# MUTAGENESIS OF COWPEA FOR EARLY MATURITY AND HIGHER YIELD

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## DECLARATION

I hereby declare that, except for references to the works of other researchers which have been duly cited, this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

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## DEDICATION

This thesis is dedicated to my dear wife Diana Mawufemor Katamany-Dorvlo and my beloved children Elena Klenam and Kemual Eyram Ahiable for their payers and well - wishes.

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## LIST OF ABBREVIATIONS

| ABBREVIATIONS | IN FULL |
|---|---|
| **ANOVA** | Analysis of variance |
| **BNARI** | Biotechnology and Nuclear Agriculture Research Institute |
| **CSIR-CRI** | Council for Scientific and Industrial Research-Crops Research Institute |
| **CSIR-SARI** | Council for Scientific and Industrial Research -Savanna Agricultural Research Institute |
| **CV%** | Coefficient of variation |
| **DAP** | Days after planting |
| ***et al.*** | Etcetera |
| **FAO** | Food and Agriculture Organization |
| **FAOSTAT** | Food and Agriculture Organization Corporate Statistical Database |
| **G** | Gram |
| **GAEC** | Ghana Atomic Energy Commission |
| **Gy** | Gray |
| **ICRISAT** | International Crops Research Institute for the Semi-Arid Tropics |
| **$LD_{50}$** | Dose rate for reducing the target population by half |
| **Lsd** | Least significant difference |
| **$M_1$, $M_2$, and $M_3$** | Mutant generations, one, two, and three |
| **MOFA** | Ministry of Food and Agriculture |
| **MVD** | Food and Agriculture organization (FAO) and international Atomic Energy Agency (IAEA) Mutant Varieties Database |
| **QTLs** | quantitative trait loci |
| **$RD_{50}$** | Dose that results in 50% reduction in plant height |
| **RTC** | Radiation Technology Centre |
| **SSA** | Sub-Saharan Africa |

## ABSTRACT

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important grain legume that is widely grown in sub-Saharan Africa (SSA) for food and feed. Its grain is composed of high levels of protein, carbohydrate, micro-nutrients and macro-nutrients which are essential for human nutrition. In Ghana, cowpea productivity is considerably low due to frequent terminal drought as a result of climatic changes. Therefore, breeding improved varieties by incorporating "farmer-preferred" traits remains an overriding consideration to boost the productivity of cowpea in Ghana. The main objective of this study was to develop early maturing and high seed yielding cowpea varieties through mutation induction using gamma irradiation. Before the commencement of the mutagenesis, it is important to determine the right dose of gamma radiation for causing genetic variability in the desired agro-economic trait. Therefore, seeds of a farmer-preferred cowpea variety 'Videza' (obtained from a farmer in Akatsi, Volta region of Ghana) were gamma irradiated using twelve irradiation doses (0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 and1100 Gy) at GAEC. Using a linear regression model, the $LD_{50}$ value of the cowpea variety 'Videza' was calculated as 240.51Gy. A dose-dependent reduction was observed in seed germinastion, seedling survival and plant height. For mutation induction, 2000 seeds of cowpea variety 'Videza' were acutely irradiated at 230 Gy, at the Radiation Technology Centre (RTC) of Ghana Atomic Energy Commission (GAEC), Accra, Ghana, using a Cobalt 60 source, delivering at a dose rate of 300 Gy/hr. Normal looking $M_1$ plants with the desired traits (early maturity and high seed yield) were advanced to $M_2$ generation and further advanced to $M_3$ generation. The planting was linear and serpentine at a seeding rate of one seed per hill using 75cm x 40 cm. Control seeds were sown in three rows after every ten rows of the irradiated seeds separated by a spacing of 1.50m. Compared to the Control (Videza),

genetic variability was recorded among plants in both $M_2$ and $M_3$ generation. The extent of genetic variability for the number of days to 50% flowering, number of days to 90% maturity, number of pod-bearing branches per plant, number of pods per plant, number of seeds per pod, 100-seed weight (g) and seed yield per plant (g) were evaluated in $M_2$ and $M_3$ generations. The number of days to 50% flowering and 90% maturity reduced in putative mutants in both $M_2$ and $M_3$ generations compared to the parental line (Control). The number of days to 50% flowering and days to 90% maturity, reduced further in the $M_3$ generation as compared to $M_2$. Increments in 100-seed weight per plant (g) and seed yield per plant (g) were observed among the putative mutants in $M_3$ generation compared to the parental line (Control). The following twelve putative mutant lines P1N06#20, P1N06#9, P1N08#13, P1N08#17, P2N09#12, P4N03#2, P4N14#7, P5N05#10, P5N07#14 and P6N10#19 were outstanding in the $M_3$ generation, exhibiting both early maturity as well as high seed yield. The twelve putative mutant lines are recommended for yield trials (preliminary and advanced), alongside the parental line (Videza) as well as a local check (as Controls), in farmer-participatory multilocational trials (including the Akatsi District of the Volta Region of Ghana) towards identifying superior lines for release as new variety or varieties.

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1. Background

Cowpea [*Vigna unguiculata* (L) Walp] is part of the Leguminosae family and the Fabaceae subfamily. It is one of the most essential nutritious grain legumes grown in the semi-arid tropics and in some temperate regions of the world (Timko & Singh, 2008). The crop has various morphological forms, such as erect, semi-erect, climbing, prostrate or creeping and is usually indeterminate under favorable environmental conditions (Timko *et al.*, 2007). Cowpea is resistant to drought, well suited to many areas of humid tropics and temperate areas. The crop responds favourably to moderate moisture compared to other leguminous crops. Cowpea grows well on a different type of soil but does best on well-drained sandy loams or sandy soils where soil pH is about 5.5 and 6.5.

About 7.5 million tons of dried cowpeas were grown worldwide in 2018, with almost 7.1 million tons of dried cowpeas grown in Africa (FAOSTAT, 2020). Nigeria, Niger, Senegal, Ghana, Mali and Burkina Faso are the leading cowpea-producing nations (Singh, 2020). Nigeria and Niger account for 66 per cent of cowpea production worldwide.

Cowpea is grown in all of Ghana's agro-ecological zones mainly by resource-poor smallholder farmers for livelihood purposes (Asare *et al.*, 2010; Odendo *et al.*, 2011). Most of the cowpea production in Ghana is found in the Savanna and Forest Transition Zones of Guinea (Quaye *et al.*, 2011)

Because of its nutritional value, cowpea cultivation is becoming increasingly popular in developing countries, especially in arid regions of the world. The dry seeds are an essential

source of protein (22-25%) and also contain significant amounts of vitamins and minerals for human consumption (Singh, 2020). Cowpea is usually prepared and eaten as a whole grain or milled and used in preparing different meals across Africa and beyond. The fresh seeds and immature pods are eaten as vegetables, while dry grains are used to make many dishes (Jackson *et al.,* 2012). The dry grains that are processed into flour and fried in cooking oil called "koose". The dry haulms of cowpea are equally essential as nutritious livestock fodder (Singh *et al.,* 2003). In developed countries the dry grains are processed into flours and used as a concentrate of proteins in the formulation of animal feed (Chinma *et al.,* 2008; Sreerama *et al.,* 2012).

Cowpea also enhances the fertility of the soil by fixing atmospheric nitrogen that leads to higher yields of nitrogen-intensive crops grown with or after them (Matusso *et al.,* 2014).

### 1.2 Problem Statement

In Sub Saharan Africa, cowpea suffers considerable damage due to frequent terminal drought as a result of climate change especially during the flowering and pod filling stage (Armah *et al.,* 2011). According to Agbicodo *et al.* (2009), cowpea crop exposed to moisture stress at early flowering and pod filling stage results in losses of up to 80 per cent of yield. Early maturing varieties escape terminal drought and some infestations of insects (Ehlers & Hall, 1997).

In spite of rich collections of germplasm available at the various national breeding institutions, cowpea genetic variability is narrow due to the self-pollinating nature of the crop. (Asare *et al.,* 2010). In both conventional and molecular methods of plant breeding,

breeders depend on the availability of sufficient levels of genetic variation for the trait of interest for crop improvement.

In Ghana, the improved cowpea varieties grown by the farmers are mostly released by the CSIR-Savanna Agricultural Research Institute (SARI) and CSIR-Crops Research Institute (CRI) using conventional breeding. Some of the early maturing varieties such as 'Sanzi'and 'Asontem', grown by Ghanaian farmers have low yield, small grain size, and light red seed coat colour that consumers do not prefer (Owusu *et al.*, 2018). Deficiency in these two varieties in terms of consumer traits (cream seed coat colour and large grain size) makes those varieties less desirable for Ghana's farmers and consumers. Therefore, there is a need to create genetic variation in cowpea genotype with desired traits such as early maturing, grain yield, drought escape and tolerant to insect pest.

### 1.3 Justification

To deal with erratic rainfall patterns in sub-Saharan Africa, many small-scale farmers purposely prefer early maturing crops to avoid total crop failure and provide food during the food shortage period in August / September (Fatokun *et al.*, 2012).

Due to the lack of sufficient genetic variability and failure of seed setting on emasculated buds due to unfavourable weather condition, the breeding of cowpea using conventional breeding methods are generally not successful, to meet the demands for cowpea improvement (Singh, 2006; Singh, 2020). In most self-pollinated crops where genetic variability is inadequate, induced mutation is the best tool for causing significant genetic variability for a desired agro-economic trait such as early maturing, grain yield, drought and insect pest tolerant cowpea.

Mutagenesis is faster, more effective than conventional breeding in generating genetic variation for crop improvement. Because of this, mutation breeding is becoming increasingly popular as a powerful tool to improve crops (Roychowdhury & Tah, 2013) and an efficient way to cause genetic variability for plant breeding. According to Ahloowalia *et al.* (2004); Roychowdhury and Tah (2013), the mutant varieties produced and released in major crops were grown by farmers on large scale and this led to increased food production, thus contributing to food security.

The induced mutation has successfully changed many agro-morphological traits of cowpea like the seed coat colour and high seed yield, protein content, fodder yield, maturity, determinate habit and high yield (Ahloowalia *et al.*, 2004). Thus, induced mutation can be used to create variability in cowpea for a desired trait.

### 1.4 Overall research objective

The main objective of the study is to develop early maturity and high seed yielding cowpea varieties using gamma irradiation.

#### 1.4.1 Specific objectives

1. To determine an effective dose of gamma radiation for inducing genetic variation in a farmer-preferred cowpea variety 'Videza'.

2. To induce genetic variability for early maturity and high seed yield in cowpea variety 'Videza' using an acute dose of gamma irradiation.

3. To screen the mutagenized population for early maturity and high seed yield through selections from the $M_2$ to $M_3$ generations.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1. Origin and distribution of Cowpea

The center of origin of cultivated cowpea [*Vigna unguiculata* (L) Walp] has been most controversial because most authors point out Asia and Africa as independent centers of origin of the crop due to the fact that cowpeas discovered in Asia are very diverse and morphologically different from the ones growing in Africa (Ibrahima, 2012; Timko & Singh, 2008). The extensive distribution of wild cowpeas is the clear that Africa is the center of origin of the cultivated cowpea. Ng (1995) stated that cowpea is a native of Africa where it evolved in cultivation with sorghum and pearl millet in the cereal farming system of the West African zone, where it is now a major grain legume.

Cowpea nowadays is widely adapted and grown throughout the world. Cowpea was introduced by the Spanish in the course of the slave trade from Africa to the new world in the 17th century and has been cultivated in the South of the USA since the early 18th century (Padulosi & Ng, 1997), to Europe through Northeastern Africa around 300 BC, to India at about 200 BC from where it underwent further diversification in India and Southeast Asia (Padulosi & Ng, 1997). It can be argued that the widespread nature of the crop globally in tropical and subtropical zones of the world can be attributed to its adaptability to a variety of soils and climatic conditions, cropping systems and to its value as a food crop for man and fodder for livestock**.**

## 2.2 Morphology and Biology

Cowpea is an annual, warm-season herbaceous plant grown in many tropical and subtropical nations (Singh & Sharma, 1996; Timko & Singh, 2008) and also a trifoliate leafy legume crop. There are four genotypes of cowpea: prostrate (trailing), ascending, erect, and semi-erect genotype. There are two types of cowpea growing habits, consisting of an indeterminate type and determined type, but usually, the plant continues to bloom and produce seed for an extended period (Padulosi & Ng, 1997). Cobbley and Steele (1976) described cowpea as an annual glabrous herb with a strong taproot and more lateral branching roots that branch to the soil surface compared to other legumes.

Germination of cowpea is epigeal but cotyledons do not survive and can lose as much as 90% of their dry matter when seedlings emerge (Bewley *et al.*, 2012). The first leaves above the cotyledons are simple and opposite at the seedling stage; subsequent trifoliate leaves alternate with the terminal leaflet, which is often larger and longer than the two asymmetric laterals (Padulosi & Ng, 1997). The leaflets are 5-18 cm long, 3-16 cm wide and are defined as linear, lanceolate or narrowly ovate, cuneate or rounded at the base and gradually tapering to a pointy tip. The stems are striated and often tinged with purple, smooth or slightly hairy. The flowers are arranged in racemes or intermediate inflorescences at distal ends with peduncles 5-60 cm long (Purseglove, 1981)

Most cowpeas exhibit sensitivity to photoperiods while others are day-neutral when it comes to the initiation and development of floral buds (Timko *et al.*, 2007; Timko & Singh, 2008). Most early maturing cowpea cultivars can start flowering at 30 days after sowing when the temperature is about 30 ° C. At the tip of the branches, flowers are borne in alternating pairs, with at least three flowers per inflorescence (Padulosi & Ng, 1997). To

attract pollinating insects, flowers are borne on short pedicels with white or purple corollas. The nature of the long peduncle is a characteristic of cowpea and this makes it easy to harvest by hand (Timko *et al., 2007)*. Cowpea is characterized by two or three pods per peduncle, but if the growing conditions are favorable, a single peduncle may have at least four pods (Timko *et al.*, 2007).

The pod of cowpea may contain 10 - 20 seeds depending on the length of the pod and size of the cowpea seed vary (Kabululu, 2008). The shape of cowpea seed is mostly related to the development of seed in the pod. Cowpea seeds are a kidney shape; when the seeds are not restrictive in the pod, the seed maintains that shape until maturity (Padulosi & Ng, 1997). But the pod has a tendency to restrict the shape of the seed to a more globular shape. The seed coat may be smooth or rough or wrinkled. The colour of the seeds varies from white, cream, brown, red and black, and is not limited to uniform colours, but can be speckled, mottled and blotchy or eye-shaped (black eye, pink eye, purple eye (Singh *et al.*, 2003). Various variables such as seed size and colour, taste, yield and time to maturity can distinguish cultivated cowpea varieties from each other (Kabululu, 2008).

### 2.3 Agronomic Requirements

Cowpea is more resilient to a wide range of soils because it can perform better in acidic soil and infertile soils than other crops. However, the crop performs best on well-drained, sandy loams or sandy soils, which tends to be much less restrictive on root development with soil pH ranges from 5.5 to 6.5 (Singh, 2020). This is because cowpea has an adaptability mechanism that involves less restrictive root growth, combined with the ability to adapt to moisture stress condition due to reduced leaf growth, less water loss via the stomata and leaf orientation to minimize the light and heat stress.

Cowpea is a subtropical crop that requires less rainfall than most crops; therefore, most of its production is in the dry savannah regions (Ehlers & Hall, 1997). Heavy rainfall promotes excessive vegetative growth and increases the incidence of disease (Purseglove, 1981). Cowpea performs well in agro-ecological areas with rainfall ranges between 500 and 1200mm / year (Ehlers & Hall, 1997). Cowpea can be cultivated under rain-fed conditions and in the dry season by irrigation or residual moisture along river or lake. Excessive irrigation promotes more vegetative growth and sometimes causes a delay in maturity.

The crop requires temperatures above 10°C for germination to take place, and temperatures varying from 21 to 33°C is best for vegetative growth. The best recommended sowing time in northern Ghana is between middle of July and mid August (Alidu, 2019).

## 2.4 Economic importance and utilization of cowpea

### 2.4.1 Food value

Cowpea is an essential legume crop in the lives of so many people, especially those living in the subtropics and tropics in less developed countries (Odendo *et al.,* 2011). Cowpea production continues to be the most essential legume crop cultivated by small-scale farmers, primarily in most sub-Saharan African nations, and a solution to the increasing demands for high-priced proteins with excellent dietary and functional properties, especially in developing and underdeveloped nations where food supplies of animal origin are scarce (Odendo *et al.*, 2011). The chemical composition of Cowpea is the same as most suitable edible legumes; it contains approximately 24% protein, 62% soluble carbohydrates, and small amounts of other nutrients. The majority of its nutritional value is proteins and carbohydrates (Lambot, 2002).

Cowpea is eaten as a vegetable crop at all stages of growth and it is a superb food security crop because it mixes well with different recipes, and the dry seed is used in many meals (Lambot, 2002). The fresh green leaves are an essential food source in Africa and are prepared as a pot herb (Sebetha *et al.*, 2010). Immature seeds are boiled or can be canned or frozen as fresh vegetables (Sebetha *et al., 2010)*. Also the dry cowpea grains are appropriate for cooking and canning (Coulibaly *et al.*, 2009).

In many parts of the world, cowpea is used as livestock feed , especially during the dry season when animal feed is scarce, making the cowpea an essential and integral part of sustainable integrated farming systems in semi-arid and arid regions of Sub-Saharan Africa (Tarawali. *et al.*, 2002). In developing countries, the price of cowpea haulms varies from 50 percent to 80 percent of the grain price on a dry weight basis and hence, haulms are a major source of income. The protein content of cowpea haulms varies from 13 to 17% and contain low fiber and highly digestible. This makes cowpea haulm is a good substitute for cereal stalks due to its high protein content for sustainable livestock production (Tarawali. *et al.*, 2002).

Besides its nutritious benefit, cowpea provides many farmers, mainly small farmers in African countries with a source of income, because cowpea is significantly cheaper compare to the price of selling rice (Odendo *et al.*, 2011).

### 2.4.2 Medicinal value

Cowpea consumption has been associated with numerous beneficial biochemical repercussions for the control of certain chronic disorders such as coronary heart problems and colon cancer (Bazzano *et al.*, 2001). The leaves can be chewed and applied to burns and headaches can also be treated with the leaves; root paste can also be used as an antidote

for snake bite (Timko & Singh, 2008). The seed of cowpea is also used in treating various diseases such as bilharzia, liver problem, and amenorrhoea (Enyiukwu *et al.*, 2018).

### 2.4.3 Soil fertility management

In addition to being a nutritious crop, cowpea can fix atmospheric nitrogen, thereby reducing the demand for nitrogen need by the crop. This makes cowpea popular and enormously valuable to Africa, especially in the rural areas where resource-poor farmers do not have access to fertilizers. Cowpea fixes atmospheric nitrogen with the aid of Rhizobia contained in root nodules, which is made available in the soil for successively grown crops, particularly in areas where there are poor soils (Matusso *et al.*, 2014). According to Zahran (1999), the amount of atmospheric nitrogen fixed biologically by cowpea ranges from 65 to 335 kg N/ha per year. The crop is mostly included in crop rotation farming systems in many areas due to its ability to restore the lost nutrient from the soil after the cultivation of cereal crops (Bationo *et al.*, 2002). It also grows and covers the ground quickly, preventing soil erosion (Matusso *et al.*, 2014).

## 2.5 Cowpea production in the world

Worldwide, approximately 14.5 million hectares of cowpea is grown annually, with 7.4 million tons of dried cowpea grain produced in 2018 (FAOSTAT, 2020) with Africa alone accounting for 7.1 million tons (Singh, 2020). The important cowpea growing countries in West and Central Africa are Benin, Burkina Faso, Cameroon, Ghana, Mali, Niger Republic, Nigeria, Senegal, and Togo (Singh, 2020). FAOSTAT (2020). Nigeria is the world's largest producer with over 2 million tons, followed by Niger with 650,000 tons and Mali with 110,000 tons, while Ghana produces around 57,000 tons of annually.

According to the Food and Agriculture Organization, the average cowpea yield in Africa has been estimated as 483 kg / ha-1 , which is still below the expected potential production. (FAOSTAT, 2020).

The majority of farmers in Africa that cultivate cowpea are mostly women who engage in subsistence cropping (Langyintuo *et al.*, 2003).

### 2.6 Cowpea production in Ghana

Cowpea is one of the most commonly grown legumes, especially in Ghana's savannah and transition zones (Egbadzor *et al.*, 2013). Although cowpea production outside of the Savanna zones in Ghana is mostly in a subsistent manner, some varieties are known to perform well across the various agro-ecological zones (Quaye *et al.*, 2011).

Cowpea is an essential source of vegetable protein and minerals for over 70% of the Ghana population and the second most significant grain legume (Adeniyan *et al.*, 2011). In Ghana, a total of 143,000 MT are produced yearly on approximately 156,000 ha of land making Ghana the fifth largest cowpea producer in Africa (SRID-MOFA, 2018)

In Ghana, most cowpea seeds for planting are obtained from the market or from the other farmer or from the seed stored by the farmer from the previous farming season. Most of the cowpea farmers use ash to preserved the cowpea seeds. (Danso, 2017; Quaye *et al.*, 2011). In Ghana, the cultivation of cowpea is mostly done by small-scale farmers who intercrop it with cassava, sorghum, millet and yams (Adeniyan *et al.*, 2011; Quaye *et al.*, 2009). Cowpea varieties cultivated by most peasant farmers are the local varieties that vary widely in various forms such as seed size and grain colour etc.

Currently, cowpea consumption is higher than its production in Ghana resulting in importation. This is because the cowpea production in Ghana is constrained by pests and diseases, drought, unavailability of improved seed, poor cultural practices, and unavailability of improved tools and equipment for large-scale production (Ibrahima, 2012). Another major factor that may be affecting the consumption and production of cowpea in Ghana could be a varietal preference (Egbadzor *et al.,* 2013). Cowpea consumers in Ghana prefer cowpea that has large grain size with cream seed coat colour (Egbadzor *et al.,* 2013; Quaye *et al.,* 2009). In Ghana, cowpea is mainly imported from Burkina Faso, Niger and Nigeria due to the high demand for cowpea and customer preference for large grain with cream colour (Egbadzor *et al.,* 2013). Breeding of novel cowpea genotype with farmers' and consumer-preferred traits could boost its cultivation in Ghana.

### 2.7 Cowpea production constraints in Ghana

The main causes of cowpea yield loss are insect pests, viruses and fungi that attack the foliage and stems, including Cowpea Aphid-Borne Mosaic Virus, and pod and seedling diseases, drought and heat, the parasitic weed *Striga gesnerioides*, the unavailability of the seed of improved varieties, and the inaccessibility to agro-chemical such as pesticides (ICRISAT, 2012; Tignegre, 2010). These major constraints to cowpea production are classified into abiotic constraints like drought, heat and low soil fertility and biotic constraints such as insect pests, diseases (Jackai *et al.,* 1985). Chiezey *et al.* (1990); (Stajkovic *et al.,* 2011) also identified that the lack of proper strains of rhizobia in the soil is one of the constraints for cowpea production.

### 2.7.1 Abiotic constraints

The most important abiotic factors influencing cowpea development and productivity are drought and heat stresses, and low soil fertility. The response of crops to water stress at different stages of growth depends on the crop species, stage of development, economic portion of the crop, period and stress intensity (Akram, 2011)). Drought stress is one of the most important abiotic constraints that limit cowpea production in Africa's arid and semi-arid zones (Abdou Razakou *et al.*, 2011). Rivas *et al.* (2016) reported that cowpea is more resistant to drought. However, in regions where rainfall is low and erratic, cowpea suffers significant damage due to recurrent droughts (Singh, 2006). According to Rivas *et al.* (2016) cowpea is prone to significant damage during the flowering and pod setting stages due to recurrent drought. Agbicodo *et al.* (2009); Akyeampong (1986) reported that when exposed to drought at the vegetative stage, cowpea can sustain good seed yield, provided that there is sufficient moisture during flowering and pod setting. An increase in temperature may cause decreased photosynthetic ability, leaf senescence, inhibition of shoot and root growth, impairment of pollen and anther development, and consequently decrease crop yields (Bita & Gerats, 2013). A major threat to crop production worldwide is heat stress due to high temperatures which result in yields reduction and dry matter production in many crops (Bita & Gerats, 2013; Teixeira *et al.*, 2013). This is because high temperatures affect flower production and pollen viability (Hall, 2004). In cowpea, heat stress above a 16 ° C threshold temperature caused a loss of 4 to 14% in the setting of pods and grain yield (Hall, 2004; Mesihovic *et al.*, 2016).

Cowpea yield is also reduced because of low fertility in the soil. The low soil fertility in the Sahelian region is said to be more restrictive than rainfall for the production of cowpea

grain and fodder, and high soil fertility would increase the quality of water usage (Bationo *et al.*, 2002).

### 2.7.2 Biotic constraints

#### *2.7.2.1 Insect Pests*

The major biotic constraint in cowpea production is the insect pests infestation. They infest cowpea both in the field and under storage thereby causing a great reduction in the grain yield. Dugje *et al.* (2009) report that aphids (*Aphis craccivora*), bean pod borer (*Maruca vitrata*), bruchids (*Callosobruchus maculatus*), leafhoppers (*Empoasca spp*), foliage beetles (*Callosobruchus maculates*) and beetles (*Ootheca mutabilis*) are the major insect pests that infest cowpea in the field. These pests feed on the leaves of cowpea and also act as agents of transmission of cowpea viral diseases. In Africa, where more than 70% of the world's cowpea is produced, insect pests are often responsible for 100 percent yield losses when insect pests are not controlled (Jackai *et al.*, 1985; Obopile, 2006)

#### *2.7.2.2 Parasitic weeds*

The major parasitic weeds that affect cowpea production in sub-Saharan Africa are Striga (*Striga gesnerioides* (Wild.) and yellow witchweed (*Alectra vogelii* (Benth)). These weeds grow and attach themselves on the root surfaces of the hosts where they absorb nutrients. The yellow witchweed causes a severe reduction in yield of cowpea in Africa (Singh & Emechebe, 1997). Alonge *et al*. (2005) reported that Striga weeds also causes a serious decrease in yield in cowpea production. The use of the traditional method of controlling parasitic weeds is quite difficult because their seeds can remain dormant in the soil for several years (Kabambe *et al.*, 2013). The best method of controlling Striga and

witchweed is hand-picking immediately they start germinating or before they start flowering.

#### *2.7.2.3 Root-knot nematodes*

Another cause major cause of yield loss in the production of cowpea is root-knot nematodes which impede soil nutrient and water flow (Sharma & Haseeb, 2007). Gheysen and Mitchum (2011); Huang *et al.* (2012) reported that root-knot nematodes have a negative effect on cowpea growth and development by interfering and restricting auxin transport and plant cell differentiation pathways. Root-knot nematode species, *Meloidogyne javanica* and *Meloidogyne incognita* are popular in cowpea fields (Oliveira *et al.*, 2012). According to Oliveira *et al.* (2012), some transgenic cowpea varieties are resistant to nematodes. Cultural practices like clearing of infected crop residues from the field after harvesting and crop rotation can also prevent nematode infestation in cowpea production (Gheysen & Mitchum, 2011)

#### *2.7.2.4 Bacterial diseases*

The most common and serious bacterial diseases of cowpea are bacterial pustules caused by *Xanthomonas campestris* pv *vigna euguiculatae* and bacterial blight caused by *Xanthomonas campestris* pv *vignicola* (Viswanatha *et al.*, 2011). Both pathogens have been reported to result in 71% yield loss in susceptible cowpea varieties in India (Viswanatha *et al.*, 2011). During moderate infection, the bacteria cause the leaves to turn yellow gradually display of irregular to round spots. This leads to senescence and the dropping of leaves. Some biocontrol agents (*Trichoderma harzianum* and *Trichoderma viride* ) were confirmed to be successful in controlling cowpea bacterial blight disease (Jindal & Thind, 1993; Reddy *et al.*, 2013).

#### *2.7.2.5 Viral diseases*

Viral diseases are among the most important and biological crop pathogens in agriculture and cause significant economic losses by reducing crop yields and quality. Eight virus strains affect cowpea production and productivity in Africa and these viruses are noted to be transmitted by aphids, beetles, and other insect pests that live and feed on the cowpea (Thottappilly & Rossel, 1992). Popular beetle-borne cowpea viruses are yellow mosaic comovirus, mosaic sobemovirus and mottle virus of southern beans (Hampton *et al.*, 1997). Aphid-borne viruses of cowpea include mosaic potyvirus and cucumber mosaic cucumovirus. Some cowpeas viruses are transmitted by whiteflies including cowpea mild mottle carlavirus and golden mosaic (Thottappilly & Rossel, 1992). The red mosaic virus inhibits bacterial growth and development of rhizobium, which results in a 15 to 35 percent reduction in root nodulation (López *et al.*, 2017). Mbeyagala *et al.* ( 2014) recommended that introducing new cowpea genotypes that are resistant to a new growing environment may reduce viral epidemics. In Uganda, it was confirmed that cowpea varieties such as WC35B, WC32, and NE43 are resilient to virus strains (Mbeyagala *et al.*, 2014).

#### *2.7.2.6 Fungal diseases*

Cowpea's most damaging fungal disease involves leaf smut (false smut or black spot), which is caused by *Protomycopsis phaseoli* (Adegbite & Amusa, 2008; Singh & Allen, 1979). In cowpea, leaf smut, stem rot, and root rot are mostly caused by Fungal diseases (Mbeyagala *et al.*, 2014) Fungal diseases cause cowpea yield losses ranging from 20 to 100 percent (Adegbite & Amusa, 2008). These yields losses due to fungal diseases have been reported in several African countries.

### 2.8 Breeding strategies

Genetic enhancement of crops is a central component of the efforts to tackle global food security and nutrition demands (Ronald, 2011). The essential component of the breeding programmes is the existence of adequate genetic variability. It is necessary to induce genetic variability for self-pollinated because the genetic makeup of self-pollinated plants such as cowpea is stable over generations unless a natural mutation occurs (Singh *et al.*, 2013). Spontaneous mutations occur at very low rates and cause mutagenesis at random. Inducing genetic variability in self-pollinated species such as cowpea for crop cultivar improvement is achieved either through hybridization and/or mutation.

#### 2.8.1 Conventional approaches to breeding for early maturing cowpea

The cowpea's large flowers and untwisted keels make it one of the easiest legumes to emasculate and pollinate. Cowpea flowers have little floral nodes per raceme and have a lower abortion rate than many other species. These properties make the cowpea easier to be crossed than many other grain legumes.

The conventional breeding method used in cowpea variety development is the same as the method for breeding self-pollinated crops. These include pedigree breeding, single-seed descent, backcrossing breeding, and bulk population breeding (Boukar *et al.*, 2016). According to Fery (1985), combination of backcross and pedigree breeding methods are mainly used in plant breeding to transfer desirable traits from un-adapted cultivars into well-adapted commercial cultivars. In this approach, only three backcrosses are performed while the rest of the breeding is handled by pedigree methods.

There are some limitations, despite the success made using conventional breeding methods. Cowpea breeding using the conventional breeding approaches takes a minimum of four to

five years to develop the desired cultivars because they involve screening and identifying appropriate genotypes and introgressing the desired trait into the farmers' preferred genotypes. Also, conventional breeding includes sequential and regular phenotypic evaluations and yield performance trials (Huynh *et al.*, 2013).

#### *2.8.1.1 Pedigree Breeding Method*

This method is largely used by modern breeders in cowpea breeding. This breeding method is effective in developing cultivars with new combinations of agronomic traits (Fery, 1985). In this method, the selection of desired traits starts with the F2 population and continue until homogeneous lines are developed through successive segregated generations. In pedigree selection, desirable superior progenies are selected and the relationship between parents and progenies is recorded. These records guide the breeders in deciding which lines to continue with and which to discard. A continuous pedigree selection may increase homozygosity, but several generations are needed to achieve homozygosity in loci linked with agronomic traits (Inagaki *et al.*, 1998). Most of the lines by generation F5 or F6 are expected to be homozygous at most loci.

#### *2.8.1.2 Single Seed Descent*

Single seed descent is particularly used for the quick generation of transgenic inbred lines and the identification of quantitative trait loci. This method involves the use of a single seed from each plant and bulking the seeds from each plant for the subsequent generation. This procedure is repeated until the optimal inbreeding standard is achieved, after which superior lines are selected and evaluated for desirable characters.

Mehta and Zaveri (1997) studied various methods of breeding cowpea for comparative purposes. They found that the mean performance of the F3 progeny obtained from this

method was higher for yield and yield components than pedigree selection. In addition, single seed descent method gives a higher estimate of heritability in broad-sense than other methods.

#### *2.8.1.3 Backcross Breeding Method*

Backcross breeding method is another most common breeding method used in breeding cowpea. This method has proven effective in transferring single-resistance genes from the resistant cowpea line to susceptible cowpea lines preferred by farmers (Boukar *et al.*, 2016). Backcrossing results in increased homozygosity which allows the selection of desirable genotype. This method is extensively used for breeding disease-resistant cultivars.

#### *2.8.1.4 Bulk Population Breeding*

To reduce the time and effort to keep records relevant to the pedigree method, the breeders have been using the bulk population method. According to Boukar *et al.* (2016), the segregating populations must be harvested in bulk over several generation under natural or artificial conditions. Some bulk seeds are used for the next generation and the selection of individual plants is often started in the F6 or later generations (Mehta & Zaveri, 1997). Bulk population breeding allows the breeder to handle a large number of individuals inexpensively.

### 2.8.2 Molecular approaches to cowpea improvement

Drought escape in cowpea is controlled by multiple genes whose effects are often masked or interact with the environment (Agbicodo *et al.*, 2009). Advanced molecular breeding has led to the discovery of multiple genes linked to genes that influence traits of interest in crop plants, including single-gene genes and quantitative trait loci (QTLs) or genomic

regions that affect quantitative traits. Molecular breeding enhances the selection response, especially for traits that are difficult to improve with conventional selection due to their low heritability or traits for which phenotype measurement is difficult and costly (Xu *et al.*, 2017).

According to Varshney *et al.* (2005) some molecular breeding techniques, such as genomic selection (GS), and marker-assisted breeding (MAB) are used for crop improvement. Marker-assisted breeding (MAB) is considered to be one of the most effective approaches due to its ability to examine the entire genomic regions of the crop. Marker-assisted breeding involves marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), and genome-wide selection (GWS) (Ribaut *et al.*, 2010). Batieno (2014) used marker-assisted backcrossing (MABC) to transfer QTLs for yield under drought and stay-green into "Moussa", a farmer preferred local cowpea landrace.

### 2.8.3 Mutation approaches for cowpea improvement

The availability of sufficient genetic variation in crop species is a prerequisite for crop improvement. In plant breeding, both conventional and molecular approaches rely on the existence of sufficient genetic variation for the traits of interest (Chahal & Gosal, 2002). In self-pollinated plants where there is inherently inadequate genetic variation, this may be created through induced mutation.

In mutation breeding, the most widely documented method for producing novel genetic variation is the treatment of plant materials with chemical or physical mutagens. The choice of the type of mutagen to be used for mutation breeding depends largely on the recorded previous successes of the species, the availability of mutagens and the cost (Bado *et al.*, 2015; Mba, 2013). The IAEA and national breeding programmes routinely use gamma

irradiation to induce genetic variation and to produce mutant cultivars (Ahloowalia *et al.*, 2004; Mba, 2013). This may be due primarily to superb penetration power and the energy level of gamma radiation. According to Mba *et al.*(2010), gamma radiation primarily targets the genetic material for the biological effects and leads to various genomic aberrations. Mutations remain the primary source of inducing genetic variability in all organisms from which new alleles emerge, some spontaneously, others as a result of exposure to mutagenic agents (Spencer-Lopes *et al.*, 2018). These new alleles become the raw material for plant breeding programs.

Mutation breeding involves three main steps; (a) inducing mutations by exposing the plant propagule to the mutagen, (b) screening for putative mutant line and (c) mutant testing and official release. In this method, it is essential to determine the right mutagen dose, which can induce 50 percent lethality before beginning a large-scale experiment for an effective mutagenic treatment. A simple, careful experiment for the emergence of seedlings and the reduction of growth is regularly used to determine the right mutagen dose, i.e. a mutagen dose that results in a 50 % decrease in growth at the seedling stage (Spencer-Lopes *et al.*, 2018). This mutagen dose causes a very high degree of sterility and lethality of $M_1$ plants and therefore plant breeding programmes aimed at enhancing only one or two quantitative traits should use a dose of a mutagen causing a reduction in the growth of less than 30 per cent (Szarejko *et al.*, 2017). According to Parry *et al.* (2009), induced mutation breeding is rapidly advancing in the development of germplasms and may take up to six generations ($M_6$) only. After the $M_6$ generations, the subsequent generation single seed descent method of breeding can be used to generate a homozygous plant.

### 2.9 Radiosensitivity and determination of the right doses of gamma radiation

The optimization of the right dose of mutagen treatment for each crop genotype is an important pre-requisite prior to large scale mutagenesis. The determination of a dose that induces 50 percent seedling growth reduction and seedling emergence is the first step in breeding crops with an induced mutation. Mutagen doses above the $LD_{50}$ will induce a high frequency of mutations in the M1 population whereas, doses below the $LD_{50}$ cause a low frequency of mutations (Brown & Caligari, 2011; Szarejko *et al.*, 2017). The dose of mutagen that causes a low mutation frequency requires a larger number of mutagenized populations associated with higher labour cost. Thus, it is important to use appropriate doses of mutagen that induce the maximum frequency in $M_2$ plants, despite the somatic effect in the $M_1$ population (Parry *et al.*, 2009). According to Acquaah (2009), it is difficult to find the appropriate dose but careful experimentation can identify the dose. According to Mba *et al*. (2010), the optimal dose is the mutagen dose that achieves the maximum mutation frequency with the least possible unintended damage.

The optimal dose, however, varies from plant to plant, within the same species, plant propagule exposed to the mutagen and the physiological state of the propagule (van Harten, 1998). It has been documented that it is recommended to carry out a seedling growth test with a range of doses to establish the optimal dose for a specific variety. Most researchers have recommended that a dose close to lethal dose 50 ($LD_{50}$), should be considered as optimal in treating plant propagule with the mutagen. It is however recommended to have a very large population of induced mutations to ensure that the gene of interest carries sufficient significant mutations.

**2.10 Dose of mutagen and responsiveness of the genotype**

To identity the right doses of gamma radiation for treating plant propagules, wide survey of radiation sensitivity becomes necessary. The right dose of gamma radiation, however, varies from plant to plant, within the same species and plant propagule. In radiosensitivity studies with any plant material, it is imperative to establish a simple field test for seedling emergence, plant survival and growth reduction to determine the dose of gamma radiation that induces 50 per cent seedling growth reduction. Many researchers are of the view that a dose close to that which could kill 50 percent of the treated plant materials should be considered as the right dose of gamma radiation.

According to Soriano (1961), the dose of gamma radiation less than 30kR has no significant reduction in percentage germination of dry seed of rice but the dose 4kR and 50kR most killed most of the seeds. Diouf *et al.* (2010) studied the effect of gamma irradiation on germination, seedling height, and survival rate of two sesame cultivars. They observed that the cultivar 38-1-7 was less sensitive to gamma irradiation than the cultivar 32-15. Horn and Shimelis (2013) conducted a radiosensitivity test on three cultivars of elite cowpea in Namibia with a varying doses of gamma radiation (100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy). It was observed that the cowpea cultivars *Nakare* and *Shindimba* were more sensitive to gamma irradiation while the cultivar *Bira* was tolerant to gamma irradiation. The lethal dose was 550 Gy and 740 Gy for 32-15 and 38-1-7 cultivars, respectively. It was found that the lethal dose ($LD_{50}$) for *Nakare* was 165.24 Gy, *Shindimba* (198.69 Gy) while the tolerant cultivar *Bira* was 689.00 Gy. Gnankambary *et al.* (2019)conduct a radiosensitivity test on three cowpea genotypes in Burkina Faso with varying doses of gamma radiation (150 Gy, 200 Gy, 250 Gy and 300 Gy). They observed

that the *Moussa* local and *Tiligré* genotypes were less radiosensitive compared to KVx396-4-5-2D.

**2.11 Previous work on mutagenesis of cowpea.**

According to FAO and IAEA Mutant Varieties Database (MVD, 2020), 3,290 crop cultivars were developed through induced mutations (FAO/IAEA, 2020). There are few records on cowpea cultivars developed using an induced mutation in the FAO and IAEA Mutant Varieties Database. Adekola and Oluleye (2007) irradiated cowpea variety *IT84S-2246* at gamma radiation doses of 196 Gy and 245 Gy observed that some of the $M_2$ plants have a higher yield compared to their parent. Girija et al. (2013) observed several unique mutants at M6 generation after treating cowpea seeds with gamma rays (20, 25, 30 KR) and ethyl methyl sulfonate (EMS) (20, 25, 30 mM). The most distinct mutants identified were the colour of the flower, large seed size, shape and seed coat colour. According to Gunasekaran *et al.*(1998) after treating cowpea seeds with gamma radiation and ethidium bromide, a great variation in agronomic traits was observed in the $M_2$ plant. They also observed that gamma radiation was more effective in inducing variability in cowpea than ethidium bromide. Horn (2016) radiated four cowpea varieties in Namibia. They observed that some of the cowpea mutants are higher yielding than the parent with an average grain of 2.83 mt/ha.

It is evident from the above review that mutation breeding has been used for cowpea improvement, and most of these cowpea mutants have met the demand of farmers and consumers.

### 2.12 Importance of extra-early maturing cowpea varieties

The preference for extra-early maturing cowpea variety by farmers in Sub-Saharan Africa is similar to other cowpea growing countries (Bozokalfa *et al.*, 2017). To cope with erratic rainfall patterns in sub-Saharan Africa, most farmers purposely prefer early maturing crops to avoid total crop failure (Fatokun *et al.*, 2012). According to Bozokalfa *et al.* (2017), most farmers preferred growing early maturing cowpea varieties because these varieties provide an early harvest than the late maturing varieties. Farmers adopt extra-early maturing varieties in Savanna regions of Sub-Saharan Africa because they provide food security during the food shortage period in August / September and much emphasis is on earliness of crop maturity rather than on yield (Kamara *et al.*, 2006). Extra early maturing cowpea varieties are ideal for intercropping with other crops because they offer less competition for growth resources than late maturing varieties (Lithourgidis *et al.*, 2011)The extra-early cowpea varieties can escape detrimental heat stress as well as the water stress during the reproductive phase.

Dugje *et al.* (2009)classified cowpea varieties that mature in less than 60 days after planting as extra-early, 61-75 days after planting as early and more than 80 days after planting as late. Hall *et al.* (2003) reported that the extra-early maturing cowpea varieties are capable of producing seeds less than 60 days after planting often providing farmers with the first source of food from the current harvest sooner than any other crop.

### 2.13 Preference for cowpea varieties in Ghana

In Ghana, different cowpea varieties are cultivated by the farmers in different agro-ecological zones due to varietal adaptability to the agro-ecological zones and consumer preference for traits such as size, grain colour, sweetness and ease of cooking. According

(Egbadzor *et al.,* 2013), most farmers in Volta region of Ghana prefer cream seeded cowpea grains to other colour due to consumer preference. Owusu *et al.* (2018) also reported that some farmers in Savanna regions of Ghana prefer early maturing cowpea varieties due to erratic rainfall patterns. Some of the common cowpea varieties preferred in Ghana are reviewed below.

*Nhyira* is high yielding cowpea variety and early maturing variety (65-68 days) with an average yield of 2,460 Kg/ha (CSIR-CRI, 2019). According to CSIR-CRI (2019), Nhyira is cultivated in all the six agro-ecological zones where cowpea is grown in Ghana. Farmers prefer it for its resistance to pest, disease and drought while consumers also prefer it due to its cream seed coat and ease of cooking and sweetness (Agyeman *et al.,* 2015). According to (Egbadzor *et al.,* 2013)due to the consumers' preference for large grain size, makes these varieties less desirable by farmers in Ghana.

*Asontem* , is an early maturing cowpea variety released for cultivation in Ghana. Though some consumers reject *Asontem* for its red colour (Egbadzor *et al.,* 2013) others prefer it because of the perception that cowpea with red seed colour contains a high amount of nutrients. Due to the consumers' preference for cream grain colour *Asontem* is less preferred by most farmers due to its red seed coat colour and its poor performance in the Sudan savanna agro-ecological zone while other farmers prefer it for its resistance to pests and diseases. (Egbadzor *et al.*, 2013)

*Zaayura* is a high yielding cowpea variety and which matures between 64-67 days with a mean yield of 600 kg/ha (Kankam *et al.*, 2018). *Zaayura* is highly resistant to aphids and moderately resistant to pest, disease and Striga (Kusi *et al.*, 2020).

*Benkpla* is an extra early maturing variety (55-60) with an average yield of 1,255 kg/ha (CSIR-CRI. (2019). *Benkpla* has high grain and fodder yield but highly susceptible to cowpea stem rot. It is only cultivated in Guinea savanna zones and humid areas.

*Marfo-Tuya* is a high seed yield and medium maturity cowpea variety (66-70days) with a mean yield of 600 kg/ha (Padi *et al.*, 2004b). *Marfo-Tuya* is cultivated in the Guinea and Sudan savannah zones of Ghana and tolerant to heat during reproductive development, and resistance to Striga gesneriodes (Padi *et al.*, 2004b)

*Apagbaala* is high grain yielding variety and early maturing (60 days) with a mean yield of 600 kg/ha (Padi *et al.*, 2004a). *Apagbaala* is resistant to Striga and heat tolerant during reproductive development but susceptible to aphid (Padi *et al.*, 2004a)

*Padi-tuya* is a high grain and fodder yield cowpea variety and matures between 64-67 days with a mean yield of 400 kg/ha (Egbadzor *et al.*, 2014) It is moderately resistant to insects, diseases and striga. According to Wahaga (2019), *Padi-tuya* is cultivated in all the six agro-ecological zones where cowpea is grown in Ghana

*Songotra* is a high seed yield cowpea variety and matures between 62-65 days with a mean yield of 600kg/ha (Egbadzor *et al.*, 2014). It is highly resistant to striga and moderately resistant to most insects, diseases but susceptible to aphids.

*Bawutawuta* is a high seed yield cowpea variety and matures between 69-75 days with a mean yield of 400kg/ha (Owusu *et al.*, 2018). It is highly resistant to striga, it is moderately resistant to most insects, diseases but susceptible to aphids '*Videza* 'is high yielding variety and it is late maturing, between 68-77 days. It is moderately tolerant to Cercospora leaf spot, disease and insect-pests especially thrips (Agyeman *et al.*, 2015)

# CHAPTER THREE

# 3.0 RADIO-SENSITIVITY OF COWPEA SEED TO GERMINATION, EMERGENCE REDUCTION AND SEEDLING GROWTH TO VARYING IRRADIATION DOSES

## 3.1 Introduction

Mutations are the primary sources of genetic variation and offer plant breeders the unique raw material for crop improvement (Shu *et al.,* 2012; van Harten, 1998). For an effective mutagenic treatment, it is necessary to determine the right dose of mutagen before the start of large-scale experiment. The determination of effective radiation dose is imperative in induced mutation breeding because its predictable value guides the researcher in the choice of the right dose of mutagen, in treating the plant materials to achieve the desired outcome (Horn & Shimelis, 2013). Mba *et al.* (2010) reported that the right dose of mutagen in mutagenesis is the dose that induced the maximum mutation frequency with the least possible lethality.

There is a wide genetic variation between and within species with regard to sensitivity to gamma irradiation (Spencer-Lopes *et al.,* 2018). According to Gnankambary *et al.,* (2019), cowpea varieties exhibit different sensitivity in response to gamma radiation and have seeds moderately resistant to gamma irradiation. Hence, pilot studies of species reaction to mutagen induction are recommended. According to Mba *et al.* (2010), gamma radiation doses ranging from 0 to 600 Gy should be tested to establish the right gamma radiation dose for treating cowpea genotype. However, they did not recommend the ideal gamma radiation dose for treating cowpea due to the variation in genotypic responses to gamma radiation. In cowpea, Horn and Shimelis (2013) tested varied gamma radiation doses (50

Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, and 600 Gy) to determine the optimum dose for mutagenesis. Also, Raina *et al.* (2018) used varied gamma radiation doses (50 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy and 1000 Gy) to determine the optimum dose for cowpea.

In general, the widely used radio-sensitivity criteria in mutation breeding are seed germination, survival and seedling height, and in some cases, $M_1$ sterility has been shown to be the appropriate criterion (Shu *et al.*, 2012).

### 3.1.1 Objective of the study

The objective of this study was to determine the ideal dose of gamma radiation to induce genetic variation for earliness and high yield in cowpea variety *Videza*

#### *3.1.1.1 Specific objectives*

The study has the following specific objectives:

1. To determine the effect of gamma irradiation on percent germination of cowpea seed.
2. To determine the effect of gamma irradiation on seedling survival 14 days after germination.
3. To determine the effect of gamma irradiation on seedling height 14 days after germination.

## 3.2 Materials And Methods

### 3.2.1 Experimental Site

A field experiment was conducted on the research farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission at

Kwabenya near Accra. The farm is located at 76 m above sea level on latitude 05 40' and longitude 0 13' W. It lies within the coastal savannah zone with an annual rainfall ranging between 750 mm to 1000 mm. The predominant soil type found at the site is a well-drained Savannah Ochrosol (Ferric Acrisol, locally called Haatso series, sandy loam), derived from quartzite schist (FAO/UNESCO, 1988).

### 3.2.2 Plant Material

A farmer-preferred variety of cowpea, *Videza* was used for the study. This was obtained from the best cowpea farmer in the Volta Region of Ghana, precisely in Avenorpedo (Akatsi-North District). This cowpea variety was first planted in the Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) between March 2018 to April 2018 and pods harvested from the best performing single plant to ensure homogeneity of seeds. The harvested seed was multiplied from the best performing single selected plant, planted in the BNARI Research Farm, between July 2018 to September 2018, to obtain approximately 8,000 seeds. Dry, healthy and quiescent seeds were conditioned for irradiation. This involved cleaning to remove all debris and drying to a moisture content of 12.8% using an electronic moisture analyzer at the Seed Division of Plant Protection and Regulatory Directorate, Pokuase. Preliminary germination and viability tests were also conducted at the Seed Division of Plant Protection and Regulatory Directorate, Pokuase.

### 3.2.3 Treatment of seeds for a radio-sensitivity test

A radio-sensitivity test was carried out following the protocols established at the Plant Breeding and Genetics Laboratory of the Joint FAO/IAEA (FAO/IAEA 2018). The study involved twelve irradiation doses (0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000

and1100 Gy) replicated three times making 36 different polythene zip lock bags of conditioned seeds. Thirty seeds per dose were irradiated using the gamma irradiation facility at the Radiation Technology Centre (RTC) of the Ghana Atomic Energy Commission (GAEC) in March 2019 using a $CO_{60}$ source. All irradiation was done at a dose rate of 240 Gy/h, determined during preliminary irradiation. The varying gamma radiation doses were used to establish the $LD_{50}$ that induced the highest mutation frequency with the least possible lethality (Mba *et al.*, 2010).

### 3.2.4 Experimental Design

Irradiated seeds were planted the following day in the field at BNARI Research Farm at a spacing of 80cm x 40cm. Each plot comprised five rows of six plants/row at a seeding rate of one seed per hill. The experiment was set up in a Randomized Complete Block design with three replications. Seeds and seedlings were watered twice a week to ensure adequate soil moisture for germination and growth.

The biological effects of the gamma irradiation on the plants were studied following the protocols established at the Plant Breeding and Genetics Laboratory of the Joint FAO/IAEA (Spencer-Lopes *et al.*, 2018).

### 3.2.5 Data Collection and Analysis

The first seedling emergence was recorded on the fourth day after planting and on the seventh day after planting the final seedling emergence was recorded and expressed in percentage of control. Survival rate and seedling height were recorded and expressed in percentage of control fourteen days after planting (DAP). These parameters are known to be sufficient for assessing the damage caused by mutagenic treatment (Spencer-Lopes *et al.*, 2018). The seedling height was measured above the soil surface to the tip of the primary

leaf using a ruler and expressed in milimetres. The data taken were used to calculate seedling emergence percentage, average seedling height and survival percentage for each treatment as a follow:

Seedling emergence percentage $= \frac{numberofgeminated\ seeds}{number\ of\ seeds\ planted} x100.$

Average seedling height$= \frac{sum\ of\ seedling\ height\ in\ cm}{the\ number\ of\ plants\ \ measured} x100.$

Survival percentage$= \frac{number\ of\ surviving\ plant}{number\ of\ seeds\ planted} x100.$

The difference in seedling emergence, average seedling height, and seedling survival were expressed as a percentage of control using the equation.

**For seedling emergence**,

Percent of control$= \frac{average\ germination\ percentage\ of\ irradiated\ treatment}{average\ germination\ percentage\ of\ control\ treatment} x100.$

**For seedling height**

Percent of control$= \frac{average\ seedling\ height\ of\ irradiated\ treatment}{average\ seedling\ height\ of\ control\ treatment} x100.$

**For seedling survival**

Percent of control $= \frac{average\ surviving\ plants\ of\ irradiated\ treatment}{average\ \ surviving\ plants\ of\ control\ treatment} x100$

A simple linear regression model was used to estimate the $LD_{50}$ and $RD_{50}$, using a straight-line equation y= mx+c;

Where

y is the response variable (percent germination).

x is the independent variable (irradiation dose).

m is the slope of the straight line.

c is the coordinate on y intercept (constant).

Duncan's multiple range test at $p \leq 0.05$ was used for means separation.

## 3.3 Results

### 3.3.1 Percent germination of induced seeds

Figure 3.1 shows the effect of treating seeds of cowpea with varying doses of gamma irradiation on seed germination. Seed germination (in percentage) decreased drastically with an increasing dose of gamma radiation from 86.81% at 100 Gy to 1.39% at 700 Gy. There was no seedling emergence at the gamma radiation doses above 800 Gy.

From Fig 3.1, the seedling emergence response of the cowpea line against the irradiation dose is given by the linear equation y = -0.1558x + 87.472. The gamma radiation dose that will induce an $LD_{50}$ in the seeds was estimated at 240.51Gy.

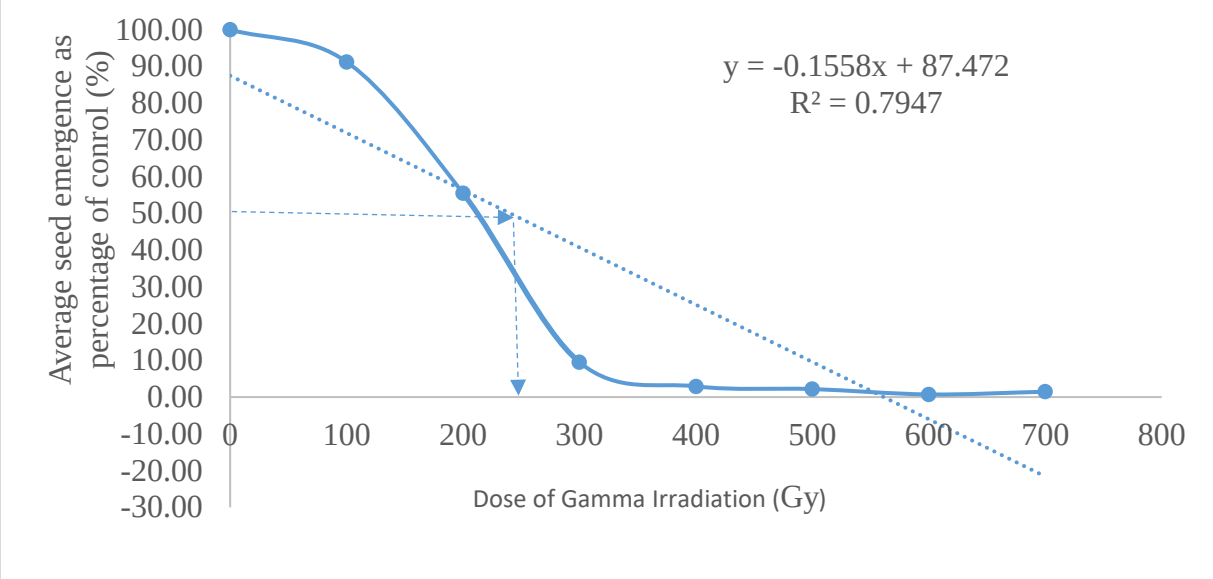



**Figure 3.1: Graph of percentage emergence reduction of cowpea seedling against irradiation doses.**

### 3.3.2. Survival reduction in induced seedling

Figure 3.2 shows the effect of treating seeds of cowpea with varying doses of gamma irradiation on seedling survival reduction. The trend observed in seedling survival is similar to the trend observed in seedling emergence. The rate of seedling survival reduced

drastically as gamma radiation dosage increased. The highest average number of surviving plants were observed at the gamma radiation dose 100 Gy (87.5%) and decreased drastically to 0.69% at 400 Gy. No seedling survival was recorded at the gamma radiation doses above 400 Gy.

From Fig 3.2, the seedling survival response of the cowpea line against the irradiation doses is given by the linear equation y = -0.1599x + 88.143. Aiming seedling survival response, y, at 50% the $LD_{50}$ value of the cowpea genotype was calculated at 238.54 Gy.

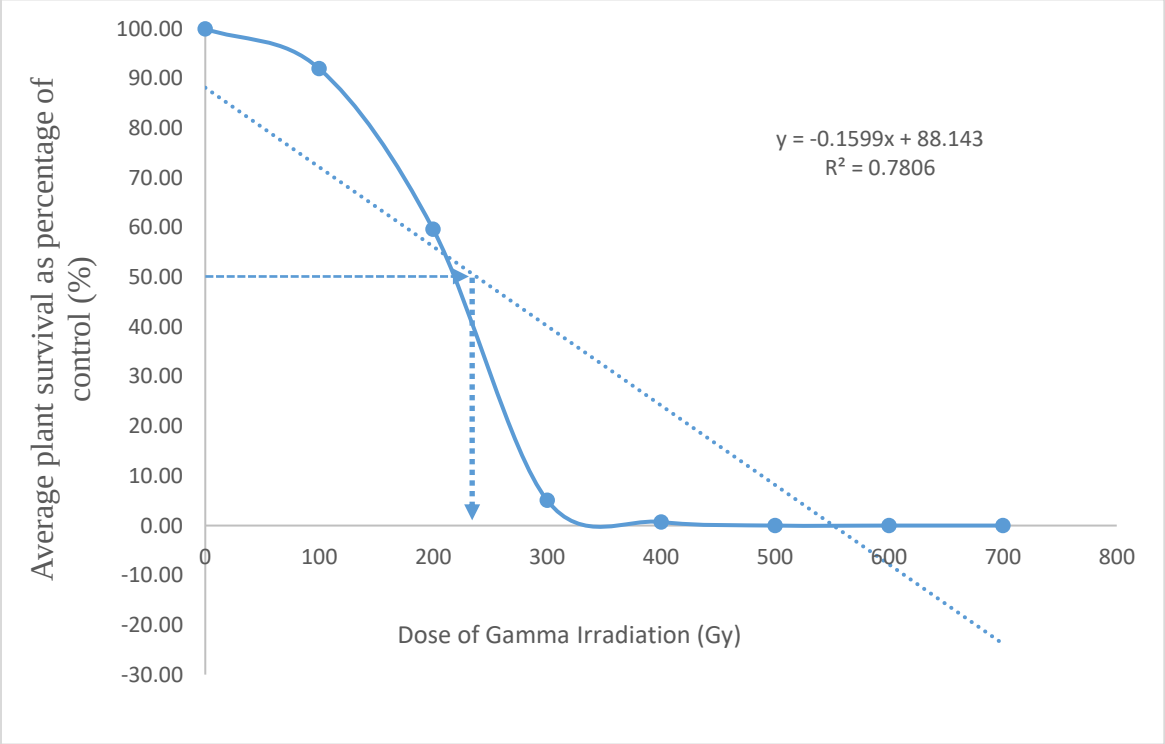



**Figure 3.2: Graph of percentage seedling survival of cowpea seedling against irradiation dose.**

### 3.3.3. Reduction in height of induced seedling

Figure 3.3 shows the effect of treating seeds of cowpea with varying doses of gamma irradiation on seedling height taken at 14 days after planting (DAP). The results showed that gamma radiation has a significant impact on plant height. The average plant height showed a decreasing trend as gamma radiation dosage increased. The tallest plants (mean height = 111.35mm) were observed for seeds irradiated at gamma radiation dose 100 Gy.

From Figure 3.3 the response of plant height the cowpea line against the irradiation dose is given by the linear equation y = -0.1544x + 87.613. The radiation dose that induced a fifty percent growth reduction ($RD_{50}$) was estimated at 243.63 Gy.

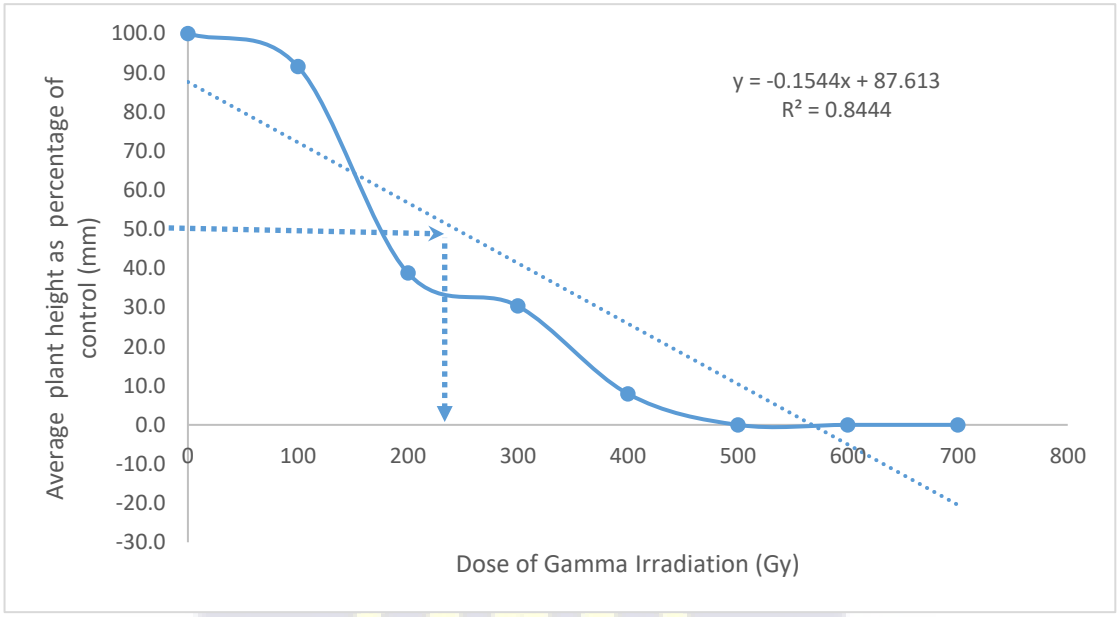



**Figure 3.3: Graph of percentage seedling height of cowpea seedlings against irradiation dose.**

## 3.4 Discussion

### 3.4.1. Percent germination of induced seeds

In this study, dose-dependent reduction of germination in the irradiated seeds was observed. Thus, seed germination decreases with an increasing dose of gamma radiation. These results are in consonance with the findings of Gnankambary *et al.* (2019) in cowpea, Horn (2016) in cowpea and (Kumar & Mishra, 2004) in Okro. The percentage decrease in seed germination at a high dose may be due to mutagen-induced disturbances in the bio-physiological metabolic pathways that play a key role in seed germination. According to De Micco *et al.* (2011), the decline in seed germination after the mutagen treatment is attributed to the abnormalities induced by mutagen in the mitotic cycles and the metabolic pathway of the cell.

### 3.4.2. Survival reduction in induced seedling

In this study, an inverse relationship between the dose of gamma radiation and seedling survival at 14 days after planting (DAP) was recorded for the cowpea variety. With an increase in the dose of gamma radiation, the seedling survival rate drastically decreased. There were significant differences among values obtained for percent seedling survival following varying doses of gamma radiation (at $p<0.001$). This observation was in consonance with (Horn & Shimelis, 2013) in cowpea, (Ramya *et al.*, 2014) in black gram. Gnankambary *et al.* (2019) who also reported that seedling survival reduced with increasing dose of gamma radiation. In this study $LD_{50}$ for induced seedling survival reduction is 213.28 Gy.

### 3.4.3. Reduction in plant height of induced seedling

All radiation doses generally caused a substantive decrease in the plant height of the cowpea line compared with the control treatment. The current findings show that the induced seedling height reduction was gradually increasing with an increase in gamma radiation. Horn and Shimelis (2013) also made a similar observation that there is a progressive increase in the mean induced seedling length reduction of epicotyl and hypocotyl in cowpea genotypes as the radiation dose increased.

## 3.5 Conclusion

From the studies carried out the following conclusion have been arrived at:

1. Percentage of seed germination decreased with increased doses of gamma radiation applied. A dose of 240.5 Gy was estimated as the $LD_{50}$.
2. Seedling survival reduced drastically with increased doses of gamma irradiation applied. A dose of 238.54 Gy was estimated as the $LD_{50}$.
3. Seedling height reduced with increased doses of gamma irradiation. A dose of 243.63 Gy was estimated as the $RD_{50}$.

# CHAPTER FOUR

# 4.0 INDUCTION OF GENETIC VARIABILITY IN COWPEA VARIETY 'VIDEZA' FOR EARLINESS AND HIGH SEED YIELD USING GAMMA IRRADIATION

## 4.1 Introduction

Genetic variability for desired morpho-agronomic traits in most self-pollinated crops can be generated either by hybridization and/or mutation. The generation of the genetic variability in cowpea using hybridization is limited on the account of the high rate of flower abortion that occurs in crops due to damage caused during emasculation and pollination. Thus, induced mutagenesis offers an alternate means of generating genetic variability in cowpea.

Mutations, whether spontaneous or induced, result in changes in gene base sequences and changes in chromosomes (Mba *et al.,* 2010; Singh *et al.,* 2006). The induced mutation has helped to widen the genetic base and therefore increased genetic variability, which has made it possible to bridge yield gaps, reduce maturity time and improve nutritional quality in many crops (Mba, 2013; Shu *et al.,* 2012). The use of induced mutation is a valuable supplementary approach to plant breeding particularly when it is desired to improve a few easily identifiable traits in a well-adapted variety (Majumder *et al.,* 2018). According to Spencer-Lopes *et al.* (2018) mutation alters the basic genotype of variety only slightly along with the introduction of a novel trait.

In general, mutations are not readily observable within the mutagenized population in the $M_1$ generation since the resulting plants would be genotypically heterogeneous (chimeric)

(Jankowicz-Cieslak *et al.,* 2017). Hence, it is important to advance normal looking and early maturing $M_1$ plants to $M_2$ generation after raising $M_1$ generation since the mutation is more visible in $M_2$ and subsequent generations due to segregation. Datta (2014) and Jankowicz-Cieslak *et al.* (2017) recommended that it is important to start phenotypic screening for observable traits from the first non-chimeric generation ($M_2$ generation) to avoid possible loss of desirable traits in subsequent generations due to the mutagenic effect. Therefore, visual screening for quantitative traits, such as the number of days to flowering, the number of pods per plant, seed yield per plant should preferably be carried out in $M_2$ generation. Horn (2016) and also Srinivas and Veerabadhiran (2010) studied the induction of variability in cowpea and observed a significant difference in the number of days to 50% flowering, days to 90% maturity, number of branches per plant, number of pods per plant, 100 seed weight, seed yield per plant in $M_2$ generation.

There have been a few reports on the use of gamma radiation in inducing variability in cowpea in Ghana. Acquaah and Klu (1983) radiated two varieties of cowpea in Ghana. However, no mutant with early flowering and/or pod maturity was observed. Instead, an erect mutant was obtained. But in Namibia, Horn (2016) irradiated four varieties of cowpea and obtained an early maturing mutant at the $M_5$ generation. In addition, early flowering and maturing cowpea mutants were developed after gamma radiation treatment with 196 Gy in Nigeria (Adekola & Oluleye, 2007). In Zimbabwe, a cowpea mutant with an increased seed size and high seed yielding ability was developed using a gamma radiation dose of 150 Gy (Matova *et al.*, 2018).

In mutagenesis, it is necessary to assess the mutagenized population for any variability induced by mutagen to confirm the effect of mutagen on the propagule. This can be

accomplished by visual screening or by using molecular methods. Visual screening is the most common, inexpensive, and efficient method used by mutation breeders to identify the phenotypic mutations (Oladosu *et al.*, 2016; Shu *et al.*, 2012).

### 4.1.1 Objective of the study

The objective of this study was to induce genetic variability for early maturity and high seed yield in cowpea variety *Videza* using gamma irradiation.

#### *4.1.1.1 Specific objectives*

The study has the following specific objectives

1. To screen the mutagenized population at $M_2$ and $M_3$ generation for lines that show promise for earliness.
2. To screen the mutagenized population at $M_2$ and $M_3$ generation for lines that show promise for high seeds yield.
3. To identify putative mutant lines that combine earliness with high seed yield.

## 4.2 Material and Method

### 4.2.1 Experimental Site

A field experiment was conducted on the research farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission at Kwabenya near Accra. The farm is located at 76 m above sea level on latitude 05 40' and longitude 0 13' W. The predominant soil type found at the site is a well-drained Savannah Ochrosol (Ferric Acrisol, locally called Haatso series, sandy loam), derived from quartzite schist (FAO/UNESCO, 1988).

### 4.2.2 Plant Material

Cowpea seeds developed from a farmer-preferred variety, *Videza* using the single seed descent method was used for the study. Dry, healthy and quiescent seeds were conditioned for irradiation. This involved cleaning to remove all debris as well as damaged and shriveled seeds before drying to a moisture content of 12.8% using an electronic moisture analyzer at the Seed Division of Plant Protection and Regulatory Directorate, Pokuase. Preliminary viability and germination tests were also conducted at the Seed Division of Plant Protection and Regulatory Directorate, Pokuase.

### 4.2.3 Treatment of seeds for raising the $M_1$ population.

A seed lot consisting of approximately 2000 cowpea seeds was acutely irradiated at 230 Gy, at the Radiation Technology Centre (RTC) of Ghana Atomic Energy Commission (GAEC), Accra, Ghana, using a cobalt 60 ($^{60}Co$) source, delivering at a dose rate of 300 Gy/hr. The non irradiated materials serve as control.

#### 4.2.3.1 **Experimental design for raising $M_1$ generation**

The irradiated cowpea seeds were planted the following day at Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI). The irradiated seeds and unirradiated seeds were established in the field using an inter-row spacing of 75cm and intra-row spacing of 40 cm with a single seed per hill. Weeds were controlled manually. The $M_1$ seeds were planted under normal growing conditions, with additional irrigation during dry spells. The $M_1$ field comprised 10 rows of irradiated seeds followed by 3 rows of control seeds separated by a spacing of 1.50m. The experiment was conducted from July 2019 to August 2019.

#### *4.2.3.2 Harvesting of $M_1$ plants*

Normal looking $M_1$ plants with the desired traits (early maturity and a large number of pods/seed size) were selected and harvested individually. All the seeds harvested from each of the selected individual $M_1$ plants formed $M_2$ seeds.

#### *4.2.3.3 Coding for the $M_2$ generation.*

Selected $M_1$ plants were each tagged with a unique code as a putative mutant. Using visual screening in the field, based on the number of days to 90 % maturity and the number of pods per plant as well as the number of seeds per plant. The codes from P1 to P30 were used to label the $M_1$ putative mutants.

### 4.2.4 Raising of the $M_2$ generation

All the seeds, derived from the selected $M_1$ lines with the desired traits were sown after drying at Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) from September 2019 to October 2019 as a single-progeny. The planting was linear and serpentine at a seeding rate of one seed per hill using a spacing of 75cm x40 cm. Control seeds were sown in three rows after every ten rows of the irradiated seeds separated by a spacing of 1.50m. Weeds were controlled manually. The $M_2$ generation was established during the offseason under irrigation.

#### *4.2.4.1 Observations recorded in $M_2$ generation*

Observations in the $M_2$ generation were recorded on ten plants in each progeny row using phenotypic appearance (early maturity and number of the pod). Procedures adopted in recording each of the observations are given below.

#### *4.2.4.1.1 Quantitative traits*

**Days to 50 % flowering:** Number of days from sowing to the stage when lines have begun to flower.

**Days to 90% maturity:** Number of days from sowing to the stage when over 90% per pods have matured and turned yellowish.

**Number of pod-bearing branches per plant:** The total number of pods bearing branches in a plant during harvesting.

**Number of pods per plant:** The number of pods per plant was recorded by counting the total number of pods per plant.

**Pod length (cm):** Pod length was measured on the pod which arose from the first fruiting node of the main axis".

**Number of seeds per pod:** The number of seeds was counted in the same pod which was used to measure the pod length and recorded as the number of seeds per pod.

**Hundred-seed weight (g):** One hundred seeds randomly counted and weighed.

**Seed yield per plant (g):** The seed yield per plant was recorded by weighing the total number of seeds produced in each sample plant."

#### *4.2.4.2 Harvesting of $M_2$ plant*

At maturity, the tagged normal-looking $M_2$ lines from each progeny line with the desired traits (early maturing and high seed yield) were selected and harvested individually. All the seeds harvested from selected individual $M_2$ plant formed $M_3$ seeds.

*4.2.4.3* ***Coding of $M_2$ and $M_3$ generation.***

Selected $M_2$ lines were tagged with unique codes as a putative mutants. The codes from P1N01 to P10N30 were used to label the $M_2$ putative mutants.

### 4.2.5 Raising of the $M_3$ generation

From March 2020 to May 2020 the $M_3$ seeds obtained from the selected $M_2$ population were sown at Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) as single-plant progenies. The planting was linear and serpentine at a seeding rate of one seed per hill using a spacing of 75cm x 40 cm. Control seeds were sown in three rows after every ten rows of the irradiated seeds separated by a spacing of 1.50m. Weeds were controlled manually. The $M_3$ generation was established during the offseason under intensive irrigation.

#### *4.2.5.1 Observations recorded in $M_3$ generation*

All observations recorded on $M_2$ were similarly recorded on selected putative mutants showing the desired traits in the $M_3$ generation.

#### *4.2.5.2 Harvesting of $M_3$ plant*

Normal looking $M_3$ plants from each progeny line with the desired traits (early maturing and high seed size) were harvested separately.

#### *4.2.5.3 Coding of surviving M3 lines.*

Selected $M_3$ pants were tagged with unique codes as a putative mutants. The codes from P1N01#01, P1N30#30, etc. were used to label selected $M_3$ lines.

### 4.2.6 Generation of codes for $M_1$, $M_2$ and $M_3$ population.

Codes generated for the $M_1$, $M_2$ and $M_3$ populations are indicated in Table 4.1 below.

**Table 4. 1 Codes generated for putative mutants in $M_1$ , $M_2$ and $M_3$ generations.**

| Generation | Selected Putative Mutant Codes |
|---|---|
| $M_1$ | P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, P11, P12, P13, P14, P15, P16, P17, P18, P19, P20, P21, P22, P23, P24, P25, P26, P27, P28, P29, P30 |
| $M_2$ | P1N02, P1N06, P1N08, P2N09, P2N11, P2N15, P3N01, P3N18, P3N21, P4N03, P4N14, P4N19, P5N05, P5N07, P5N13, P6N10, P6N12, P6N22, P7N17, P7N23, P7N27, P8N16, P8N24, P8N26, P9N14, P9N20, P9N25, P10N28, P10N29, P10N30. |
| $M_3$ | P1N02#1, P1N02#4, P1N06#20, P1N06#9, P1N08#13, P1N08#17, P2N09#12, P2N09#16, P3N01#15, P3N01#5, P4N03#2, P4N03#3, P4N14#11, P4N14#7, P5N05#10, P5N05#6, P5N07#14, P5N07#8, P6N10#18, P6N10#19. |

## 4.3 Results

### 4.3.1 Variation within the M2 and M3 generations for quantitative traits

#### *4.3.1.1 Variation in number of days to 50% flowering at M2 and M3 generations.*

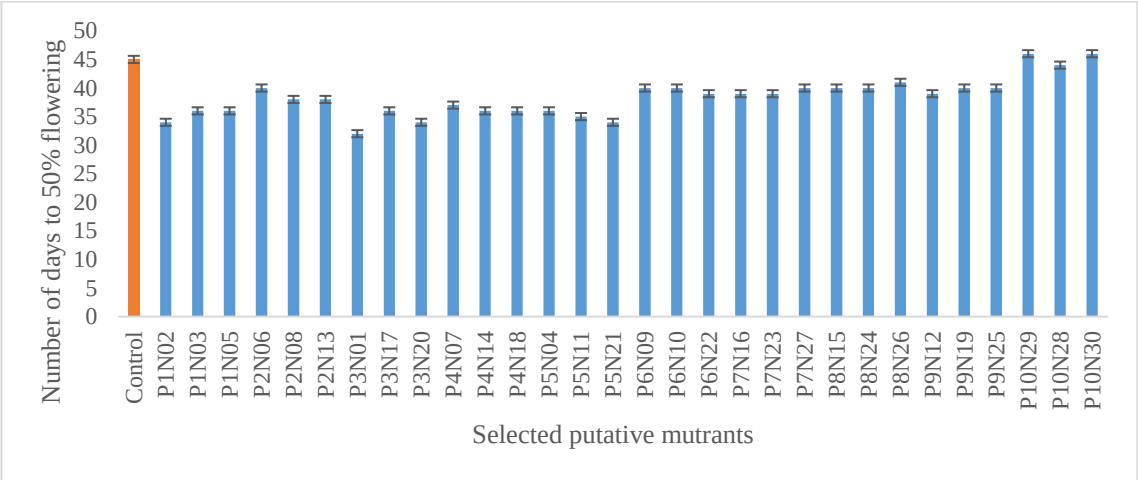
The number of days to 50% flowering at $M_2$ and $M_3$ generations are presented in figure 4.1. In the $M_2$ generation, number of days to 50% flowering ranged from 32 days to 46 days among the $M_2$ lines. The earliest to attain 50% flowering was observed in putative mutant P3N01 while the latest was observed in putative mutant P10N29. The control plant flowered at 47 days after planting.

In the $M_3$ generation, number of days to 50% flowering ranged from 30 days to 36 days among the $M_3$ lines. Putative mutants P1N02#4, P3N01#5, P4N03#2 and P4N14#7 flowered on the 30$^{th}$ day after planting while the latest to attain 50% flowering were P2N09#16 and P6N10#19. The control plants in $M_3$ generations attained 50% flowering at 46 days after planting.

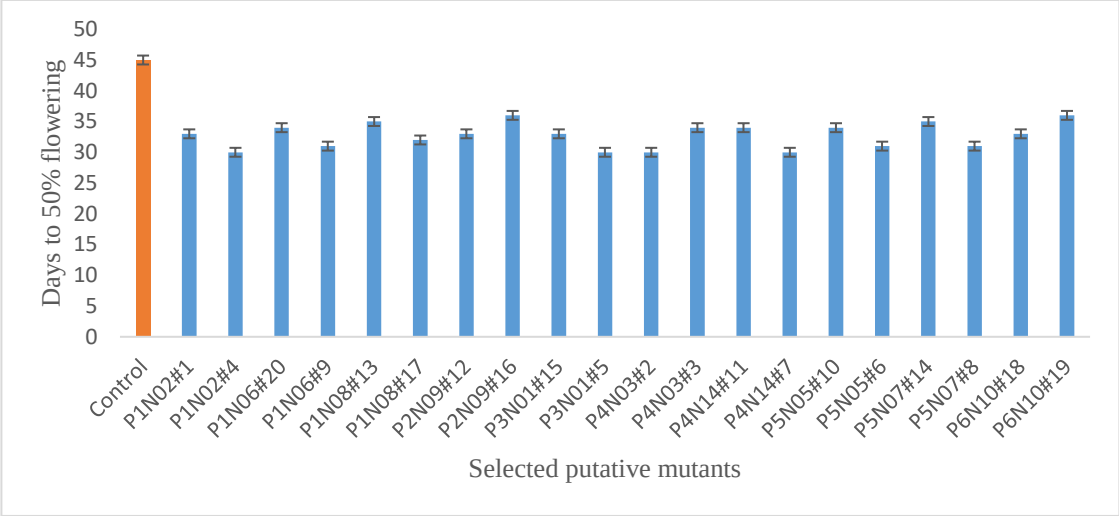
#### *4.3.1.2 Variation in number of days to 90% maturity at $M_2$ and $M_3$ generations.*

Figure 4.2 displays the number of days to 90% in $M_2$ and $M_3$ generations. Days to 90% maturity ranged from 50 days to 66 days among the putative mutant lines in the $M_2$ generation. The earliest maturing putative mutants were P1N02, P3N01, and P4N03 while the latest was putative mutant P10N30. The control plant in $M_2$ generation attained 90% maturity at 71 days after planting.

In $M_3$ generation, the number of days to 90% maturity ranged from 48 days to 60 days among the putative mutant lines. The earliest to attain 90% maturity was putative mutant P1N02#1, while the latest was putative mutants P2N09#16 and P6N10#19. The control plant in $M_3$ generation attained 90% maturity at 70 days after planting.

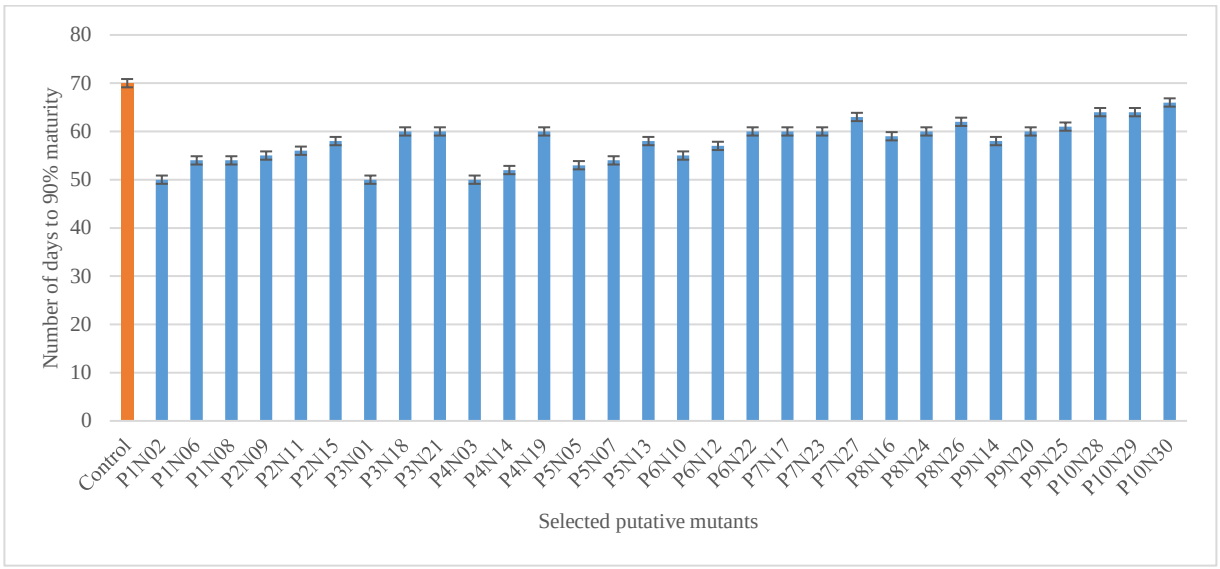



$M_2$ generation

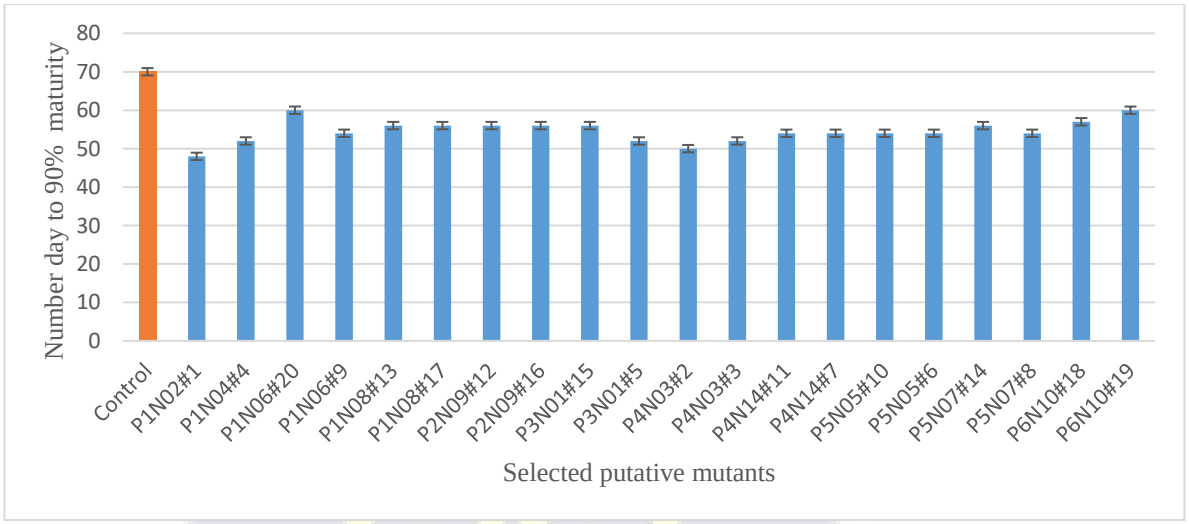



$M_3$ generation

**Figure 4.1: Number of days to 50% flowering of $M_2$ and $M_3$ generations."**




$M_2$ generation




$M_3$ generation

**Figure 4.2: Number of days to 90% maturity of $M_2$ and $M_3$ generations**

***4.3.1.3 Variation in number of pod-bearing branches per plant at $M_2$ and $M_3$ generations.***

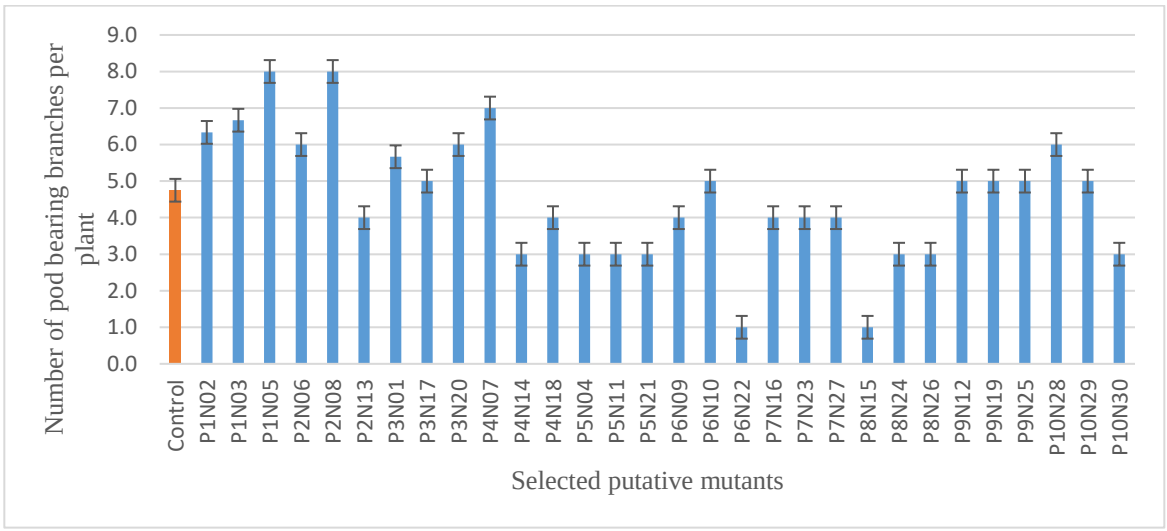
The number of pod-bearing branches at $M_2$ and $M_3$ generations are shown in figure 4.3. In the $M_2$ generation, number of pod-bearing branches ranged from 1.00 to 8.00 among putative mutant lines. Putative mutant P1N05 and P2N08 recorded the highest number of pods bearing branches while P8N15 and P6N22 had a single pod bearing branch. By comparison, the control lines had a mean of 4.75 pod-bearing branches per plant.

Number of pod-bearing branches ranged from 3.00 to 7.00 among putative mutants in $M_3$ generation. The highest number of pod-bearing branches (7.00) was observed in putative mutants P3N01#15, P1N02#1 and P4N03#3 while the lowest number of pod-bearing branches (3.00) was observed in P6N10#19. The control line had a mean of 5.00 pod-bearing branches per plant at the $M_3$ generation.

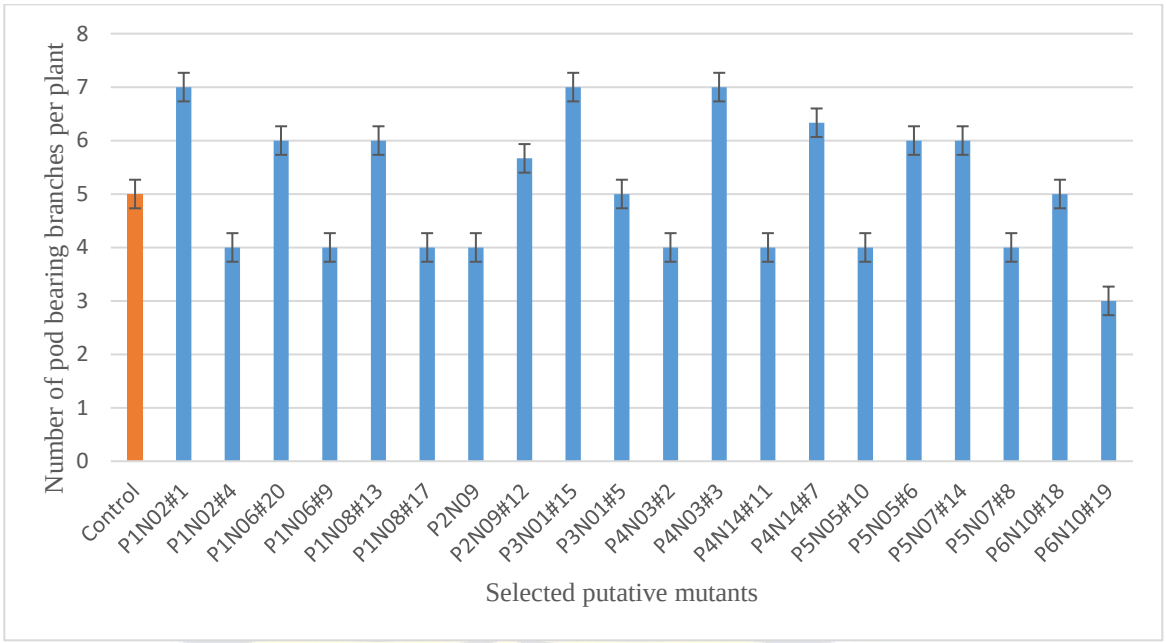
***4.3.1.4 Variation in number of pods per plant at $M_2$ and $M_3$ generations.***

Figure 4.4 presents the number of pods per plant at the $M_2$ and $M_3$ generations. The number of pods per plant ranged from 10.00 to 45.00 among the putative mutants in $M_2$ generation. The highest number of pods per plant was observed in P1N05 while the lowest number of pods was recorded in P8N15. By comparison, the control lines a mean number of pods per plant of 28.90.

In $M_3$ generation, the number of pods per plant ranged from 26 to 47 among selected putative mutants. Putative mutant P4N03#2 had the highest number of pods per plant (47) while the lowest number of pods (26) was observed in P1N08#13. The control line planted alongside the $M_3$ generation had a mean of 30 pods per plant.

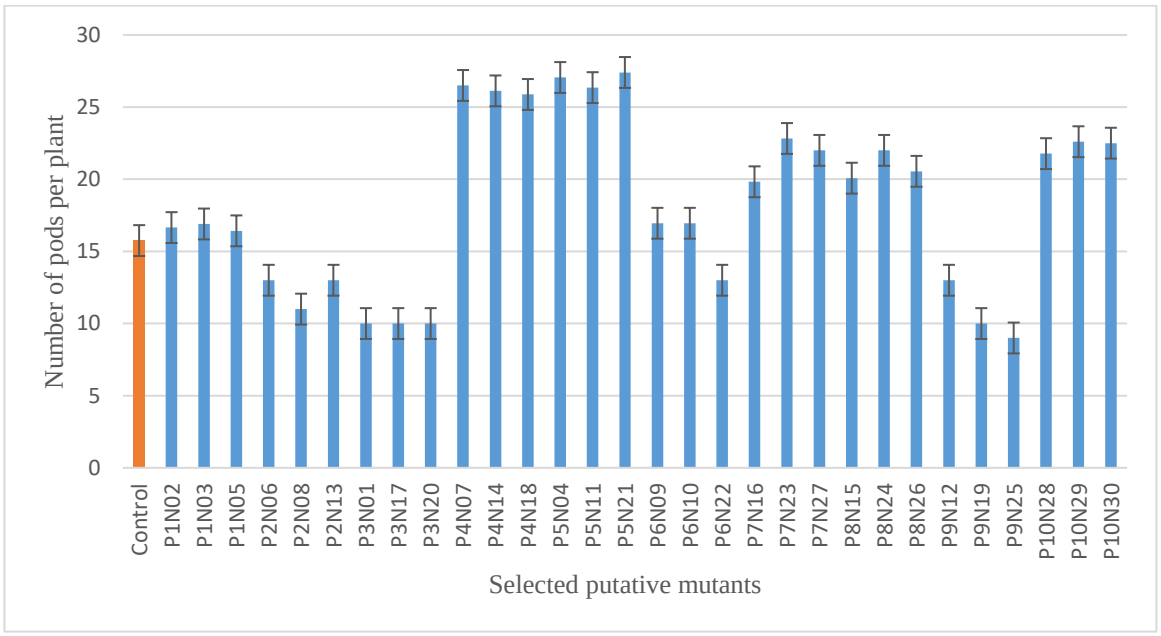



$M_2$ gerneration

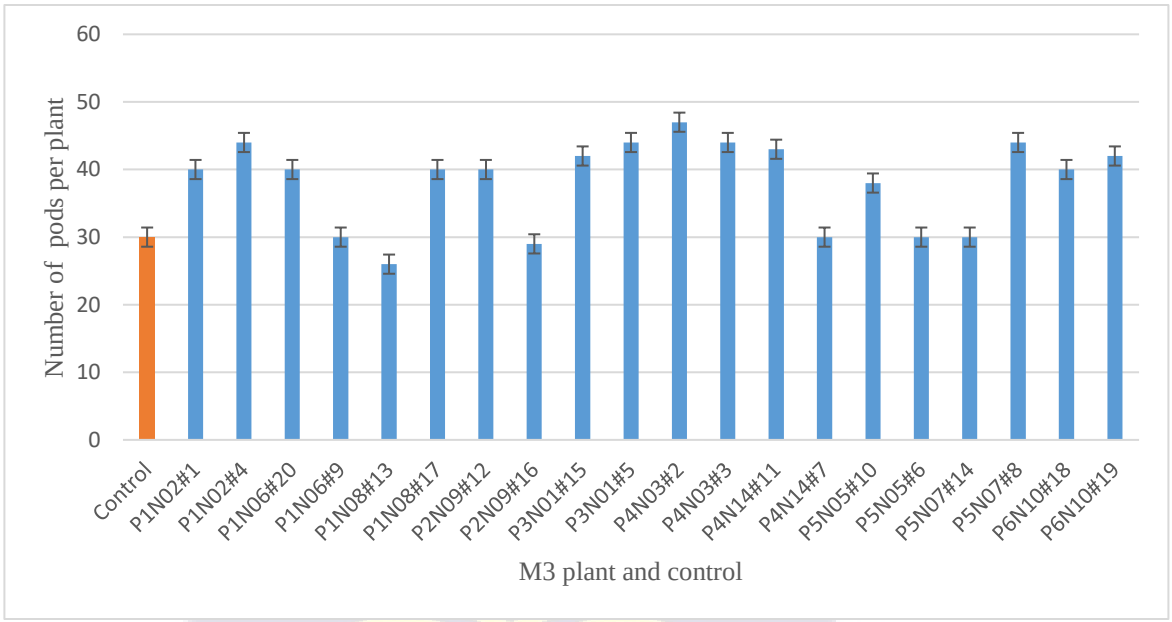



$M_3$ generation

**Figure 4.3: Number of pod-bearing branches per plant of $M_2$ and $M_3$ generations**




**$M_2$ generations**




**$M_3$ generation**

**Figure 4.4: Number of pods per plant of $M_2$ and $M_3$ generations**

#### *4.3.1.5 Variation in pod length at $M_2$ and $M_3$ generations.*

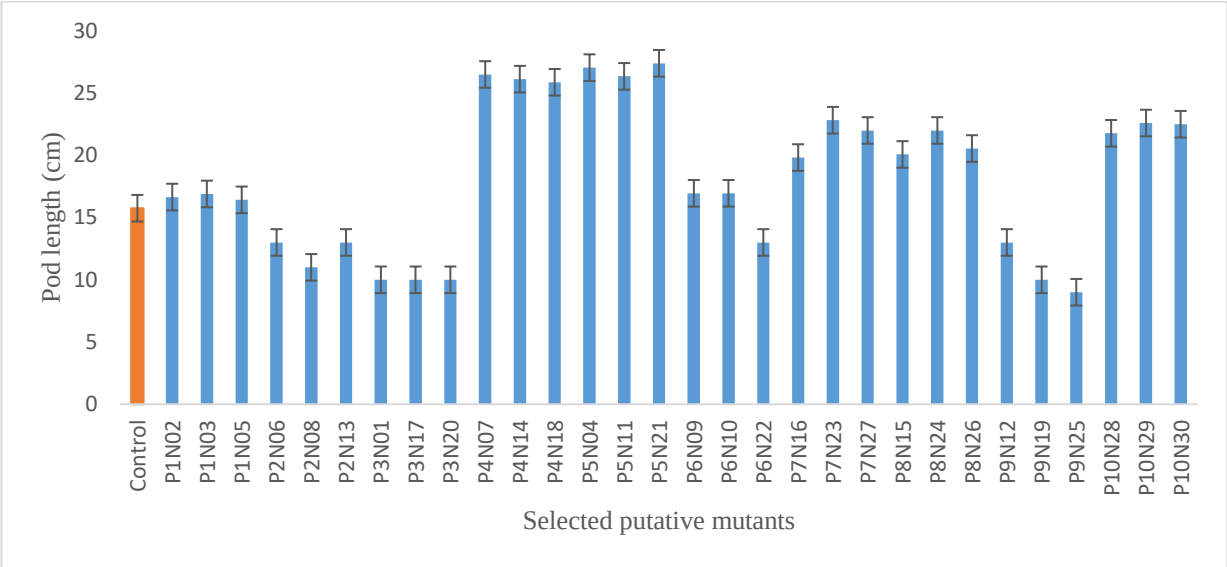
The pod lengths among the selected putative mutant in $M_2$ and $M_3$ generations are presented in figure 4.5. In the $M_2$ generation, pod length ranged from 14.30 cm to 25.40 cm among the selected putative mutants. The putative mutant P5N21 had the longest pod length while the shortest pod length was observed in P9N25. The control lines planted along side of the $M_2$ generation had a mean of 15.75 cm for pod length.

In $M_3$ generation, the pod length ranged from 14.30 cm to 26.80 cm among the putative mutants. The longest pod length was observed in putative mutant P1N06#9 while putative mutant P3N01#5 had the shortest pod length. The control lines planted along side of the $M_3$ generation had a mean of 16.50 cm for pod length.

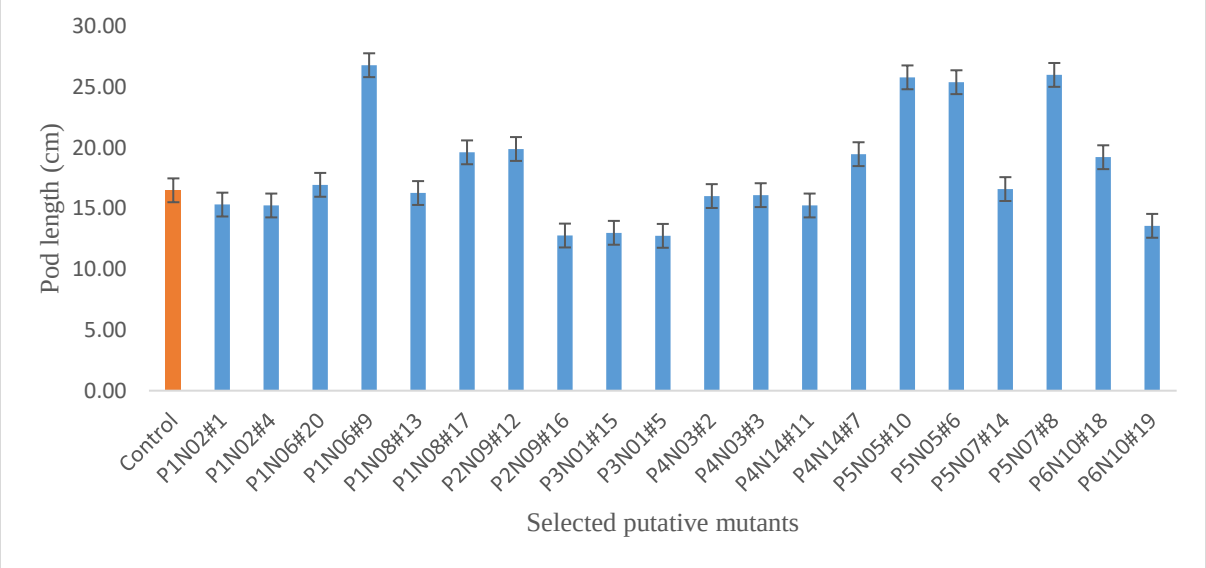
#### *4.3.1.6 Variation in number of seeds per pod at $M_2$ and $M_3$ generations.*

Figure 4.6 shows the number of seeds per pod of the selected putative mutants at $M_2$ and $M_3$ generations. In the $M_2$ generation, the mean number of seeds per pod varied from 10.25 to 22.50 among putative mutants. The putative mutant P5N11 had the highest number of seeds per pod while putative mutant P9N12 and P8N24 had the lowest number of seeds per pod. The control lines planted along side of the $M_2$ generation had a mean of 12.25 for number of seeds per pod.

The number of seeds per pod varied from 12.50 to 23.00 among selected putative mutants in $M_3$ generation. The highest number of seeds per pod was observed in P5N07#8 while P1N06#9 had the lowest number of seeds per pod. The control lines planted along side of the $M_3$ generation had a mean of 13.00 for number of seeds per pod.

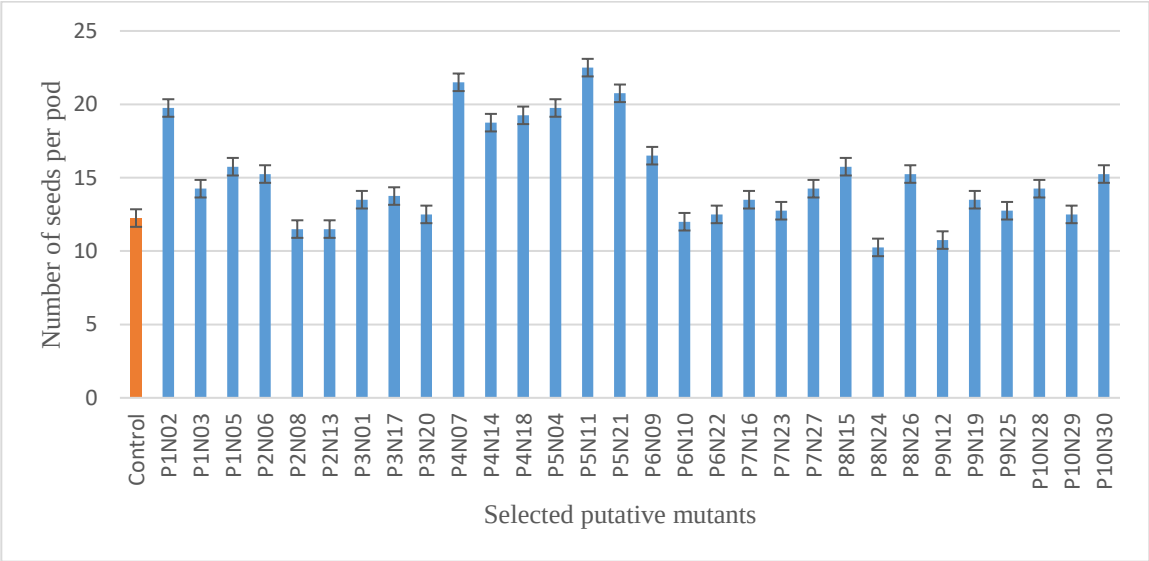



**$M_2$ generation**

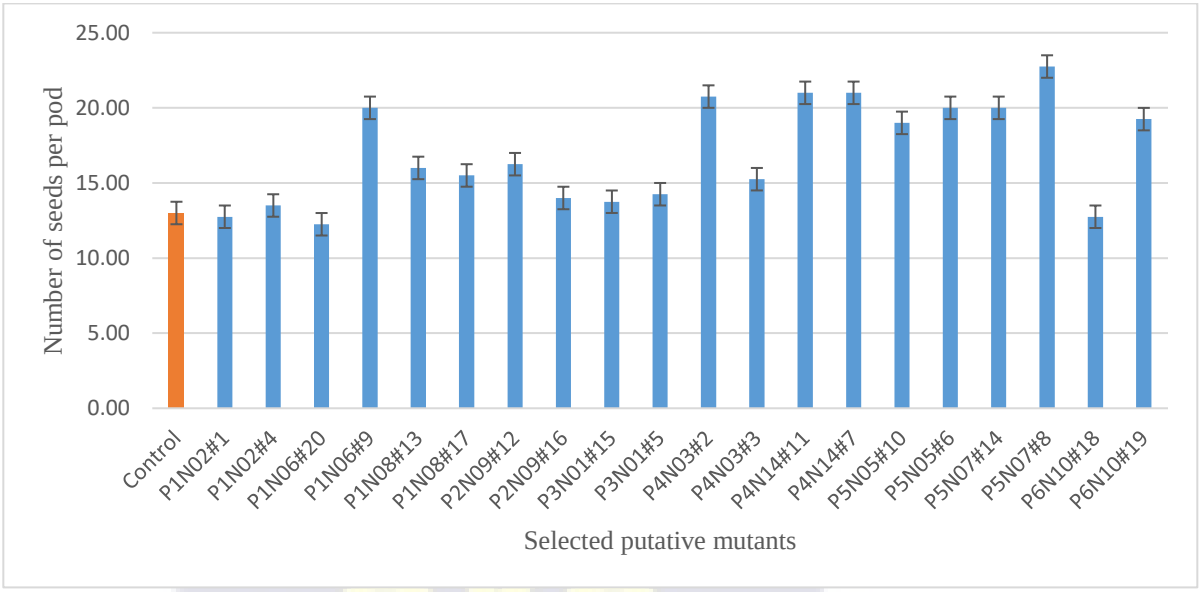



**$M_3$ generation**

**Figure 4.5: Number of seeds per pod of $M_2$ and $M_3$ generations.**




**$M_2$ generation**




**$M_3$ generation**

**Figure 4.6: Number of seeds per pod of $M_2$ and $M_3$ generations**

#### *4.3.1.7 Variation in hundred-seed weight per plant at $M_2$ and $M_3$ generations.*

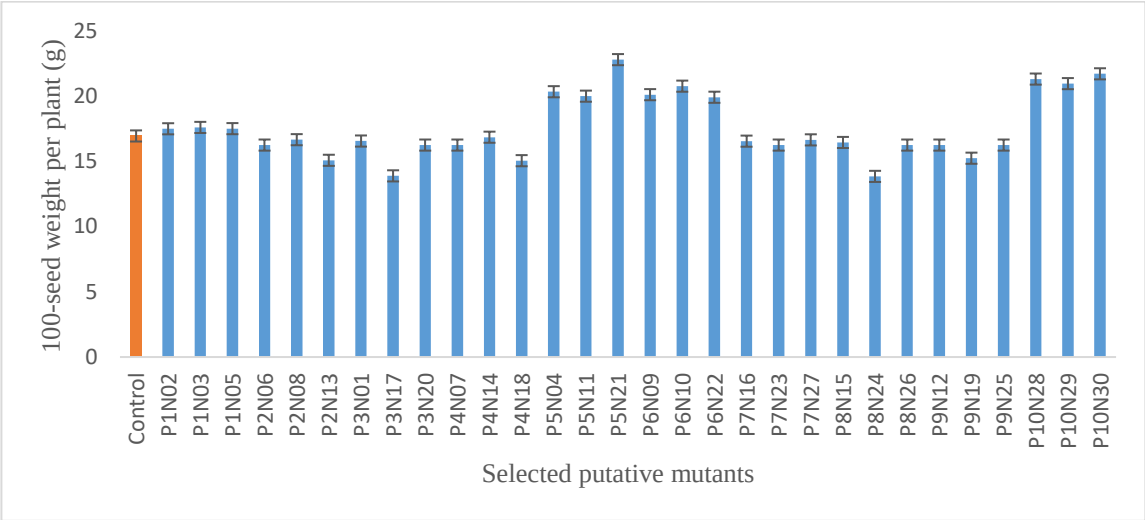
The hundred-seed weight per plant among the selected putative mutants at $M_2$ and $M_3$ generations are presented in Figure 4.7. In the $M_2$ generation, the 100- seed weight per plant varied from 13.85g to 22.80g among the putative mutants. The putative mutant P5N21 had the highest 100-seed weight per plant (22.80g) while the lowest 100-seed weight per plant was observed in P8N24 (13.85g). The control line planted alongside the $M_2$ generation had a mean of 16.95g for 100-seed weight per plant.

The hundred-seed weight ranged from 12.69g to 21.71g among selected putative mutants in the $M_3$ generation. The highest hundred seed weight was observed in the putative mutant P6N10#19 and the lowest in P4N14#11. The control line planted alongside the $M_3$ generation had a mean of 15.28g for 100-seed weight per plant.

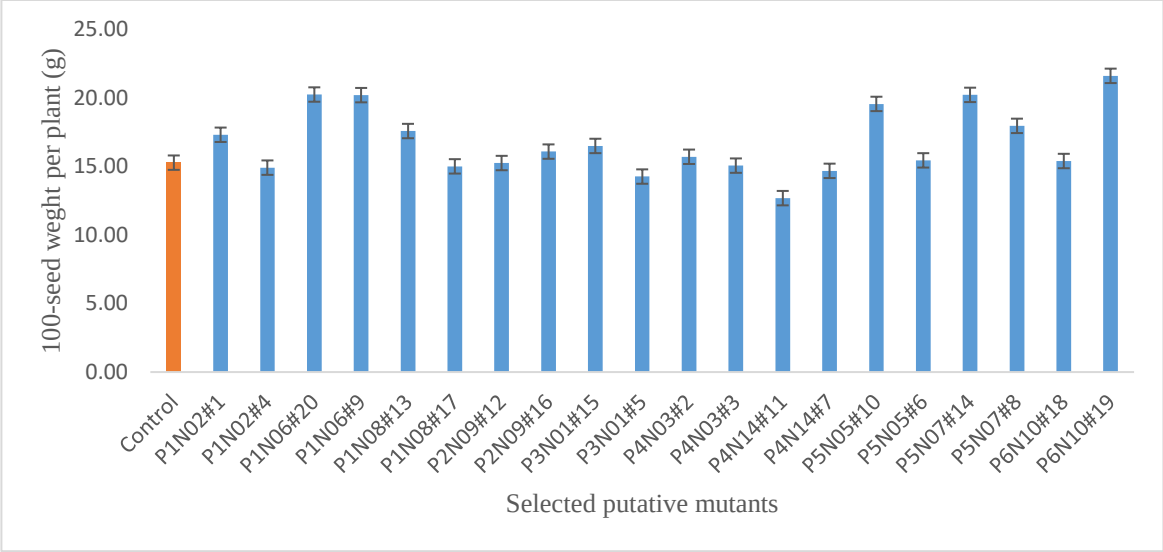
#### *4.3.1.8 Variation in seed weight per plant at $M_2$ and $M_3$ generations.*

Figure 4.8 displays the seed weight per plant among the selected putative mutants at $M_2$ and $M_3$ generations. Seed weight per plant exhibited a wide range of variation from 24.66g to 89.28g among the selected putative mutants in $M_2$ generation. Putative mutant P5N11 had the highest seed weight while the lowest seed weight per plant was recorded in P2N08. The control line planted alongside the $M_2$ generation had a mean of 45.60g for seed weight per plant.

The seed weight per plant varied from 19.12g to 95.90g among selected putative mutants in $M_3$ generation. Putative mutant P1N06#9 recorded the highest seed weight per plant while the lowest was observed in P4N03#3. The control line planted alongside the $M_3$ generation had a mean of 46.65g for seed weight per plant.

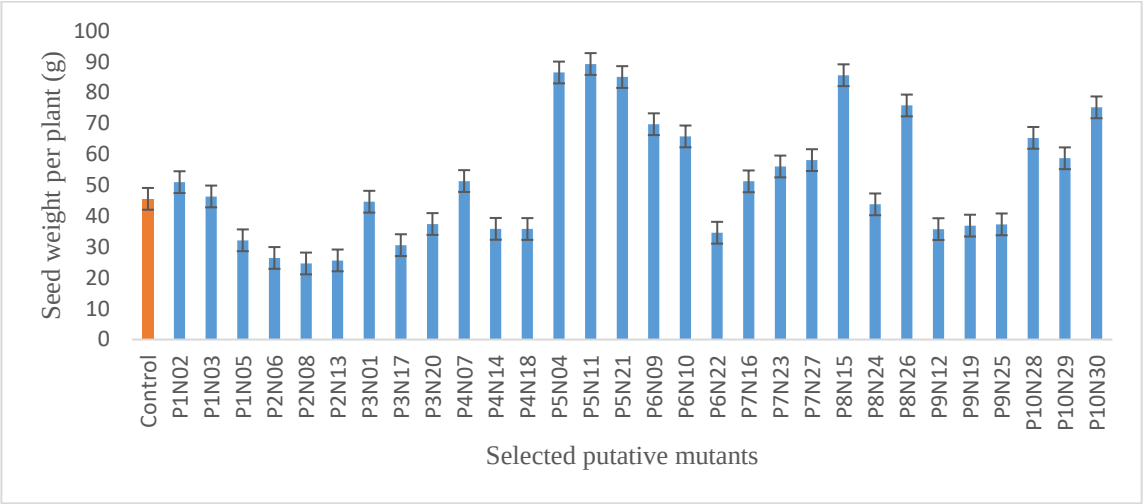



$M_2$ Generation

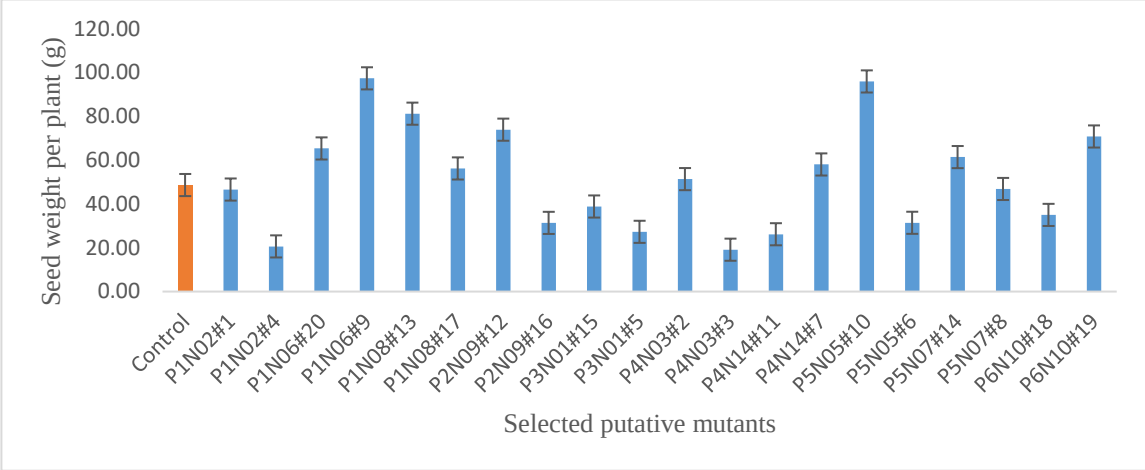



$M_3$ Generation

**Figure 4.7: 100- seed weight per plant of $M_2$ and $M_3$ generations**




**$M_2$ Generation**




**$M_3$ Generation**

**Figure 4.8: Seed weight per plant of $M_2$ and $M_3$ generations**

### 4.3.2 Putative Mutant lines exhibiting earliness and high seed yield at the $M_3$ generation

Rankings of putative mutants exhibiting both earliness and high seed yield at the $M_3$ generation are represented in Table 4.2. Putative mutants P1N06#9, P1N02#1, P1N08#13, P1N08#17, P1N06#20, P1N08#17 P2N09#12, P4N03#2, P5N05#10, and P5N07#8 were observed to exhibit early maturity as well as high seed yield.

**Table 4.2: Putative Mutant lines exhibiting earliness and high seed yield of the $M_3$ generation.**

| No. | Control/ Putative Mutant | Earliness (Days to 90% Maturity | | Seed Yield per Plant | | Summary of Rankings | |
|---|---|---|---|---|---|---|---|
| | | Value recorded | Ranking | Value recorded | Ranking | Sum | Final Ranking |
| *X* | *Control* | *70* | *8* | *48.65* | *11* | *19* | *13* |
| 1 | P1N02#1 | 48 | 1 | 46.60 | 13 | 14 | 10* |
| 2 | P1N02#4 | 52 | 3 | 20.62 | 20 | 23 | 19 |
| 3 | P1N06#20 | 60 | 7 | 65.36 | 6 | 13 | 9* |
| **4** | **P1N06#9** | **54** | **4** | **97.38** | **1** | **5** | **1*** |
| 5 | P1N08#13 | 56 | 5 | 81.24 | 3 | 8 | 3* |
| 6 | P1N08#17 | 56 | 5 | 56.23 | 9 | 14 | 10* |
| 7 | P2N09#12 | 56 | 5 | 73.94 | 4 | 9 | 4* |
| 8 | P2N09#16 | 56 | 5 | 31.38 | 17 | 22 | 18 |
| 9 | P3N01#15 | 56 | 5 | 38.85 | 14 | 19 | 13 |
| 10 | P3N01#5 | 52 | 3 | 27.28 | 18 | 21 | 16 |
| 11 | P4N03#2 | 50 | 2 | 51.36 | 10 | 12 | 5* |
| 12 | P4N03#3 | 52 | 3 | 19.12 | 21 | 24 | 21 |
| 13 | P4N14#11 | 54 | 4 | 26.18 | 19 | 23 | 20 |
| 14 | P4N14#7 | 54 | 4 | 58.05 | 8 | 12 | 5* |
| 15 | P5N05#10 | 54 | 4 | 95.97 | 2 | 6 | 2* |
| 16 | P5N05#6 | 54 | 4 | 31.41 | 16 | 20 | 15 |
| 17 | P5N07#14 | 56 | 5 | 61.42 | 7 | 12 | 5* |
| 18 | P5N07#8 | 54 | 4 | 46.86 | 12 | 16 | 12* |
| 19 | P6N10#18 | 57 | 6 | 35.01 | 15 | 21 | 17 |
| 20 | P6N10#19 | 60 | 7 | 70.83 | 5 | 12 | 5* |

*** Indicates putative mutants exhibiting both earliness and high seed yield**

### 4.3.3 Potential yield of ten top putative mutants compared to some officially released cowpea varieties

For each of the twenty selected putative mutants at the $M_3$ generation, expected seed yield (on per hectare basis) was estimated using a standard spacing (60 cm x 20cm) (Dugje *et al.,* 2009). Results for the top ten putative mutants are displayed in Table 4.2, compared with cowpea varieties recently released by the National Variety Released Committee (CSIR-CRI, 2019).

The putative mutants P1N06#9, P5N05#10, P1N08#13, P2N09#12, P4N03#2, P4N14#7, P5N07#14, P6N10#19, P1N06#20, P1N02#1, P1N08#17 and P5N07#8 outperformed the local varieties (recently released cowpea variety) in term of earliness as well as seed yield per plant.

**Table 4.3: Potential yield of six top putative mutant compared to some officially released cowpea varieties**

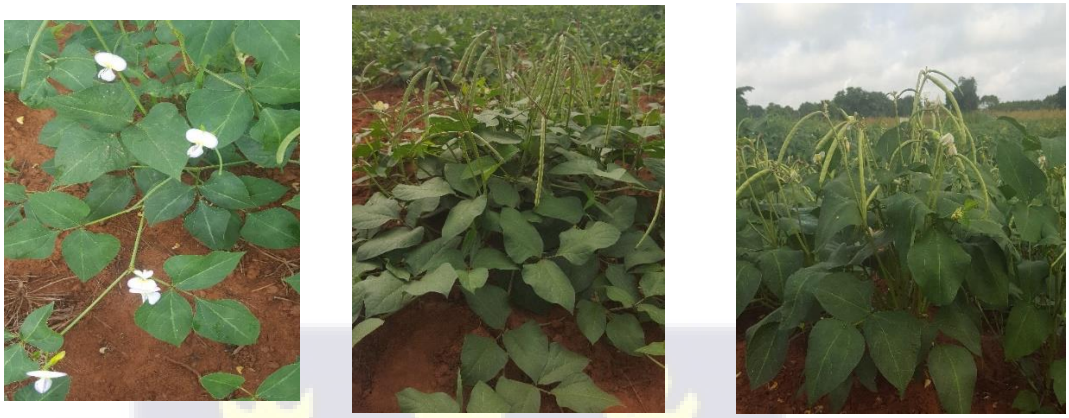
| Checks /Putative Mutant | Source | Days to Maturity | Yield Per Hectare (Estimated kg/ha) |
|---|---|---|---|
| P1N02#1 | Putative mutant-BNARI | 48 | 2796 |
| P1N06#20 | Putative mutant-BNARI | 60 | 3921 |
| P1N06#9 | Putative mutant-BNARI | 54 | 5842 |
| P1N08#13 | Putative mutant-BNARI | 56 | 4874 |
| P1N08#17 | Putative mutant-BNARI | 50 | 3374 |
| P2N09#12 | Putative mutant-BNARI | 56 | 4436 |
| P4N03#2 | Putative mutant-BNARI | 50 | 3081 |
| P4N14#7 | Putative mutant-BNARI | 52 | 3483 |
| P5N05#10 | Putative mutant-BNARI | 54 | 5758 |
| P5N07#8 | Putative mutant-BNARI | 54 | 2811 |
| P5NO7#14 | Putative mutant-BNARI | 56 | 3685 |
| P6N10#19 | Putative mutant-BNARI | 60 | 4249 |
| Videza | CSIR-Crops Research Inst. | 68-77 | 3043 |
| Hans Adua | CSIR-Crops Research Inst. | 65-67 | 3500 |
| Agyenkwa | CSIR-Crops Research Inst. | 62-64 | 3300 |
| Nketewad | CSIR-Crops Research Inst. | 62-65 | 3200 |
| Asare-moya | University of Cape Coast | 51 | 3500 |
| Aduapa | University of Cape Coast | 58 | 4050 |

**Source: Catalogue of crop varieties released and registered in Ghana (2019).**

### 4.3.4 Variation in some morphological characteristics at $M_3$ generation

#### *4.3.4.1 Growth habit*

Figure 4.9 presents growth habit variations observed in some of the putative mutants at the $M_3$ generation compare to the control. The control cowpea variety *Videza* had a semi-erect growth habit. In the $M_3$ generation, some of the induced lines changed from the semi-erect growth habit to prostrate or erect growth habit.



A. Semi-erect B. Prostrate C. Erect

**Figure 4.9:** ***Growth habit variations from the Control (Videza) of $M_3$ generation.*** Control (A) and $M_3$ plants (B and C).

#### *4.3.4.2 Flower colour*

Flower colour variations observed among some putative mutants at the $M_3$ generation compared to the Control are shown in Figure 4.10. The control cowpea variety *Videza* had white flower. At $M_3$ generation, some of the mutagenized plants changed from white flower to violet, and white with violet flower colour.

A. White flower B. Violet C. White with violet keel

**Figure 4.10: *Flower colour* variations from the control of $M_3$ generation**

Control (A) and $M_3$ plants (B and C).

***4.3.4.3 Seed eye***

Seed eye colour variations observed among some putative mutants at the $M_3$ generation compared to the control are shown in Figure 4.11. The control cowpea variety 'Videza' had blackeye. At $M_3$ generation, some of the induced lines changed the seed eye colour from a black eye to brown or brown splash seed eye.

A. Black B. Brown C. Brown splash

**Figure 4.11: Seed eye colour *deviations from the control of $M_3$ generation.***

Control (A) and $M_3$ plants (B and C).

#### *4.3.4.4 Seed coat colour*

Figure 4.12 presents Seed coat colour variations observed among some putative mutants at the $M_3$ generation compared to the control. The control cowpea variety 'Videza' had a white seed coat. In the $M_3$ generation, some of the mutagenized plants changed from white seed coat colour to brown or grey speckled or brown holstein or grey holstein,

A. White B. Brown C. Grey speclked

D. Grey Holstein E. Brown holstein,

**Figure 4.12: *Seed coat colour* variations from the control of $M_3$ generation.**

Control (A) and $M_3$ plants (B, C, D and E).

#### *4.3.4.5 Seed shape*

Figure 4.13 presents seed shape variations observed among some putative mutants at the $M_3$ generation compared to the control. The control cowpea variety 'Videza' had a kidney-shape. At $M_3$ generation, some of the induced lines were observed to change from kidney shape to globose, and Rhomboid seed shape.

A. Kidney B. Globose C. Rhomboid

**Figure 4.13: *Seed shape* variations from the control of $M_3$ generation.**

Control (A) and $M_3$ plants (B and C).

#### *4.3.5.6 Seed testa texture*

Seed testa texture variations observed among some putative mutants at the $M_3$ generation compared to the control are presented in Figure 4.14. The control cowpea variety 'Videza' had smooth to rough seed testa texture, while some of the induced lines had smooth or rough to wrinkled or wrinkled seed shape.

A. Smooth to rough B. Wrinkled C. Smooth

**Figure 4.14: *Seed testa texture* variations from the control of $M_3$ generation.**

Control (A) and $M_3$ plants (B and C).

### *4.3.5.7 Pod length*

Pod length variations observed among some putative mutants at the $M_3$ generation compared to the control are shown in Figure 4.15. In the $M_3$ generation, some of the Putative mutants had pod length longer than the pod length of the Control.


**Figure 4.15: *Pod length* variations from the control of $M_3$ generation.**

## 4.4 Discussion

### 4.4.1 Number of days to 50% flowering of M2 and M3 generations

In this study, number of days to 50% flowering reduced among the selected putative mutants compared to the control in the $M_2$ generations. Some of the putative mutants were observed to have flowered 10-15 days earlier than the control. The putative mutant P3N01 was first to have flowered at 32 days after planting while the control line flowered at 47 days after planting. In the $M_3$ generation, further reduction in days to 50% flowering was observed among some of the selected putative mutants compared to what was observed in the $M_2$ generation. A similar result was reported by Tulmann and Alves (1997) in soybeans and Karimi *et al.* (2008) in chickpea, Horn (2016) in cowpea, Shamsun *et al.* (2018) in chickpea. The reduction in flowering time would offer the possibility of selecting early maturing mutants. The reduction in the number of days to 50% flowering proves that gamma radiation stimulates gibberellin and brassinosteroids biosynthesis which activate the LEAFY (LFY) gene and causes the putative mutants to flowered earlier than the control.

### 4.4.2 Number of days to 90% maturity of $M_2$ and M3 generations.

Earliness in the maturity period of crops has been an imperative aim of plant breeders due to frequent climatic changes. In this study, number of days to 90% maturity decreased among the selected putative mutants in $M_2$ generation compare to the control line. In the $M_3$ generations, a further reduction in the number of days to 90% maturity was observed among some of the selected putative mutants compared to what was observed in the $M_2$ generation. In the $M_3$ generations, some of the putative mutants were observed to have matured 10-22 days earlier than the control. Reductions in days to 90% maturity due to gamma radiation treatment have been reported earlier in many crops including cowpea (Horn, 2016), chickpea (Shamsun *et al.*, 2018), soybean (Wang *et al.*, 2003) and chickpea (Karimi *et al.*, 2008; Wani, 2011). Early maturity is desirable for cowpea cultivation in

Akatsi District due to the loss of yield as a result of frequent drought especially during the flowering and pod filling stage. Early maturity will enable the cowpeas to escape drought during the reproductive phase (Armah *et al.,* 2011). The reduction in days to 90% maturity and the increased variability in yield characters depicted that variability induced by gamma reduction is in the desired direction and would offer the chance of selecting early maturity and high seed yield putative mutants**.**

### 4.4.3 Number of pod-bearing branches per plant of $M_2$ and $M_3$ generations.

The number of pod-bearing branches increased in most of the selected putative mutants compared to the control. The number of pod-bearing branches increased further in the $M_3$ generation. A similar result was reported by Wani (2011) in chickpea; Tulmann and Alves (1997) in soybeans and Horn (2016) in cowpea. The increase in the number of pod-bearing branches per plant observed in this study provides an opportunity for selecting putative mutant(s) with an increased number of pods per plant. The increase number of pod-bearing branches indicates that gamma irradiation caused a significant increase in the putative mutant's cytokinin-responsive genes.

### 4.4.4 Number of pods per plant of $M_2$ and $M_3$ generations.

In this study, number of pods per plant increased in some of the selected putative mutants compared to the control in the $M_2$ generation. The number of pods per plant increased further in the $M_3$ generation. It is fascinating to note that the some of the selected putative mutants with an increased number of pod-bearing branches also increased with the number of pods per plant, and this signified a close association between the two quantitative traits. Increase in the number of pods per plant after mutagenic treatment has also been reported by Horn (2016) in cowpea, Wani (2011) in chickpea and Tulmann and Alves (1997) in soybeans. Selvam *et al.* (2000); Suganthi and Murugan (2008) in cowpea reported that an increase in number of pods per plant is attributable to an increase in number of flowers.

The selection of this trait would play a vital role in increasing the seed yield per plant in cowpea.

### 4.4.5 Pod length of $M_2$ and $M_3$ generations.

In this study, some of the putative mutants had a mean pod length longer than the pod length of the control in $M_2$ generation. The mean pod length of some of the selected putative mutants increased further in the $M_3$ generation. A similar result was reported by Raina and Khan (2020) in cowpea and Pathak (1991) in cowpea. Wani (2011) also reported that gamma radiation causes an increased in pod length. The increase in pod length may be due to the incidence of favourable mutations in the irradiated population. The increase in pod length provides opportunity for selecting putative mutants with a high number of seeds per pod.

### 4.4.6 Number of seed per pod of $M_2$ and $M_3$ generations

In this study, number of seeds per pod increased in some of the selected putative mutants compared to the control. Data on the mean number of seeds per pod clearly shows that the putative mutant with the highest number of seeds per pod also exhibited longest pod length, signifying a close relationship between these two traits. Even though the mean pod length increased slightly in the $M_3$ plants, no substantial changes in the mean number of seeds per pod were observed. Khan and Wani (2006) in green gram, Wani (2006) in *Vigna radiata* Justin *et al.* (2012) in soybean, Laskar and Khan (2017) in lentil. Khan and Wani (2006) also observed no significant differences in the number of seeds per pod in $M_2$ and subsequent generation after gamma radiation treatments. The increase in the number of seeds per pod provides opportunity for selecting high seed yielding putative mutants.

### 4.5.7 Hundred seed weight per plant of $M_2$ and $M_3$ generations

Hundred seed weight is considered as one of the most reliable traits for predicting seed yield in grain legumes. In this study, an increase in 100-seed weight was recorded in some

of the selected putative mutants compared to the control in $M_2$ generation. The hundred seed weight per plant increased further in some of the selected putative mutants at $M_3$ generation. Horn (2016) in cowpea also reported significant improvement in the 100-seed weight per plant in cowpea mutants after gamma irradiation. Yuliasti and Reflinur (2018) in Soybean also reported an increase in 100-seed weight in soybean mutants. Increase in 100-seed weight per plant observed in this study indicates the possibility of further selection for comparatively large-seeded putative mutants.

### 4.4.8 Seeds weight per plant of $M_2$ and $M_3$ generations

One of the major objective of plant breeders is to improve yield of a crop. In grain legumes, seed weight per plant is influenced by quantitative traits such as the number of pod-bearing branches, number of pods per plant, seeds per pod, and 100-seed weight. Seed weight per plant increased greatly among some of the selected putative mutants compared to the control in the $M_2$ generation. In the $M_3$ generation, increase in seed weight per plant was observed. These results are in agreement with the findings of Horn (2016) in cowpea and Laskar and Khan (2017) in *Lens culinaris*. Khan et al. (2004) in *Vigna radiata* also reported a significant increase in seed weight per plant after mutagenic treatment. Increase in seed weight per plant may be due to the selection of normal looking plants in $M_1$ and further in $M_2$ and $M_3$ which led to the elimination of aberrant plants and also due to the changes induced at the genetic level after gamma irradiation treatment. Also, it may be due to an increase in other yield contributing traits especially the number of pods per plant and number of seeds per pod. The increase in seed weight per plant observed in this study indicates the possibility of further selection for comparatively higher seed yielding putative mutants.

### 4.4.9 Morphological variation

A broad range of morphological mutants such as modified growth habit (erect, prostrate) seed coat colour (brown, brown holstein, grey holstein, and grey speckled), flower colour (violet and white with violet) and longer mature pods were observed in both the $M_2$ and $M_3$ generations. Horn (2016) in cowpea also reported that a broad range of morphological mutants was observed in the $M_2$ generation after gamma irradiation treatment. The chromosomal aberrations have been attributed to be the main cause for the development of morphological mutants (Wani *et al*., 2011).

## 4.5 Conclusions

From the studies carried out the following conclusion have been arrived at:

1. Gamma radiation dose of 230Gy was effective in inducing genetic variability for earliness and high seed yield in cowpea variety 'Videza'.
2. Twenty putative mutant lines outperformed the parental line (Control) by taking between 50 and 60 days to attained 90% pod maturity compared to the former which took 70 days. These were P1N02#1 (48days), P1N02#4 (52days ), P1N06#20 (60days), P1N06#9 (54days) , P1N08#13 (56days) P1N08#17 (56days), P2N09#12 (56days), P2N09#16(56days), P3N01#15 (56days), P3N01#5 (52days), P4N03#2 (50days), P4N03#3 (52days), P4N14#11 (54days), P4N14#7 (54days), P5N05#10 (54days), P5N05#6(54days), P5N07#14 (56days), P5N07#8 (54days), P6N10#18 (57days), P6N10#19 (60days).
3. Ten putative mutant lines produced higher seed yield per plant (51.36g -97.38g) than the parental line (Control) which has a mean of 48.65g for seed yield per plant. These were P1N06#20 (65.36 g), P1N06#9 (97.38 g), P1N08#13 (81.24g), P1N08#17 (56.23g), P2N09#12 (73.94g), P4N03#2 (51.36g), P4N14#7 (58.05), P5N05#10 (95.97), P5N07#14 (61.42g) and P6N10#19 (70.83g).

4. Twelve putative mutant lines outperformed the parental line (Control) in earliness and seed yield per plant. These were P1N02#1 (48days; 46.6g), P1N06#20 (60days; 65.36g) , P1N06#9 (54days; 97.38g), P1N08#13 (56days; 81.24g), P1N08#17 (56days; 56.23g), P2N09#12 (56days; 73.94g), P4N03#2 (50days; 51.36g), P4N14#7 (54days;58.05g), P5N05#10(54days; 95.97g), P5N07#14 (56days; 61.42g), P5N07#8 (54days; 46.86g) and P6N10#19 (60days; 70.83g).

### 4.6 Recommendations

1. Twelve putative mutants P1N06#9, P5N05#10, P1N08#13, P2N09#12, P4N03#2, P4N14#7, P5N07#14, P6N10#19, P1N06#20, P1N02#1, P1N08#17 and P5N07#8 derived from the cowpea variety 'Videza' which exhibit superior earliness and high seed yield compared to the parental line(Control) are recommended for yield trials (preliminary and advanced) alongside the parental line (Videza) as well as a local check (as Controls), in farmer-participatory, multilocational trials (including the Akatsi District of the Volta Region of Ghana) towards identifying superior lines for release as new variety or varieties.
2. These putative mutants are also recommended for low-cost TILLING to screen for the genes responsible for early maturity and high seed yield.
3. Other agro-morphological variants observed in this study may also be included in some research work by future breeders.

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# APPENDICES

**Appendix 1 Average emergence of cowpea at eleven different doses of irradiation expressed as percentage of control.**

| Radiation Doses (Gy) | Average Germination (%) | Percentage of Control |
|---|---|---|
| Control (0) | 95.14 a | 100.00a |
| 100 | 86.81 b | 91.24b |
| 200 | 52.78 c | 55.48c |
| 300 | 9.03 d | 9.49d |
| 400 | 2.78 de | 2.92de |
| 500 | 2.08 de | 2.19de |
| 600 | 0.69 e | 0.73e |
| 700 | 1.39 e | 1.56e |
| 800 | 0.00 e | 0.00e |
| 900 | 0.00 e | 0.00e |
| 1000 | 0.00 e | 0.00e |
| 1100 | 0.00 e | 0.00e |

**Averages sharing the same letter do not differ significantly according to Duncan's multiple range tests at p≤0.05**

**Appendix 2. Average survival plant percentage of cowpea genotype at eleven different doses irradiation expressed as a percentage of control.**

| Radiation Doses (Gy) | Average survival Plant (%) | % of control |
|---|---|---|
| Control (0) | 95.14 a | 100.00a |
| 100 | 87.5 b | 91.97b |
| 200 | 56.73 c | 59.63c |
| 300 | 4.86 d | 5.11d |
| 400 | 0.69 d | 0.73d |
| 500 | 0.00 d | 0.00d |
| 600 | 0.00 d | 0.00d |
| 700 | 0.00 d | 0.00d |
| 800 | 0.00 d | 0.00d |
| 900 | 0.00 d | 0.00d |
| 1000 | 0.00 d | 0.00d |
| 1100 | 0.00 d | 0.00d |

**Averages sharing the same letter do not differ significantly according to Duncan's multiple range tests at p≤0.05**

**Appendix 3. Average Plant height (mm)of cowpea genotype at eleven different doses and expressed as a percentage of control.**

| Radiation Doses (Gy) | Average Plant height (mm) | % of control |
|---|---|---|
| Control (0) | 121.60 a | 100.00a |
| 100 | 111.35 a | 91.57a |
| 200 | 47.18 b | 38.80b |
| 300 | 36.92 b | 30.36b |
| 400 | 9.67 c | 7.95c |
| 500 | 0.00 c | 0.00c |
| 600 | 0.00 c | 0.00c |
| 700 | 0.00 c | 0.00c |
| 800 | 0.00 c | 0.00c |
| 900 | 0.00 c | 0.00c |
| 1000 | 0.00 c | 0.00c |
| 1100 | 0.00 c | 0.00c |

**Appendix 4. Seed germination and survival in $M_1$ generation**

| | Number of seeds sown | Number of seeds germinated | Germination % | Plant survival at maturity % |
|---|---|---|---|---|
| $M_1$ seeds | 1800 | 1130 | 62.78 | 51.61 |
| Control | 180 | 174 | 96.67 | 92.78 |

**Appendix 5. Seed germination and survival in $M_2$ generation**

| | Number of seeds sown | Number of seeds germinated | Germination % | Plant survival at maturity % |
|---|---|---|---|---|
| $M_2$ seeds | 16200 | 14629 | 90.30 | 88.03 |
| Control | 1800 | 1710 | 95 | 91.67 |

**Appendix 5. Seed germination and survival in $M_2$ generation**

| | Number of seeds sown | Number of seeds germinated | Germination % | Plant survival at maturity % |
|---|---|---|---|---|
| $M_2$ seeds | 600 | 575 | 95.83 | 91.67 |
| Control | 120 | 118 | 98.33 | 95.33 |