A., Maluszynski M., and Donini B. (1985). Plant cultivars derived from mutation induction or the use of induced mutants in cross breeding. *Mutation Breeding Review* No.3. [AEA, Vienna.
- Micke A. (1996). Seventy years induced mutation to be reconsidered? *Mutating Breeding News Letter*, 42:22-25.
- Ministry of Food and Agriculture/Statistics/Research and Information Directorate (MoFA/SRID). (2010). "Agriculture in Ghana: Facts and Figures.
- Moghaddam S. S., Jaafar H., Ibrahim R., Rahmat A., Abdul Aziz M. and Philip E. (2011). Effects of acute gamma irradiation on physiological traits and flavonoid accumulation of *Centella asiatica*. *Molecules*, 16: 4994-5007.
- Mohammad M. R. and Abdollah B. (2011). Influence of Gamma Irradiation on Some Physiological Characteristics and Grain Protein in Wheat (*Triticum aestivum* L.). *World Applied Sciences Journal*, 15 (5): 654-659.
- Moussa H. R. (2011). Low dose of gamma irradiation enhanced drought tolerance in soy bean. *Acta Agronomica Hungarica*, 59:1-12.

uduli K. C. and Misra R. C. (2007). Efficacy of mutagenic treatments in producing useful mutants in finger millet (*Eleusine coracana* Gaertn.). Indian Journal of Genetics, 67(3): 232-237.

Muhammad R. and Afsari S. (2001). Quantitative variations induced by gamma irradiation and gibberellic acid in M<sub>1</sub> generation of Chick pea. Sarhad Journal of Agriculture, 17(3), 367-372.

Muthusamy A., Vasinth K. and Jayabalan N. (2003). Response of physiological and biochemical components in *Gossypium hirsutum* L. to mutagens". Journal of Nuclear Agriculture and Biology, 32 (1): 44-51.

Munns R., James R. A. and Lauchli. A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany, 57: 10251043.

National Academy of Science (NAS). (1996). Lost crops of Africa. 1. Grains. National Academy Press, Washington, DC, USA.

National Population Council (NPC). (2011). Ghana population stabilization report, pp 11.

Norfadzrin F., Ahmed [ O. H., Shaharudin S. and Rahman D. A. (2007). A preliminary study on gamma radio-sensitivity of tomato (*Lycopersicon esculentum*) and okra (*Abelmoschus esculentus*). International Journal of Agricultural Research, 2, (7), 620-625.

Obilana A. B. and Manyasa E. (2003). Millets. In 'Pseudocereals and less common cereals: grain properties and utilization potential' (P.S. Belton and J.R.N. Taylor eds), Springer-Verlag, Berlin Heidelberg, New York, pp 177-217.

Omonona B. T. and Agoi G. A. (2007). An Analysis of food security situation among Nigerian urban households. Journal of central European Agriculture, 8 (3): 397,401 & 45.

Park J. O., Cho H. S., Park M. Y., Jo Y. S and Lim D. G. (2008). Characteristics of *Hosea plantaginea* mutants derived from -ray treatment. International symposium on induced mutations in plants (ISIM), Book of Abstracts, 207: 127.

Parry M. (2007). The implications of climate change for crop yields, global food supply and risk of hunger. ICRISAT, Vol. 4, pp 1-44.

Payne W. A., Wendt C. W. and Lascano R. J. (1990). Root zone water balance of three low-input millet fields in Niger, West Africa. Agronomy Journal, 82(4), 813-819.

Prasad P. V. V. and Staggenborg S. A. (2011). Soil, plant growth and crop production- Vol. II. Growth and production of sorghum and millets. Encyclopaedia of Life Support Systems.

Preussa S. B. and Britta A.B. (2003). A DNA-damage- induced cell cycle checkpoint in Arabidopsis. Genetics, 164: 323-334.



Rachovska G. (1996). Investigation on effects of sodium azide, gamma rays and their combine action in bread winter wheat. 5th International Wheat Conference, June 10-14, Ankara, Turkey, 75.

Radcliffe D., Cabrera M., Hanna W., Dale N. and Gascho G. (2001). Manure phosphorus: Problems and solutions. Broiler Industry, WATT Poultry USA. July: 4-5.

Rao S. R., Hosney R. C., Finney K. F. and Dhogren M. D. (1975). Effect of gamma irradiation of wheat on breadmaking properties. Cereal Chemistry Journal, 52: 506-512.

Rao B. R., Nagasampige M. H. and Ravikiran M. (2011). Evaluation of nutraceutical properties of selected small millets. Journal of Pharmacy and Bioallied. Science, 3:277-279.

Rehman A., Das M. L., Howlidar M. A. R. and Mansur M. A. (1987). Promising mutants in *Birassica campestris*. Mutation Breeding Newsletter, 29: 1415.

Rohrbach D. D. (2004). Improving the commercial viability of sorghum and pearl millet in Africa. Series Report. ICRISAT, Bulawayo, Zimbabwe.

Rosegrant M. W (2011). Impacts of climate change on food security and livelihoods. *In* Food Security and Climate Change in Dry Areas, Proceedings of an International Conference, Amman, Jordan, Solh, M., Saxena, M.C., Eds.;



International Center for Agricultural Research in the Dry Areas: Aleppo, Syria, pp. 24-26.

Roychowdhury R. and Tah J. (2011). Mutation breeding in *Dianthus caryophyllus* L. for economic traits. *Electronic Journal of Plant Breeding*, 2(2):282-286.

Roychowdhury R. (2011). Effect of chemical mutagens on carnation (*Dianthus caryophyllus* L.): a mutation breeding approach. LAP Lambert Academic Publishing, Germany, pp 1-276.

Roychowdhury R., Bandyopadhyay A., Dalal T and Tah J. (2011). Biometrical analysis for some agro-economic characters in M1 generation of *Dianthus caryophyllus*. *Plant Archives* 11(2):989-994.

Roychowdhury R., Datta S., Gupta P. and Tah J. (2012). Analysis of genetic parameters on mutant populations of mung bean (*Vigna radiata* L.) after ethyl methane sulphonate treatment. *Notulae Scientia Biologica*, 4(1):137-143.

Ryan J. G. and Spencer D. C. (2001). Future challenges and opportunities for agricultural R&D in the semi-arid tropics. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. pp 83.

SADC/ICRISAT (Southern Africa Development Community/ICRISAT). (1987). Proceedings of the sorghum and millet seed production workshop for Eastern and Southern Africa, 13-19. September 1987, Nairobi, Kenya.



Rachovska G. (1996). Investigation on effects of sodium azide, gamma rays and their combine action in bread winter wheat. 5th International Wheat Conference, June 10-14, Ankara, Turkey, 75.

Radcliffe D., Cabrera M., Hanna W., Dale N. and Gascho G. (2001). Manure phosphorus: Problems and solutions. Broiler Industry, WATT Poultry USA. July: 4-5.

Rao S. R., Hoseney R. C., Finney K. F. and Dhogren M. D. (1975). Effect of gamma irradiation of wheat on bread making properties. Cereal Chemistry Journal, 52: 506-512.

Rao B. R., Nagasampige M. H. and Ravikiran M. (2011). Evaluation of nutraceutical properties of selected small millets. Journal of Pharmacy and Bioallied. Science, 3:277-279.

Rehman A., Das M. L., Howlidar M. A. R. and Mansur M. A. (1987). Promising mutants in Brassica campestris. Mutation Breeding Newsletter, 29: 1415.



Rohrbach D. D. (2004). Improving the commercial viability of sorghum and pearl millet in Africa. Series Report. ICRISAT, Bulawayo, Zimbabwe.

Rosegrant M. W (2011). Impacts of climate change on food security and livelihoods. *In* Food Security and Climate Change in Dry Areas, Proceedings of an International Conference, Amman, Jordan, Solh, M., Saxena, M.C., Eds.;

International Center for Agricultural Research in the Dry Areas: Aleppo, Syria, pp. 24-26.

Roychowdhury R. and Tah J. (2011). Mutation breeding in *Dianthus* 2(2):282-286.

Roychowdhury R. (2011). Effect of chemical mutagens on carnation (*Dianthus caryophyllus* L.): a mutation breeding approach. LAP Lambert Academic Publishing, Germany, pp 1-276.

Roychowdhury R., Bandyopadhyay A., Dalal T and Tah J. (2011). Biometrical analysis for some agro-economic characters in M1 generation of *Dianthus caryophyllus*. Plant Archives 11(2):989-994.

Roychowdhury R., Datta S., Gupta P. and Tah J. (2012). Analysis of genetic parameters on mutant populations of mung bean (*Vigna radiata* L.) after ethyl methane sulphonate treatment. Notulae Scientia Biologica, 4(1):137-143.

Ryan J. G. and Spencer D. C. (2001). Future challenges and opportunities for agricultural R&D in the semi-arid tropics. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. pp 83.

SADC/ICRISAT (Southern Africa Development Community/ICRISAT). (1987). Proceedings of the sorghum and millet seed production workshop for Eastern and Southern Africa, 13-19. September 1987, Nairobi, Kenya.





Sharma D. K. and Rana D. S. (2007). Response of castor (*Ricinus communis*) Sciences, 77 (7): 467-469.

Siddiqui B. A. and Khan S. (1999). Breeding in crop plants: mutations and in *vitro* mutation breeding. Kalyani, India, pp 402.

Singh S. D. and Barsaul C. S. (1976). Replacement of maize by coarse grains for growth and production in white leghorn and Rhode Island Red birds. Indian Journal of Animal Sciences, 46: 96-99.

Singh R. S., Josh N. L. and Singh H. P. (1998). Pearl millet phenology and growth in Relation to thermal time under arid environment. Journal of Agronomy and Crop Science, 180 (2), 83-91.

Soeranto H., Nakanishi T. M and Razzak M. T. (2001). Mutation Breeding in Sorghum in Indonesia. Radioisotopes, 5:169-175.

Song H. S. and Kang S. Y. (2003). Application of natural variation and induced mutation in breeding and functional genomics: Papers for International Symposium; Current Status and Future of Plant Mutation Breeding. Korean Journal of Breeding Science, 35 (1): 24 34.

Subi M. I. M. and Idris A. E. (2013). Genetic variability, heritability and genetic advance in Pearl Millet (*Penisetum glaucum* [L.] R. Br.) Genotypes. British Biotechnology Journal, 3 (1): 54-65.



Suhas K., Deshumukh V. K. and Saoji A. N. (1976). Stimulating effect of gamma radiation on growth of senna (*Cassia angustifolia*) vahl. Referativny Zhurnal., 8: 882-7.

Sumathi P., Sumamth M. and Vearabadhiran P. (2010). Genetic Variability for Different Biometrical Traits in Pearl Millet Genotypes (*Pennisetum glaucum* L. R. Br.).  
Electronic Journal of Plant Breeding, 1 (4): 347-440.

Mohan T. S. (1973). Mutagenic action of nitroso methyl urea in pearl millet, Current Science, 42: 474.

Taylor J. R. N. (2003). Overview importance of sorghum in Africa (Online). Available from: <http://www.sciencedirect.com/science?> (Accessed 31 July 2008).

Taylor J. R., Schober T. J. and Bean S. (2006). Novel and non-food uses for sorghum and millets.  
Journal of Cereal Science, 44: 252-271.

Tester M. and Langridge P. (2010). Breeding technologies to increase crop production in a changing world. Science, 327, 818-822.

Thapa C. B. (2004). Effect of acute exposure of gamma rays on seed germination and seedling growth of *Pinus kesiya* Gord and *Pinus wallichiana* A.B. Jacks. *Our nature*. 2 :13-17.

Token C. B., Uzun H. and Canci C. F. O. (2005). Effects of gamma irradiation on the shoot length of Cicer seeds. Radiation Physics and Chemistry, 73 (6), 365367.

Toker C., uzen B., canci H and ceylan F.O. (2005) Effect of gamma irradiation on the shoot length of Cicer seeds. Radiation Physics and Chemistry, 73:365-367.

United Nations Secretariat (UN). (2013). Population Division of the Department of Economic and Social Affairs. World Population Prospects: The 2012 Revision. New York: United Nations.

van Oosterom E. J., O'Leary G. J., Carberry P. S. and Craufurd P. Q. (2002). Stimulating growth, development, and yield of tillering pearl millet. III. Biomass accumulation and partitioning. Field Crops Research, 79:85-106.

Vasline Y. A., Vennila S., Ganesan J. (2005). Mutation-an alternate source of variability. UGC national seminar on present scenario in plant science research. Department of Botany, Annamalai University, Annamalainagar, pp 42.

Veeresh L. C., Shivashankar G., Shailaga H. and Hittalmani S. (1995). Effect of seed irradiation on some plant characteristics of winged bean. Mysore Journal of Agricultural Sciences, 29: 1-4.

Vijendra Das L. D. (1978). Effects of radiation on the R progenies of *Pennisetum typhoides*. Environmental and Experimental Botany. 18: 121.

von Grebmer K., Torero M., Olofinbiyi T., Fritschel H., Wiesmann D., Yohannes Y. and Schofield L. (2011). Global Hunger Index: The Challenge of Hunger (Bonn; Washington, DC; Dublin: Deutsche Welthungerhilfe, International



## APPENDICES

### **Variate: Germination percentage (Dosage response studies)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	322.75	161.38	8.92	
Rep.*Units* stratum					
Irradiation	7	1694.50	242.07	13.38	<.001
Residual	14	253.25	18.09		
Total	23	2270.50			

### **Variate: Percentage survival (Dosage response studies)**

### **Variate: Germination percentage (M<sub>2</sub> and M<sub>3</sub> generations)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	76.49	38.24	3.21	
Rep.*Units* stratum					
Irradiation	7	27052.74	3864.68	324.62	<.001
Residual	14	166.67	11.91		
Total	23	27295.90			
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	14.068	4.689	0.84	
Rep.*Units* stratum					
Irradiation	3	62.613	20.871	3.75	0.027
Generation	1	1170.070	1170.070	210.23	<.00
Irradiation. Generation	3	67.951	22.650	4.07	0.020
Residual	21	116.879	5.566		
Total	31	1431.582			





**Variate: Percentage survival (M<sub>2</sub> and M<sub>3</sub> generations)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	64.631	21.544	16.56	
Rep.*Units* stratum					
Irradiation	3	6.381	2.127	1.63	0.212
Generation	1	14.311	14.311	11.00	0.003
Irradiation. Generation	3	2.381	0.794	0.61	0.616
Residual	21	27.324	1.301		
Total	31	115.029			

**Variate: Plant height**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	572.5	190.8	0.98	
Rep.*Units* stratum					
Irradiation	3	4698.4	1566.1	8.03	<.001
Generation	2	21544.0	10772.0	55.22	<.001
Irradiation. Generation	6	5491.1	915.2	4.69	0.001
Residual	33	6437.8	195.1		
Total	47	38743.8			

**Variate: Tillering**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	11.061	3.687	0.67	
Rep.*Units* stratum					
Irradiation	3	40.957	13.652	2.49	0.077
Generation	2	29.790	14.895	2.72	0.081
Irradiation. Generation	6	12.427	2.071	0.38	0.887
Residual	33	180.604	5.473		
Total	47	274.840			

**Variate: Productive tillers**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.2093	0.0698	0.21	
Rep.*Units* stratum					
Irradiation	3	9.3228	3.1076	9.36	<.001
Generation	2	11.5915	5.7958	17.45	<.001
Irradiation. Generation	6	3.6456	0.6076	1.83	0.124
Residual	33	10.9595	0.3321		
Total	47	35.7287			

**Variate: Earliness**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	8.062	2.688	0.40	
Rep.*Units* stratum					
Irradiation	3	445.729	148.576	22.27	<.001
Generation	2	561.167	280.583	42.05	<.001
Irradiation. Generation	6	121.333	20.222	3.03	0.018
Residual	33	220.188	6.672		
Total	47	1356.479			

**Variate: Head length**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	10.609	3.536	1.45	
Rep.*Units* stratum					
Irradiation	3	59.628	19.876	8.17	<.001
Generation	2	14.169	7.085	2.91	0.068
Irradiation. Generation	6	34.616	5.769	2.37	0.052
Residual	33	80.302	2.433		
Total	47	199.324			



Variate: Head width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.22591	0.07530	2.18	
Rep.*Units* stratum					
Irradiation	3	0.79302	0.26434	7.64	<.001
Generation	2	0.13704	0.06852	1.98	0.154
Irradiation. Generation	6	0.88301	0.14717	4.25	0.003
Residual	33	1.14234	0.03462		
Total	47	3.18132			

Variate: Head weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	14.16	4.72	0.38	
Rep.*Units* stratum					
Irradiation	3	59.24	19.75	1.57	0.215
Generation	2	607.96	303.98	24.21	<.001
Irradiation. Generation	6	173.43	28.90	2.30	0.058
Residual	33	414.33	12.56		
Total	47	1269.11			

Variate: 100 seed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.8865	0.2955	2.34	
Rep.*Units* stratum					
Irradiation	3	1.1354	0.3785	2.99	0.045
Generation	2	2.1738	1.0869	8.59	<.001
Irradiation. Generation	6	2.2631	0.3772	2.98	0.019
Residual	33	4.1748	0.1265		
Total	47	10.6335			



Variate: Grain yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1476691.	492230.	2.73	
Rep.*Units* stratum					
Irradiation	3	5566553.	1855518.	10.28	<.001
Generation	2	5423808.	2711904.	15.03	<.001
Irradiation. Generation	6	4550062.	758344.	4.20	0.003
Residual	33	5954387.	180436.		
Total	47	22971502.			



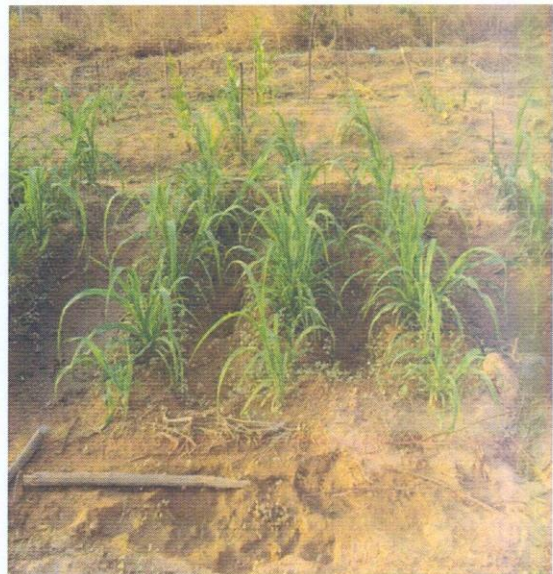
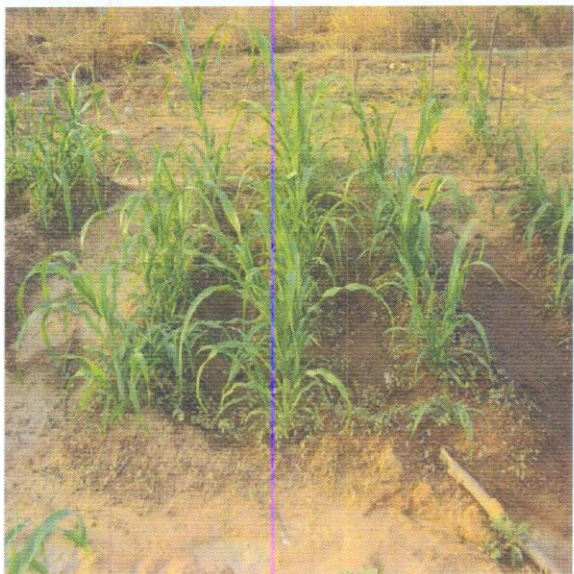




(a)



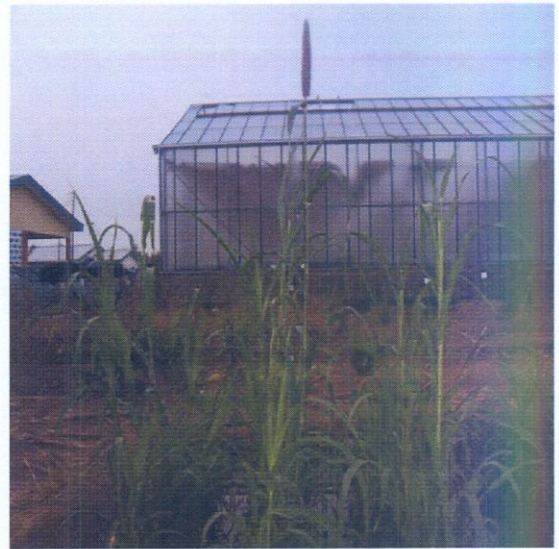
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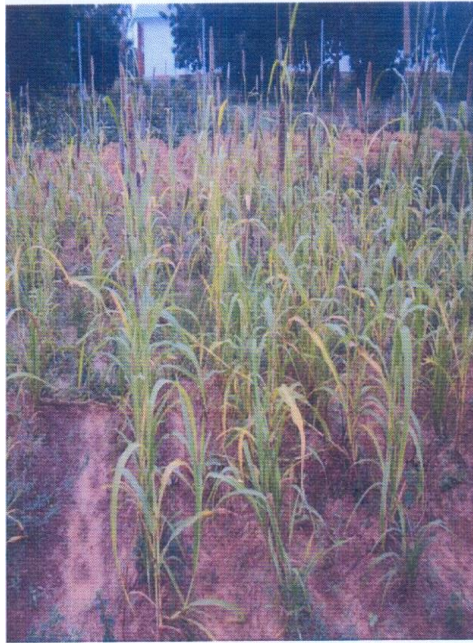
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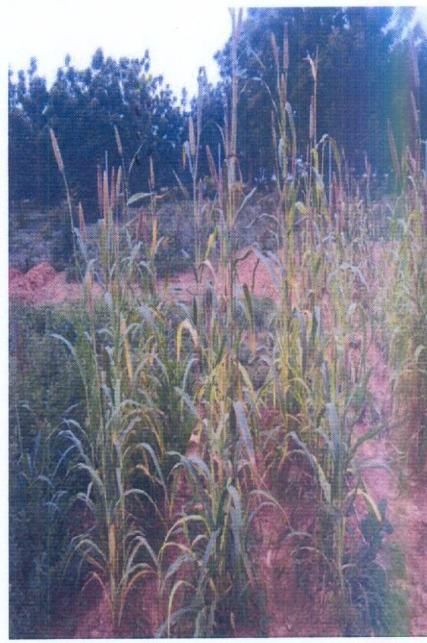
(b)







(a)



(b)







Plate 5: Heads of respective treatments in the  $M_3$  generation