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WIDENING THE GENETIC BASE OF COWPEA GERMPLASM THROUGH GAMMA RAYS MUTAGENESIS

PROSPER DEO-DONNE LUMORH

2022

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WIDENING THE GENETIC BASE OF COWPEA GERMPLASM THROUGH GAMMA RAYS MUTAGENESIS

BY

PROSPER DEO-DONNE LUMORH

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

DECEMBER, 2022

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DECLARATION

Candidate's Declaration

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

Candidate's Signature …… Date ……

Name: Prosper Deo-Donne Lumorh

Supervisors' Declaration

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

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ABSTRACT

The genetic diversity of cowpea is narrow because the crop is self-pollinated. Gamma rays were used to increase genetic diversity of Hansadua, WC-36, ACC122WxWC-10, IT97K-819 and WC-10 parental genotypes. Selecting for earliness, high yields, disease resistance and high nutritional contents compared to parental controls and checks were done at M_4 generation. The results showed significant wide variations in the responses of cowpea genotypes to gamma ray doses as LD_{50} and RD_{50}. The estimated LD_{50} values were Hansadua (452.0 Gy), WC-36 (662.0 Gy), ACC122WxWC-10 (694.0 Gy), IT97K-819 (590.5 Gy) and WC-10 (591 Gy). Hansadua was the most sensitive to gamma radiation. The mass irradiation at the respective LD_{50} and RD_{50} values induced plant architecture to vary from indeterminate to determinate, semi-erect to acute erect, prostrate, spreading and some twinning. An increase in yields from 3.7t/ha in parent to 5.8t/ha in HanM4(12)(25) was observed. The ash values ranged from 2.93-3.56%, with HanM4(17)(1W) being highest. Carbohydrates ranged from 58.27-69.73% with HanM4(12)(5) being highest and protein ranged from 19.09-30.53% from the parental control with the highest in HanM4(12)(3). All the putative mutants were early maturing with days to 50% maturity of 42 to 61 days. HanM4(17)(1W) had 21.7% incidence of brown rust while HanM4(41)(HY31) recorded 10% incidence of golden mosaic disease. The results confirmed gamma ray induction can enhance yields, nutritional components, early maturity and disease tolerance/resistance of cowpea.

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KEYWORDS

High yield

Disease resistance

Mutant

Radio-sensitivity

Mutagens

Mutagenesis

Proximate

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DEDICATION

This work is dedicated to RUFORUM for providing the platform and financial support to help contribute to knowledge in broadening the genetic base of cowpea to provide food for the sustenance of humanity.

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LIST OF ABBREVIATIONS AND ACRONYMS

^{60}Co	Cobalt – 60
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive Main Effect and Multiplicative Interaction
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
AT	Adenine Thymine
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CRI	Crop Research Institute
CRPs	Coordinated Research Programmes
CSIR	Council for Scientific and Industrial Research
DNA	Deoxyribonucleic Acid
EI	Ethylene imine
EMS	Ethyl Methane Sulfonate
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FASDEP	Food and Agriculture Sector Development Project
GAEC	Ghana Atomic Energy Commission
GC	Guanine Cytosine
GDP	Gross Domestic Product
GEI	Genotype by Environment interaction
GGE	Genotype by Genotype by Environment interaction
GxE	Genotype by Environment
Gy	Grays
HCl	Hydrochloric acid

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HTPPs	High-throughput Phenotyping Platforms
IAEA	International Atomic Energy Agency
IBPGR	International Board for Plant Resources
IITA	International Institute of Tropical Agriculture
INV	Interphase nuclear volume
LD_{50}	The LD_{50} represents the dose in induced plants that is equivalent to that which kills half of an M_0 population by 50%.
MEA	Millennium Ecosystem Assessment
METs	Multi-environmental trials
MoFA	Ministry of Food and Agriculture
MVD	Mutant Variety Database
NARC	Nuclear Agriculture Research Centre
NEU	N-ethyl-N-nitrosourea
NRC	Nuclear Regulatory Commission
OER	Oxygen Enhancement Ratio
PBGS	Plant Breeding and Genetic Section
PPMED	Policy Planning Monitoring and Evaluation Directorate
RAPD	Randomly Amplified Polymorphic DNA
RD_{50}	The dose in induced plants that is equivalent to that which reduces the growth and seed production of an M_0 population by 50%.
SA	Sodium Azide
SNPs	Single Nucleotide Polymorphism
SRID	Statistics, Research and Information Directorate

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SSA	Sub-Sahara Africa
SSR	Simple Sequence Repeat
SVD	Singular Value Decomposition
TCPs	Technical Cooperation Programmes
TILLING	Targeting Induced Local Lesions in Genomes
UV	Ultraviolet Light

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Cowpea [*Vigna unguiculata* (L) Walp] (2n = 2x = 22) is an important crop for food in many tropical and sub-tropical countries (Fatokun *et al*., 2002). In Africa, majority of the world's cowpea production, consumption and trade occur. Cowpea is recognized for its high protein, which can be up to 36.75% in certain varieties (Olotuah & Fadare, 2012). It also contains fibre, folate, iron, potassium, magnesium and B vitamins. Cowpea is consumed in many different forms and more than 50 different dishes are made from it (Boukar *et al.,* 2011; Quaye *et al.,* 2011). Therefore, improving cowpea quality is important in the fight against malnourishment and improved livelihoods in Africa.

Almost all breeding programmes in cowpea target improved grain quality and yields as the major objectives during cowpea breeding programmes (Timko & Singh, 2008). Seed size, number of seeds in each pod, eye and seed coat colour are factors that influence consumer preferences (Hall *et al.,* 1997). In general, cowpea largely remains underexploited but modest investments into its breeding can unleash its potential to supply cheap protein for its consumers (Timko *et al*., 2007; Timko & Singh, 2008).

Ghana has a deficit in cowpea consumption and imports to supplement the demand (FAOStat, 2011), although the environmental conditions are suitable for producing cowpea in most agro-ecological regions of the country. Ghana's inability to produce enough cowpea affects its food security and foreign exchange earnings. Majority of the cowpea varieties imported into

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Ghana have larger seeds than those varieties cultivated locally. However, due to their genetic composition and genotype-environment interactions, these introduced cowpea varieties are not well adapted to environmental conditions of Ghana (Padi, 2007).

In the tropics, cowpeas are mainly used for food, fodder and as vegetables (Steele, 1972). In Ghana, cowpea has several vernacular names such as 'ayi in Ewe,' 'adua' or 'atedua' in Akan, 'waakye' in Hausa, 'yoor' in Ga, 'asɛ' in Akuapem and others. It is consumed as a legume and as a supplement to the daily protein requirement (Bressani, 1985); consequently, it remains one of the most important legume crops grown by farmers, primarily in the majority of sub-Saharan African nations. Cowpea is cultivated primarily due to its natural resistance to moderate dry spell and its ability to adapt to growing in nutrient-deficient soils. Through biotic symbiosis with soil microbes, cowpea can also add atmospheric nitrogen in depleted soils where farmers are unable to adequately fertilize their crops due to the expense or lack of fertilizers (Steele, 1972).

The cowpea plant is self-pollinating, and, in most cases, the fertilization process is complete before the flower opens. As a result, there is very limited natural variation in the crop. On the other hand, cowpea has a high abortion rate of flower buds, as 70-88% of its 100-500 flower buds may be aborted before anthesis. Under certain environmental conditions, approximately fifty percent of the remaining flower buds may abort, and only six to sixteen percent of all flower buds will produce mature fruit (Ojehomon, 1968).

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The top priority of plant breeders is to create superior-yielding and -quality varieties from the genetic diversity present in the natural gene pool. If genetic diversity in the existing gene pool, is narrow, new genetic material must be acquired or induced through mutagenesis. In theory, mutations are inheritable changes in the DNA sequence that led to changes in expressed protein produced. Artificially inducing genetic variation is one of the quickest and cheapest modes of increasing genetic variation in crops (Gregory, 1955). Successful mutation induction often relies on a radio-sensitivity test as a basic requirement since its predictive value of LD_{50} guides the investigator in selecting the ideal dose to be applied acutely in mass irradiation.

Some negative effects of radiation overdose include inactive protein products, deletions in DNA nucleotide sequences that cause reading frame shifts, defective transcripts leading to null or abnormal mutations, where a particular gene may be inactivated. Optimal mutagenic dose is the dose that achieves the optimal mutation frequency (Mba *et al*., 2010). The LD_{50} is a significant parameter for measuring potential toxicity in the short-term (acute toxicity) and is generally used to estimate the optimal mutation frequency to minimize unintended harm (Owoseni *et al.*, 2007). The LD_{50} or RD_{50} values are considered the best optimal guide for determining radiation doses (Mba *et al.*, 2010). For seed-applied crops such as cowpea, preliminary gamma radiation doses up to 600 Gy has been proposed to be useful in determining the optimal treatment for experimental genotypes (Mba *et al*., 2010). However, an optimal dose was not recommended for cowpea because of the variability in genotypes' responses to radiation treatment.

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Mutations can be induced by physical and chemical mutagenic treatments on seeds and vegetative propagules of plants. However, several practical challenges are associated with chemical mutagenesis, including seed soaking, target cell penetration, treatment safety and disposal (Micke *et al.*, 1990). Among the radiation-based techniques of inducing mutations, gamma rays and fast neutron bombardment have become substitutes for X-rays in most applications. Gamma radiation is the most effective physical mutagen and has been used to induce a wide variety of mutations (Bado et al., 2015). Gamma rays penetrate deeper into target tissues and are less destructive than other forms of irradiation (Mba et al., 2012), whereas fast neutron bombardments cause chromosome losses, large deletions, and translocations (Sikora et al., 2011). There are few data evaluating the response of various cowpea varieties to varying gamma doses. Therefore, prior to any huge mutagenesis, the appropriate radiation dose for the target genotypes should be determined (Tshilenge-Lukanda *et al.*, 2012).

1.2 Background to the Study

Cowpea is a significant source of food, money, animal feed, and nitrogen for the soil. The annual worldwide production of cowpea was approximately 3.6 million tonnes, with Africa accounting for more than 60 percent (Mbene *et al.*, 2000). Similarly, Nigeria, the world's largest cowpea producer, was reported to produce over 2 million tonnes, accounting for about 50% of its total annual production of cowpea (Singh *et al.*, 2002). In Ghana, the yield of available varieties of cowpea is remarkably low, with an average yield of 1.41 tonnes per hectare and a potential yield of 2.5 tonnes per hectare (MoFA, 2016), far below the achievable yield of 1.5-3.0 Mt/h

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(Dzemo *et al.*, 2010) and the 2.7mt/ha grain yield in Egypt in 2009 (FAOSTAT, 2010). Over the years, many developing countries, including Ghana, have struggled to meet their populations' food requirements (FAO, 2000). As a result, widespread food shortages, hunger and malnutrition have persisted, especially among low-income groups.

Genetic diversity in organisms has always been the basis of biodiversity, a key element in plant breeding programmes. The majority of traditional crop improvement techniques rely on the natural genetic diversity of germplasm pools (Ceccarelli & Grando, 2007). Over the years, breeders have used conventional breeding approaches to exploit the existing germplasm pool. Under this natural breeding strategy, the genetic pool is very stable and adamant to change, there is therefore the need to use artificial force in mutagens to induce new useful inheritable traits.

Artificially inducing genetic variation allows physiological and chromosomal changes to be made in crops transmitted from one generation to the next. Mutations can be induced in various ways by exposing plant propagules, such as seeds, tissues, and organs, to chemical and physical mutagens (Mba *et al.*, 2010). Unlike natural mutation or controlled crosses between unrelated parents and matched gametes, stimulated mutagenesis can generate genetic diversity for genotypic improvement and breeding in a comparatively shorter amount of time. (Singh et al., 2006; Wani, 2006; Tulmann Neto et al., 2011). Gnanamurthy et al. (2012) also report that since the 1940s, stimulated mutations have been utilized effectively in the breeding of seed-propagated crops. Numerous plant characteristics, including plant height, maturity time, resistance to seed fragmentation, resistance to

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disease, oil quality and quantity, malt quality, size, seed coat colour, and starch granule quality, have been successfully modified by induced mutagenesis, according to studies (Horn et al., 2016). Moreover, gamma irradiation was utilized in Nigeria to create cowpea mutants with tendinous leaflets, broad leaves, and pale green pods that matured early (Adekola & Oluleye, 2007). It has been reported that various doses of gamma irradiation alter the proximate and antinutrient proportions of legumes (Udensi et al., 2012). This study's main objective was to use gamma irradiation to generate mutant lines as a basis for the development of early maturing, high-yielding, quality and resistance to some major cowpea diseases in Ghana.

1.3 Statement of the Problem

The Food and Agriculture Organization (FAO) predicts that the global population will exceed 9 million by 2050 and that food production will need to increase by approximately 70 percent to meet the demand (FAO, 2009). However, the task is as daunting as the magnitude of the unprecedented projected increase. This assertion, coupled with the scarcity of land resources, implies that future crop production should be intensified per unit area, with the cultivation of superior genotypes playing a crucial role. Thus, higher yielding and more effective crops must be bred for human use. However, less than 5 percent of global crop species' biodiversity is utilized in agriculture (Tanksley & McCouch, 1997), and cowpea's genetic diversity is limited and insufficient to meet the ever-increasing human population's food demands.

In Ghana, cowpea consumption exceeds its production and there are multiple reasons for this (Egbadzor *et al.*, 2013). Studies demonstrate that

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both producers and consumers of cowpea face distinct challenges (Egbadzor et al., 2015). While farmers have interest in varieties that are early maturing, less vegetative, high yielding and resistant to drought, diseases and pest, consumers on the other hand prefer cowpea varieties with large cream seeds, sweet taste, high swelling abilities and easy to cook (Langyintuo *et al.*, 2003; Egbadzor *et al.*, 2013). However, available varieties that can satisfy both desires of farmers and consumers are limited because of the narrow gene pool in cowpea in Ghana. According to Egbadzor *et al.* (2013), this limitation causes cowpea traders in Ghana to explore other markets in the West African sub-region (Togo, Nigeria, Niger & Burkina Faso) to fulfil consumer preferences. The lack of harmonisation between the preferences of farmers and consumers also contributes to Ghana's import of cowpea and this is a disincentive to Ghana's economy.

The miscellany of growth habits in cowpea influence adaptability and adoption among farmers (Timko & Singh, 2008; Boukar *et al.*, 2018). The type of cropping system a farmer practices is greatly influenced by the growth nature of available cowpea cultivars. For instance, determinate/erect types of cowpeas may be suitable for use as intercrops while erect varieties with synchronised pod maturity enhance mechanization during harvesting. Also, indeterminate cowpea varieties that are semi-erect or creeping may be suitable in monocropping. However, if a farmer has access to only one variety, he may have problems with increased cost of production and/or marketing when the seeds produced do not satisfy consumer preferences. This may limit the farmer's choice of cropping system with respect to his resources.

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In cowpea improvement, using conventional breeding techniques such as crosses to create variability in available cowpea lines is laborious and time-demanding (Gwata *et al.*, 2016). Timing correctly for controlled crossing in self-pollinated plants like cowpea can be almost impractical. Thus, inducing genetic variability through crosses among such genotypes is less effective. Also, inducing genetic diversity with chemical mutagens have practical dysfunctions including handling, safety and disposal (Olasupo *et al.*, 2016). More so, selecting genotypes free from seed-borne and seed-transmitted pathogens is the surest way to reducing crop epiphytotics (De Tempe & Binnerts, 1979) because diseases reduce field establishment and yield (FAO & AfricanSeeds, 2018).

1.4 Justification

Cowpea is multipurpose and can be used as food, feed and as soil amendment (Akpan & Mbah, 2016; Mshelmbula *et al.*, 2019) and therefore is a food security crop (Dube & Fanadzo, 2013). The high protein content which is relatively cheap makes its development a worthy course to help improve malnutrition in Africa. Although cowpea is one of the most widely cultivated legumes in Ghana, its cultivation is constrained by droughts, diseases, pests, grain quality, and adoption of new varieties (Egbadzor *et al.*, 2013, 2015) and therefore its production has achieved slightly above 50% of its potential (MoFA; SRID, 2017). These factors reduce cowpea's potential to be used to fight hunger in Ghana and around the world where similar conditions exist.

To meet the global demands for food resources in the future, it is estimated that current annual growth rates in cowpea production be increased

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about 37% (Tester & Langridge, 2010). Since cowpea is self-pollinated, its genetic pool is adequately stable for rapid change in itself. Therefore, there is a need to induce mutation artificially to enhance variations in cowpea's useful traits using mutagens because mutagens have played a key role in improving crop varieties (Ahloowalia & Maluszynski 2001; Jain 2005). Many mutant varieties with superior performances have been developed and released for commercial production in various crops to demonstrate the economic value of the technology (Kharkwal & Shu 2009).

The overabundance of benefits derived from cowpea production and uses has led to the breeding of cowpea through various crop improvement techniques to constantly serve the needs of farmers and consumers alike (Boukar *et al*., 2016, 2018). The effectiveness of optimal mutagenesis through irradiation has been exploited to generate diversity in crop species (Mba *et al.*, 2010) since over 3200 mutant varieties in more than 200 crop species have been breed and released for commercial production worldwide. The use of ionising irradiation in mutagenesis is a better and quick environmentally friendly tool to induce genetic variability in crop species than conventional breeding and crossing techniques (Mba *et al*., 2010; Olasupo *et al*., 2016). Mutation breeding, especially with irradiation, has proven successful in improving in self-pollinated crops (Gnanamurthy & Dhanavel, 2014) because easily overcomes cross incompatibility mechanisms, high crossing barrier and poor seed setting of some self-pollinated species.

The many achievements of optimal irradiation use include mutants with higher yield, quality produce and resistance to drought, diseases and pests (Horn *et al*., 2016; Horn & Shimelis, 2013; Olasupo *et al*., 2016). With these,

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using ionising irradiation to breed for cowpea genotypes of high yield and morphological traits desired by farmers without threatening its nutritive and physical qualities preferred by consumers is highly achievable (Horn *et al.*, 2016; Gnanamurthy, Dhanavel, & Girija, 2019). Breeding for cowpea varieties through optimal irradiation for such desired attributes in Ghana will increase the adoption, cultivation and use of the mutant cowpea varieties. Furthermore, the cultivation and use of these mutant varieties would increase farmers' incomes, create employment, alleviate poverty, reduce malnutrition and improve cowpea trade imbalances in Ghana.

Despite these advantages of radiation use in cowpea breeding, little research has been done on mutation breeding exercises in Ghana involving cowpea and other legumes. There is therefore the need to use gamma radiation to induce cowpea grain quality, high yield and resistance to diseases to profit both farmers and consumers.

1.5 Objectives

1.5.1 Main objective

The objective of the study was to increase knowledge for the development of cowpea varieties that are high-yielding, early maturing and resistant to Fusarium wilt, Cercospora leaf spot, Anthracnose and Pythium diseases of cowpea.

1.5.2 Specific objectives

The specific objectives of the study are:

1. To determine LD_{50} and RD_{50} values for cowpea genotypes derived from the progeny of a single seed

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2. To produce selected genotypes at a mass level based on LD_{50} and RD_{50} data
3. To screen M_1-M_3 generations for early maturity, disease resistance and yield compared to parental controls
4. To evaluate the elite M_4 generation for earliness, disease resistance and high yield compared to parental controls using a commercial variety as a reference variety at one location.

1.6 Hypothesis

1.6.1 Null hypotheses

H_0: Ghana's current cowpea genotypes do not produce yields that meet international standards, early maturing and are not resistant to cowpea Fusarium wilt, Cercospora leaf spot, Anthracnose and Pythium disease.

That the LD_{50} and RD_{50} values are not the same for all cowpea genotypes.

1.6.2 Alternate hypotheses

H_1: The mutant cowpea elite genotypes will be high-yielding, early maturing and resistant to Fusarium wilt, Cercospora leaf spot, anthracnose and Pythium diseases of cowpea.

That the LD_{50} and RD_{50} values are the same for all cowpea genotypes.

1.7 Significance of the Study

The induction of genetic diversity in cowpea for higher yield and disease resistance will solve some challenges in agriculture such as narrow genetic diversity varieties, less spraying of insecticides, cost effective disease management in both pre- and post-harvest storage and marketing. Thus,

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improved varieties would control some biotic and abiotic stresses. The study will contribute to satisfying consumers' preference of larger grains thereby saving resources on importation from neighbouring countries.

1.8 Organization of the study

The study was divided into six chapters. The first chapter briefly described the study's background, the problem to be studied and its importance. It also discussed the study's purpose and aim, its relevance, the beneficiaries of the results and some of the challenges. Chapter two dealt with the literature review. It focused on previous researchers' works, both primary and secondary, that are relevant to the study. Chapter three established the methodology used to conduct the research. It described all the techniques and experimental designs, statistical tools, software and other scientific methods to obtain the results. The fourth chapter presented the analysed results in tables, graphs, charts, diagrams and figures. The fifth chapter of the thesis constitutes the discussion section where the main findings were compared with works done by other researchers. The sixth chapter summarized the primary findings of the study, drew general conclusions predicated on the study's findings, and made the necessary suggestions for future research.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background to cowpea

2.1.1 Origin and distribution of cowpea

Cowpea is believed to have originated in southern Africa, before spreading to East and West Africa and then Asia (IITA, 2004). The origin, geographical distribution, cultural practices, and historical records were discussed by Faris (1965), Steele and Mehra (1980), and Ng and Marèchal (1985). In West Africa, various weedy and wild species abound on both the savanna and fringes of the forest zones (Leaky & Wills, 1977) supportive of the belief that both origination and domestication had occurred first in West Africa through selecting around 300 BC (Pratap & Kumar (2011). Carbon dating of cowpea or its wild relatives from the Kintampo rock shelter in central Ghana represents the earliest archaeological evidence of cowpea in Africa (Stahl, 1985). West and Central Africa have the greatest variety of cowpea cultivars and landraces (Boukar *et al*., 2018).

It is known that cowpea was introduced to Europe through North-Eastern Africa about 300 BC and to India around 200 BC. It is also believed that cowpea reached South-Eastern Asia about 230BC and southern Europe to be grown by Greeks and Romans under the names *Phaseolus* and *Phaselus.* Cowpea was introduced into tropical America (New world) from Africa in the 17th century by the Spanish slave traders (Steele, 1976). Since the early 18th century, cowpea has been grown in the southern USA (Wolfe & Kipps, 1959).

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2.1.2 Cowpea taxonomy

Cowpea [*Vigna unguiculata* (L) Walp.] is dicotyledonous and a diploid with 22 pairs of chromosomes. It belongs to the *Fabaceae* family, sub-family *Faboideae* (Syn. *Papillionoideae*), tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna* and section *Catiang*. The genus is classified into sub-genera based upon morphology, genetic hybridization and the species' geographical distribution. The influential groups consist of the African sub-genera *Vigna* and *Haydonia*, the Asian sub-genus *Ceratotropis and* the American sub-genera *Sigmoidotropis* and *Lasiopron* (Timko & Singh, 2008).

Based on pod and seed features, cowpea cultivars can be divided into five cultivars (Timko *et al*., 2007); *Unguiculata*, *Sesquipedalis* (yard-long-bean), Biflora, Textilis (long peduncles), and Melanophthalmus. *V. unguiculata* subspecies unguiculata comprises four cultivars: *unguiculata, biflora* (or *cylindrical*), *sesquipedalis* and *textilis* (Ng & Maréchal, 1985). The *Vigna unguiculata* subspecies *dekindiana*, *stenophylla*, and *tenuis* are intermediate wild progenitors of the domesticated cowpea and make up the core of its primary gene pool. Due to a degree of pollen sterility, wild subspecies such as pubescence do not quickly interbreed, thereby forming a secondary gene pool (Fatokun & Singh (1987).

2.1.3 Cowpea genomics and genetics

Cowpea has an estimated nuclear genome size of 620 million base pairs which is considered relatively small (Boukar *et al*., 2018; Timko & Singh, 2008). Extensive genomic sequencing and analysis in cowpea have been reported by Timko *et al*.; (2007). According to Egbadzor *et al*. (2015), there are contradictory reports on number of genes that regulate the

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inheritance of seed size and gene action in cowpea and has thus contributed to the complexity of the trait's genetic control. Although numerous national institutions and the IITA have access to abundant cowpea germplasm collections, cowpea has a limited genetic base for attributes like yield and yield-related traits, drought and disease and pest tolerance (Horn & Shimelis, 2013; Mudibu *et al*., 2012).

Advances in biotechnology have made genetic resources available in several informatics applications developed to manage cowpea genomics. These include databases such as Breedlt sodium nitroprusside (SNP) Selector (http:// breedit.org/),HarvEST:Cowpea (Windows software HarvEST:Cowpea (http://harvest.ucr.edu), HarvEST:Web (http://harve st-web.org/) and ParentChecker which have been developed to assist cowpea genomic resources accessibility (Boukar *et al*., 2018). These greatly influence marker-assisted selection and hence marker-assisted breeding (Timko & Singh, 2008). Quantitative trait loci (QTL) mapping for leaf shape, seed weight, seed size, seed coat colour and patterns and maturity are known (Boukar *et al*., 2016).

2.1.4 Morphology and biology

Cowpea is an annual crop but those with indeterminate growth habits are mostly perennial (Haizel, 1972). Padulosi (1987) and Padulosi and Ng (1997) reported that cowpea has morphological variations based on different species of wild cowpeas and their primitive characteristics, including hairiness, small sized pods and seeds, pod shattering with noticeable exine on the pollen surfaces of the pollen, outbreeding, and stigma beardedness.

Cowpea can be a prostrate, trailing, bushy, climbing, erect or sub-erect annual plant that grows between 15 and 80 cm in height. The tap root system

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is well developed with numerous laterals and large nodules, globular and often in groups (Masangwa, (2012). The seeds vary in size and shape. Noticeable cowpea shapes include globose, kidney, crowder, rhomboid and ovoid.

2.2 Cowpea production and consumption

2.2.1 World production

Cowpea is mainly produced in the tropics and sub-tropics (Clark & Raidaza, 2017). On approximately 12.8 million hectares of land, an estimated 7.6 million tons of cowpea are produced globally every year. About 64 percent is located in Africa, 21 percent in the Americas, and the remainder in Europe and Asia (Akpan & Mbah, 2016; FAOstat, 2017). Nigeria is the highest global producer of cowpea (2.14 million metric tonnes) yet it domestically consumes more than three (3) metric tonnes (FAOstat, 2017). Other important cowpea producing counties in sub-Saharan Africa are Niger and Burkina Faso with 1.59 and 0.57 million metric tonnes respectively (Boukar *et al*., 2018).

Cowpea yields in most Sub-Saharan African countries is less than 1000 kg/ha. Comparing the top 20 world producers of cowpea, Serbia has the highest yield per are (3,389 kg/ha) and Mozambique has the least; 275 kg/ha (FAOstat, 2017). The actual yields obtained in West Africa are lower (0.025 - 0.100 tons per hectare) due to severe attacks from an extensive pest and disease complex, inadequate inputs and the environment (www.Ishs.org/acta). However, grain yields between one and four metric tons per hectare are possible under optimal conditions and if products are protected against insect attack (Siddique *et al*, 2012).

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2.2.2 Cowpea production and seed characteristics in Ghana

Cowpea is an integral part of Ghana's sustainable cropping system. Apart from maize, cowpea is the second most important crop grown in Ghana. It is currently classified as a food security crop, especially across Northern, Savanna and Coastal Ghana regions (MoFA, 2010). The majority of cowpea cultivation in Ghana is confined to the Guinea Savanna and, to a lower extent, the Sudan and Coastal Savanna agro-ecological zones (Asafo Adjei et al., 2005). Outside of savanna zones, cowpea production is primarily subsistent. Certain species are bred to thrive in particular agro-ecological zones (Addo-Quaye *et al.*, 2011).

Cowpea ranks second among the major leguminous crops grown in Ghana in aspects of acreage of cultivation, volume produced, and yearly intake (Egbadzor, 2014). Ghana produced 206,380 Mt on 147,000 ha in 2016 (MoFA; SRID, 2017). While cowpea production has been increasing over the years, the area under cultivation has been slightly declining since 2003 to just above 163, 000 ha in 2010. This decline in farm size may be attributable to factors such as decreased farmer interest, difficulties with land tenure systems, urbanization, and the availability of varieties preferred by consumers. The increases in cowpea production could be as a result of improved agronomic practices and superior released varieties. Ghana consumes more cowpea than it produces. The deficit in cowpea production is supplemented by imports from neighbouring West African states. Reports show that Ghanaians prefer large cream cowpea grains (Egbadjor *et al* 2013; Quaye *et al.*, 2009).

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The production of varieties of cowpea preferred by consumers could increase cowpea cultivation in Ghana. Asontem is the most popular cowpea variety in Ghana. It was developed by the International Institute of Tropical Agriculture (IITA) and introduced by the Council for Scientific and Industrial Research – Crop Research Institute of Ghana (CRI) (Asafo-Adjei *et al.,* 2005). The hard seed coat of Asontem makes it cook for a relatively long time. This together with its red seed coat may be regarded as limitations of Asontem to the Ghanaian consumer. "Nhyira" was also a variety bred and released by CRI. Despite the cream seed coat of "Nhyira", its relatively small seededness makes it undesirous by consumers. The most favourite and therefore imported types of cowpea grain are large and white or cream-coated. Therefore, varieties with these essential characteristics are typically imported and expensive.

Along the coastal savannah agro-ecological zone of Ghana, there are reasonable indications for increased cowpea production. The cowpea crop is relatively drought-resistant and has a short growing season (Muchero *et al.,* 2009). The crop is primarily cultivated by farmers with limited resources. Hence, most markets in south are flooded with cowpea produced in northern Ghana or other West African states Togo, Burkina Faso, Nigeria and Niger. Again, due to genetic by environment interactions, most imported types are not typically grown in Ghana (Langyintuo, 2004). Cowpea breeding for preferred consumer traits and climatic adaptation would increase its cultivation to sustain its existing market potential in Ghana.

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2.2.3 Cowpea yields in Ghana

Ghana produced 7,200 metric tons of cowpea in 1991 and there has been a slight improvement since then (PPMED, 1999). The average production of the crop in 1991 was 0.9 metric tons per hectare. However, yields of 2.0 metric tons per hectare have been reported in some isolated areas where effective agronomic and crop protection practices (PPMED, 1999), especially on Guinea's loamy soils Savannah and Transition zones (CRI,1998). The low tonnage production of cowpea in Ghana is due to poor integrated management practices, such as unimproved varieties, use of susceptible varieties, poor soils and cultural practices, pre-harvest pests and diseases and post-harvest losses (FASDEP, 2002). The yield of cowpea is positively associated with the number of pods per plant per unit area, seeds per pod and weight of 100 hundred seeds (Amoatey, 1987). Characteristics such as days to flowering, days to maturity, peduncle length, and pod length, which were secondary traits, also affect seed yield (Doku, 1970). Growth habits do influence the reproductive efficiency and outcome of cowpea. The plant's height is determined by the primary shoot and the number of nodes formed to affect the plant's reproductive parts. It has been observed that the erect cultivars of cowpea have higher reproductive efficiency while semi-erect cultivars have higher total pod yield than spreading types (Ezedinma, 1965). Ebong (1972) showed that pods per plant and 100 seed weight were independent of each other but were significantly associated with cowpea's grain yield. New studies highlight more on the pods per plant as the major yield component rather than seeds per pod because seeds per pod is negatively correlated with pods per plant (Turk *et al*., 1980).

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2.2.5 Cowpea production systems

In Western Africa, fodder and grain type cowpea varieties are sometimes grown as a pure crop and commercial production is mainly done in states like Nigeria, Niger, Burkina Faso and Ghana. The available different varieties with unique growth habits (erect, semi-erect and creeping) influences the cropping systems (mono-cropping or mixed cropping) practised by most small-scale farmers (Dugje *et al.*, 2009; Boukar *et al.*, 2018). The cultivation of cowpea is mechanized in developed countries Fery, (2002). Synchronized maturity, growth habit and pod characteristics affect mechanization in cowpea production.

2.2.6 Cowpea consumption in Ghana

In Ghana, cowpea consumption exceeds production (Langyintuo *et al.*, 2003; Al-Hassan & Diao, 2007). This is so because the consumption rate is higher than production since Ghanaians prepare different meals for consumption. Despite importing cowpea, Ghana is regarded as one of the most significant producers of the crop (Singh *et al.*, 2003; Egbadzor, 2014). There was an importation of some 3,380 metric tons of cowpea to supplement Ghana's 219,257 metric tons in 2010/2011 fiscal year. However, this achievement was a significant advance over the deficit of 1990, which was about 113,000 metric tons (Al- Hassan & Diao, 2007).

The problem of Ghana's production deficit is complex. Although cowpea can be produced in other agroecological zones, commercial production occurs predominantly in regions of the northern hemisphere characterized by Savannahs. In 2010, when the national total was 219,300 metric tons, the Upper West and northern regions were indicated to have

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produced 75,969 and 105,841 metric tons, respectively (Egbadzor, 2014). Unlike the north, cowpea production in the south is mainly subsistence. In addition, varietal preference may influence cowpea production and intake in Ghana (Langyintuo *et al.,* 2003). The development of consumer and farmer preferred varieties of cowpea has become a more paramount breeding objective in recent times in Ghana.

2.2.6 Importance of cowpea production

Cowpea is important in diverse ways to African societies. The cowpea plant forms a major staple in the diet of many Sub-Saharan Africans especially those of the savannah agro-ecologies (Dugje *et al*., 2009). The seeds are frequently harvested and stored for later consumption, as wholly cooked or in the form of flour after milling because the seed is considered in most countries as the most important part (Akpan & Mbah, 2016; Timko & Singh, 2008). Cowpea leaves which are tender and non-lignified are consumed in various African cultures in diverse dishes (Boukar *et al*., 2016). In Ghana, the grains can be boiled and consumed with "Gari," a cassava product. Additionally, it can be boiled with rice and a colouring agent to produce "Waakye." The seeds may also be served with fried ripe plantain (Quaye *et al.,* 2009). The seeds contain a total protein content that is about two to four times higher than most cereals and tubers (Timko & Singh, 2008) and therefore provides a cheap protein source to complement the dietary requirements of most Sub-Saharan Africans (Dube & Fanadzo, 2013; Gnankambary *et al*., 2019). The forage and fodder of cowpea usually after harvest are used as animal feed (Timko & Singh, 2008). Cowpeas are an important source of minerals and vegetable protein for more than 70% of Ghanaians.

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Cowpea can serve as a cover crop (Timko & Singh, 2008; Singh, 2002). Its tolerance to drought makes it fit for cultivation in tropical soils where moisture, poor soil fertility and erosion are major factors limiting crop production (Langyintuo *et al.*, 2003). The cultivation of cowpea improves soil fertility through biological nitrogen fixation by nitrogen-fixing bacteria such as rhizobia (Gwata *et al*., 2016; Yirzagla *et al*., 2016). The resourcefulness of cowpea to be used as food, feed and as soil amendment concludes its potential to be nicknamed the "poor man's crop" (Dube & Fanadzo, 2013).

The value chain of cowpea production has greatly contributed to employment, poverty alleviation and revenues in Sub-Saharan Africa (Dube & Fanadzo, 2013; Langyintuo *et al*., 2003). Cowpea has also defined both formal and informal regional trades in market systems of Sub-Saharan Africa (Langyintuo *et al*., 2003). Due to the multiple benefits derived from cowpea, best qualifies it as a food security crop (Dube & Fanadzo, 2013).

2.2.8 Nutritional Composition of cowpea

Cowpea protein contains amino acids like tryptophan and lysine in higher quantities compared to cereals. However, cowpea lacks methionine and cysteine when compared to proteins from animal sources. In a study of 100 cowpea lines from the IITA, the range for protein content was between 23 and 32% of seed weight (Hall *et al.*, 2003). Similarly, 12 West African and USA cultivars had protein content ranges from 22 to 29%, where most accessions had protein content proportions from 22 - 24% (Hall *et al.*, 2003).

The cowpea seed is also rich in minerals and vitamins (Hall et al., 2003), and it contains one of the highest levels of folic acid, an essential B vitamin that aids in the development of the spinal tube in unborn children.

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Cowpea leaves are extremely nutritious and can be grown as a vegetable crop (Singh et al., 2003). The tender green leaves are an important food source in Africa and can be prepared similarly to spinach. The immature green pods are used similarly to snap beans and are frequently combined with cooked cowpea seeds or other foods. Fresh-shelled cowpea grains that are nearly mature are processed as fresh vegetables and frozen or canned. The mature, dry seeds can be processed and preserved. Cowpea foliage is widely regarded as an excellent source of high-quality hay for animal feed (Audu *et al.*, 2019).

Cowpea consumption is anticipated to increase as consumers in developed nations seek "new" healthy foods and find "traditional" diets that are low in fat, high in fiber, and offer other health advantages. The fat content of IITA's new breeding lines ranged from 1.4% to 2.7%, while the fibre content was around 6%. (Essem, 2017). Although cowpea is low in fat and high in fiber, its protein has been found to decrease heart disease-associated low-density lipoproteins (Alayande et al., 2012). In addition, because starch from grain legumes is digested more slowly than starch from cereals and tubers, consuming grain legumes such as cowpea results in less pronounced fluctuations in blood glucose levels (Alayande et al., 2012). Utilizing dry cowpea grains, processed foods such as cowpea-enhanced baked goods, extruded snack foods, and weaning foods have been developed (Alayande et al., 2012). Functional properties of cowpea protein isolates, including solubility, emulsifying, and foaming activities, make cowpea a superior alternative to soy protein for individuals with soy protein allergies (Rangel *et al.*, 2004).

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In the USA, varieties of cowpea with a “persistent-green” grain have been bred for use as a versatile product for frozen vegetable applications (Duah, 2017). These varieties remain green upon drying. Also, when they sodden in water for hours, the grains closely resemble freshly shelled cowpea that is used in frozen vegetable products by adding colour and variety.

2.2.9 Constraints of cowpea production

Cowpea yields are limited by many biotic and abiotic factors. In Ghana, cowpea production is limited by several factors. These include drought, diseases and pests, poor-performing genotypes among others (Egbadzor *et al*., 2013). The scantiness of cowpea genotypes that concurrently suit the farmers' cropping system as well as the consumer' grain quality is the major research area for cowpea breeders in Ghana. Disease incidences predominant in Ghana include anthracnose (*Colletotrichum lindemuthianum*), cowpea fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*), Cercospora leaf spot (*Cercospora canescens*) and soft stem rot (*Pythium aphanidermatum*). These diseases are seed-borne and seed transmitted (Gupta & Singh, 2010) and they cause huge losses in cowpea production (Van Gastel *et al*., 2002). Recently, participatory breeding programmes are developed to enhance the value chain of cowpea breeding. Thus, farmers and consumers alike are deliberately involved in the selection within segregating populations of cowpea to mutually serve their respective desired traits (Egbadzor *et al*., 2015).

Several socioeconomic factors that negatively impact cowpea production in sub-Saharan Africa (SSA) have been identified (Horn et al., 2015). These constraints include high operational costs for farmland, outdated

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cultivation and harvesting equipment, high costs of labour, inadequate labour availability, high pesticide costs and adulteration, low harvest prices, underdeveloped marketing channels, and a lack of market-preferred varieties. In addition, the absence of a defined value chain and the poor development of cowpea as a commodity crop continue to hinder cowpea production in many SSA nations (Sabo *et al.*, 2014). Also, inefficient transportation systems, and poorly organized cowpea trading affect value addition and cowpea enterprises development (Fakayode *et al*., 2014). The full economic potential of cowpea will be achieved if other value-added products, are introduced to the ever-growing urban population.

Increasing the mean output per hectare of cowpea will boost annual global production and income. If most of the constraints are removed, cowpea's potential gains could reach and exceed 3,000 $kgha^{-1}$(Aboki & Yuguda, 2013).

2.3 Mutation

Mutation is a heritable change in an organism's gene structure and it can be produced by a change in the sequence of base genes (Bind & Dwivedi, 2014; Gnanamurthy *et al*., 2019). Thus, mutation is the heritable change to the genetic constituents of an individual and it can occur naturally or be induced artificially (Mba *et al*., 2010). Natural mutation occurs once in 1000 years and therefore is very rare to meet increasing demands. Apart from natural mutation (spontaneous mutation), there is artificial mutation (induced mutations), following the discoveries of X-rays (1895), radioactivity (1896) and radioactive elements in 1898. Radiation was found to cause mutations in fruit flies and in crops such as maize and barley. This resulted in large - scale usage

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of induced mutation techniques for crop improvement. This revolutionized the discovery of desirable off-type plants since man did not have to wait for natural variations in species.

Mutation breeding continuous to record tremendous successes since the release of the light green tobacco mutant, being the first induced mutant variety in the 1930s in Indonesia. Mutagenesis is the artificial induction process of altering DNA of an organism with physical and chemical mutagens (Roychowdhury & Tah, 2013). These ensure genetic variation and production of beneficial traits for crop improvement (Novak *et al.*, 1992). The most critical stage in mutation breeding is screening and confirmation of putative mutants (Forster & Shu, 2012). Mutant screening entails selecting from an induced plant population and an individual induced useful plant that meets specific breeding objectives such as disease resistance and early flowering compared to the parent. Modern genotypic screening has the ability to accelerate both the detection of mutations and the development of mutant lines. Such tools can even detect single base changes which affect protein synthesis (Mba, 2013). More than 3,200 mutant varieties have officially been released for commercial use across more than 210 plant species in over 70 countries (FAO/IAEA Mutant Varieties Database).

2.3.1 Macro and Micro mutations

The visible changes, such as leaf colour, in a plant's morphology are called macro-mutations. Macromutations can have large phenotypic effects and are easily identified on individual plants. These are naturally oligomeric and have observable traits such as alternating leaf and pod shapes and sizes (Gaul, 1964).

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Micro-mutations produce small phenotypic effects that are not recognizable on an individual plant basis but are only detected in the flora and require statistical data to be processed. They are polygenic, and sampling is delayed until the M3 generation. These mutations cause imperceptible phenotypic alterations. At the M2 and M3 mutant generations, morphological and chlorophyll macromutations, also known as chlorins, are possible. Chlorophyll mutations are the most accurate indicator of the phenotypic efficacy of mutagens and are regarded as the most common type of macromutations. Chlorophyll mutations in cowpea may be classified as albina, albo-xantha, xantha, chlorine, albo-viridis, viridis, virido-albina, striata, etc. (Gnanamurthy & Dhanavel, 2014).

2.3.2 Mutagens

Mutagens are chemical or physical agents that cause mutations. Mutagens produce a DNA lesion which is not simply reversed and can induce various levels of small mutations (Ferguson & Von Borstel, 1992).

2.3.2 Physical Mutagens

There are many physical mutagens which have been employed in many ways to induce mutations in crops. These physical mutagens include gamma rays, x-rays, ion beam irradiation and implantation, ultra-violet light, beta particles, cosmic irradiation and laser beam irradiation. However, gamma and x-rays have been extensively utilized in generating mutations in crop species.

Gamma-rays are emitted by the decay of an atom's unstable nucleus. These possess more energy per photon than X-rays because gamma-rays have shorter wavelengths. Monoenergetic gamma radiation is obtained from radioisotopes such as Cobalt-60 (^{60}Co) and Caesium-137 (^{137}Cs). Artificial

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gamma rays may be produced using cyclotrons (IAEA, 2004). Gamma irradiation facilities can be used for acute or semi–acute exposures. Gamma cells are the most frequently used irradiators for plant mutation induction worldwide (IAEA, 2004). Gamma radiation sources have distinct advantage for prolonged treatments. They may be placed in an environmentally controlled chamber, in a greenhouse, or in the field to be exposed at different times and or developmental stages of the plant. The establishment of more Cobalt-60 irradiation facilities has made gamma radiation a popular mutagen.

In species such as coriander, mung bean, red palm and tomato, new species have been developed through the applications of gamma rays (Sangsiri *et al.*, 2005). Low rates of gamma irradiation together chemicals such as sodium azide has been effective in producing mutants in species like *Vigna muno* (L). This combination has been successfully applied to apples, date palms, potatoes, pineapples and sweet potatoes. The use of radiation technology in in vitro propagation in asexual plants is an effective procedure to obtain disease-free mutants rapidly. Potato seedlings raised under in vitro conditions showed a higher resistance to late blight in the field when the seedlings were irradiated between 20 and 40 Gy (Gosal *et al.*, 2001). Gamma radiation of 0.05Kr applied to sugarcane stems showed a positive effect on sugar content parameters of yield such as green leaves per plant, plant height, cane thickness and tiller volume (Khan *et al.*, 2000).

Studies by Saleem *et al.*, (2005) showed that gamma irradiance of 50 Gy applied to embryonic callus of Basmati rice (*Oryza sativa* L.) resulted in salt tolerance in the M_2 generation mutants. Similar results were obtained when gamma rays were used to develop salt-tolerant potatoes (Yaycılı &

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Alikamanoğlu (2012). According to Jain (2010), gamma rays can be used to develop biotic and abiotic stresses as well as plant characteristics in different plant propagules.

Malek *et al.*, (2012) applied 700 Gy of gamma rays to develop two efficient new mustard varieties. Through the application of 20 and 40 Gy gamma radiation, Sutarto *et al.*, (2009) identified two citrus and one watermelon genotypes with the potential to become seedless. A high-yielding rice variety was bred by applying 150 Gy (Balloch *et al.*, 2002). Javed *et al.*, (2003) also reported successes in using gamma radiation for breeding high-yielding *Brassica campestris* (L). Kavithamani *et al.*, (2010) developed high-protein and low fibre content varieties of soybean using this approach. Breeding studies on the application of radiation on ornamentals such as roses (Yamaguchi *et al.*, 2003), chrysanthemums and African violets have reported positive feedbacks.

2.3.3 Chemical Mutagens

Chemical mutagens are classified into four groups, viz. 1) alkylating agents, 2) base analogs, 3) acridine dyes and 4) others (Table 1). A brief description of some commonly used chemicals of these groups is presented below.

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Table 1: Chemical mutagens and their mode of action

Sr. No.	Group of mutagens	Name of chemical	Mode of action
1	Alkylating Agents	*Ethyl Methane Sulphonate* *Methyl Methane Sulphonate* *Ethyl Ethane Sulphonate* *Ethylene Imines*	AT ↔ GC Transitions Transitions GC ↔ AT Transitions
2	Base Analogues	*5 Bromo Uracil* *2 Amino purine*	AT ↔ GC Transitions AT ↔ GC Transitions
3	Acridine Dyes	*Acriflavine, Proflavin*	Deletion, Addition and Frameshift
4	Others	*Nitrous Acid* *Hydroxylamine* *Sodium Azide*	AT ↔ GC Transitions GC ↔ AT Transitions

2.3.4 Breeding through mutation

Mutations are the definitive sources of genetic variations, a fundamental requirement for plant breeding programmes (van Harten, 1998). Induced mutation is a powerful crop genetic improvement and breeding tool that can be derived from physical or chemical mutagens or both. In commercial mutagenesis, the optimal dose of radiation for the target genotypes is determined (Tshilenge-Lukanda *et al.*, 2012). The first step is to generate an optimal radiation dose. This is important because the predicted value assists in determining the optimal dose based on desired outcome and the plant material in use (Horn & Shimelis, 2013).

Induced mutations provide important genetic variations in a short time when the crop has minimal genetic variability for breeding. Mutagens produce desirable biochemical, physiological, genetical, or morpho-genetical changes

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in plants (Girija & Dhanavel, 2009). Mutation breeding process is advancing the development of diverse germplasm from a single seed decent even as a complement for conventional breeding techniques (Parry *et al.* 2009). However, vast populations of induced plants are recommended during screening to ensure that adequate significant variations of the gene of interest exist in the population.

The successes of mutation breeding are the numerous varieties released globally and indexed by the FAO and IAEA Mutant Varieties Database (MVD); 2,252 crop cultivars from 59 countries worldwide (Maluszynski, 2001; Nielen, 2004). As reported by Maluszynski (2001) and Maluszynski et al., these include cultivars from continental Asia (1142), Europe (847), and North America (160). (2009). Several research shows that stimulated mutagenesis has altered plant characteristics including plant height, tolerance to seed shattering, maturity, disease resistance, oil quality and quantity, and malting quality (Horn *et al*., 2016).

While induced mutation has made important contributions to plant breeding and genetics, little is known about its potential harmful effects on the environment or organisms. Moreover, studies typically focus solely on the favorable results, ignoring any potential drawbacks (Mba *et al.*, 2010; Tulmann Neto *et al.*, 2011). Induced mutation techniques have been in use for decades (Shu, 2008), indicating that the method has been accepted because its use or application are safe. The release of mutant varieties has increased food production hence contributed to improved food security (Suprasanna *et al*., 2015).

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2.3.5 Mutagenesis in Legume crops

2.3.5.1 Cowpea (Vigna unguiculata)

Nanda et al. (1997) applied ethyl-methyl sulphonate (EMS) to the M1 generation of V. unguiculata subsp. sesquipedalis, which resulted in a decrease in survival value, days to 50 percent flowering, days to first picking, and plant height, but an increase in branches/plant, pod size, pods/plant, pod weight, and yield/plant. High dosages of EMS induced an increase in chlorophyll and foliar mutations in the M2 generation. Cowpea seeds treated with gamma rays and ethidium bromide exhibited considerable changes in the M2 population for many agronomic parameters, and gamma rays were shown to be more successful than ethidium bromide at inducing mutations (Gunasekaran *et al.*, 1998).

Adekola and Oluleye (2007) subjected cowpea to 60Co gamma irradiation at 196 and 245 Gy and discovered that the mutants exhibited promising agronomic features, such as simple harvesting, increased yield, and pest tolerance. Girija and Dhanavel (2009) exposed cowpea to gamma rays and EMS and found EMS to be more efficient and effective in producing mutations as compared to gamma rays alone or the treatments combined. Studies by Kumar *et al.,* (2009) showed that cowpea treated with 30 mM EMS generated a dwarf mutant that was spreading, semi sterile, single and tri-cotyledonary. The mutants also showed variations in leaf, basal branching, white flower and white seed coat colour at the M_2 generations.

2.3.5.2 Chickpea (Cicer arietinum L.)

Four types of chickpea were exposed to gamma rays, and the results indicated the possibility of concurrently improving protein content, grain

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weight, density, and yield (Kharkwal, 1998). Shah et al. (2008) treated one Kabuli (Pb1), two desi (Pb2000 and C44), and one desi x Kabuli introgression line (CH40/91) of chickpea with 300 to 400 Gy of gamma rays and EMS [0.3 to 4%]. Inducing mutations, the data demonstrated that EMS was more successful than gamma radiation.

2.3.5.3 Pigeon pea (Cajanus cajan)

Rao and Reddy (1993) treated seeds of three *Cajanus cajan* varieties with gamma rays at a dose rate of 438 rad/min or varying concentrations of diethyl sulphate (DES); EMS; and hydrazine hydrate (HZ) for varying times and observed a broad spectrum of sensitivity to the mutagenic treatments in the mutants. Pandey *et al.*, (1996) treated three varieties pigeon pea varieties (Bahar, DA 11 and Pusa 9) with chemical mutagens sodium azide (0.025, 0.05, 0.1 & 0.125%) and streptomycin sulphate (0.1, 0.5,1 1.0 & 1.5 $molm^{-3}$) and reported that streptomycin sulphate showed better germination capacity, plant survival and pollen fertility compared to sodium azide.

2.3.5.4 Mung bean [Vigna radiata (L.) Wilczek]

Sodium azide (SA) and hydrazine hydrate (HZ) concentrations of 0.01 to 0.04% were used to pre-treat mung bean seeds (Mehraj-ud-din *et al.*, 1999). The highest spectrum of mutations in chlorophyll content was highest at lower concentrations with HZ being a more efficient and effective mutagenic agent to induce this biological damage in chlorophyll contents. Also, Singh *et al.*, (2001) exposed mung bean seeds to doses of gamma rays (200, 300 & 400 Gy), EMS (0.05, 0.1, 0.2 & 0.3 %) and Epichlorhydrin (ECH) (0.1, 0.2, 0.3 and 0.4 %) and they found that the mean and ranges of measured traits such as seeds/pods, 100-seed weight and seed yield showed significant increases for

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all four traits. Goyal and Khan (2009) induced mutations in two mung bean varieties using a from ^{60}Co (20kR), EMS (0.2%) and SA (0.02% SA). Six mutants with early maturity were selected at M_2. At M_5, the morphologically stable mutants showed high heritability for days to maturity but seed yield was the similar as the parental varieties.

2.3.5.5 Black gram [Vigna mungo (L.) Hepper]

Studies by Deepalakshmi and Anandakumar (2003) on black gram showed gamma rays produced more chlorophyll mutations than EMS in M_1 plants and M_2 seedlings. The EMS was more injurious to chlorophyll formation than gamma radiation. Dry and soaked black gram irradiated with gamma ray dose ranges of 50 to 500 Gy showed the higher doses (350 – 500 Gy) causing high lethality under both treatment conditions (dry & soaked). The 400 Gy irradiance was most mutagenic in the dry seeds. However, the lower dose (i.e., 150 Gy) was quite efficient and effective in causing mutations in the soaked seeds.

2.3.5.6 Lentil (Lens culinaris Medikus)

Gamma ray doses (5, 10 & 20 kR) and two chemical mutagens [ethylene imine (EI) and N-ethyl-N-nitrosourea (NEU)] at 0.005, 0.01and 0.02 % concentrations were used to study the germination, plant survival and seed fertility in the M_1 generation of lentil (Solanki & Sharma (1994). The results demonstrated that the mutagenic efficiency of gamma radiation increased as the dose increased, but the chemical mutagens exhibited a peak efficiency of 0.01% for both EI and NEU. Similarly, EMS and sodium azide (SA) showed increasing mutagenic efficiency with increasing concentration when applied to two lentil cultivars (L-4611 & L-4639): EMS was more efficient than SA

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(Gaikwad & Kothekar (2004). Khan *et al.*, (2006) treated seeds of lentil SA and found that the treated population revealed a high range of variability for numerous quantitative traits observed.

2.3.5.7 Common bean (Phaseolus vulgaris L.)

Individually and in combination, Gautam et al. (1998) exposed seeds to EMS and gamma radiation. Mutations in chlorophyll increased proportionally with increasing gamma radiation and EMS doses (Singh & Verma, 1998). On two cultivars of common bean, the effect of gamma rays on seed germination, lethality, damage, plant survival, fertility loss, and chlorophyll mutation was studied (Hur-120 & Giant Stringless). It was found that biological damage was dependent on dose of the mutagen in both M_1 and M_2 generations but complete recovery occurred in the M_3 generation.

2.3.5.8 Grass pea [Lathyrus sativus (L.)]

Grass pea exposed to gamma radiation doses (5, 10, 15, 20, 25, 30, 35 & 40 kR) and EMS (0.5 & 1.0 %) produced a significant magnitude of genetic variability in yield-related traits in both M_2 and M_3 generations. From M_2 to M_3,

days to flowering and maturity, number of primary branches, seeds per pod and pod length showed reduced variability. Significant variations among the M_3 families led to the identification of numerous beneficial traits related to yield.

2.3.5.9 Cluster bean [Cyamopsis tetragonoloba (L.)]

Velu *et al.*, (2007) studied the effectiveness of gamma rays (20, 40, 60, 80 and 100 kR) and EMS (0.2, 0.4, 0.8 and 1.0 %) in inducing mutations in cluster bean seeds. The results showed that the EMS induced more mutations

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than gamma rays in the M_2 generation. occurrence of mutations was more in EMS than the gamma rays. Also, the EMS treatment was more injurious and lethal than the gamma rays.

It is evident from the mutagenesis in legumes that every genotype responds differently to varying mutagen doses. Physical as well as chemical mutagens can be used in legume mutagenesis, but the most important thing is to determine the effective dose rate (LD_{50} or RD_{50}) of any genotype before undertaking mass irradiation of same. The effect of mutagens on legumes is more observable at M_2 generation and beyond.

2.4 Mutation rates detection and confirmation

The occurrence of mutations, naturally or induced, is random. Thus, the kind or magnitude of genetic variability is unpredictable. However, there are realistic approximations on the mutation frequencies. A gene is expected to mutate once in about 10000 treated cells only when the effective dose treatment is administered. Mutation rates for certain plants are known. For example, *Arabidopsis* is known to have 14 base mutations per 1.5 kb fragment length for 3000 plants induced. Also, hexaploid wheat has about 1 per 25 kb while *Brassica rapa* has1 per 60 kb as low as 1 per 500 kb for barley and maize.

Despite its ability to pinpoint the precise location and type of mutation, genome sequencing is expensive and rarely employed in practical breeding efforts for large populations. Recent advancements in plant genomics, particularly genome sequencing on a broad scale, have led to countless applications of mutation induction techniques in crop enhancement programmes. Enhanced genetic engineering techniques, like Targeting

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Induced Local Lesions in Genomes (TILLING) and next-generation sequencing methods, are utilized to produce and validate novel mutants (McCallum et al., 2000). Molecular biological methods, such as simple sequence repeat (SSR), facilitate the screening of mutants at the gene level for the presence of viral DNA in generated populations.

Mutants with any sort of nonconformity to inbred lines are called "putative," indicating that they are not absolutely "real mutants." Disease resistance may be one of those deceptive mutant traits because non-infection may due to the pathogen's absence. Mutant verification involves standardized procedures by FAO/IAEA (2009). This evaluation is mostly carried out at the M_3 to M_4 stage to meet the selection criteria.

2.5 Radio-sensitivity

Multiple environmental, biological, and chemical elements invariably influence the response of plant cells to chemical and physical mutagens. These parameters affect the efficiency and efficacy of mutagens at the cellular level in higher plants. By a wide margin, the factors underlying are poorly known, yet it is necessary to research these factors in depth because they continue to impede the radiation process. Therefore, radiosensitivity describes the response of plant components to mutagens.

2.5.1 Factors that affect radiosensitivity of seeds

The two most important components that determine a seed's radiosensitivity are its oxygen and water levels. DNA synthesis, stage of development, and dosage rate are significant factors for active tissues. For dormant and active tissues, chromosomal volume in the nuclear and interphase states is crucial. Environmental (oxic versus anoxic), seed water content, post-

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irradiation storage, and temperature parameters are typically classed as (1) environmental; and (2) biological (genetic differences, nuclear and interphase chromosome volumes).

2.5.1.1 Environmental factors

2.5.1.1.1 Oxygen

Oxygen is one of the most influential radiodensity modifiers due to the fact that most biological processes are reflective in the presence of oxygen. Radiosensitivity is further affected by secondary factors like temperature, moisture content, and post-irradiation storage conditions. Nairy et al. (2014) established a complete study of Oxygen Enhancement Ratio (OER) and its fluctuations as a function of radiation doses using yeast (Saccharomyces cerivisae).

Higher mutagenic efficiency is achieved with reduced seedling injury and chromosomal aberrations when there is minimal oxygen availability. Dehydrated seeds with less than 3% moisture content have the most outstanding oxygen enhancement ratio. The degree of enhancement varies across species. Various studies show that irradiating seeds under anoxic atmosphere, under partial vacuum, in a nitrogen-filled medium or adjusting the seed moisture content (12 ~ 14%) can control oxygen enhancement. Nevertheless, oxygen regulation is typically unfeasible for producing mutations during plant breeding, and is therefore disregarded. Furthermore, under pressure, oxygen can be mutagenic.

2.5.1.1.2 Moisture content

Moisture content is essential but is an easily controlled factor (van Harten, 1998). The difference in fresh seed weight and the corresponding

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dehydrated seed weight may be a cheaper way to estimate moisture content. Minor differences, even 0.2 to 0.3%, in moisture content can influence significantly and affect biological processes such as radiosensitivity. Optimal dose is needed to induce mutation because a too low dose may not induce mutation while too high doses may cause sterility or lethality in seeds. However, there must be an equilibrium between moisture content and dose rate varies among seeds of different species at a specific relative humidity in radiosensitivity testing (IAEA, 1977). Desiccating seeds before irradiation is a routine seed treatment in radiosensitivity test.

2.5.1.1.3 Temperature

Total genetic damage through mutation induction by gamma or X-rays is highly influenced by the plant cells' temperature before, during and after irradiation. However, temperature is not yet clearly understood as a modifying factor of irradiation and therefore appears to be insignificant to plant breeders. In terms of seedling injury and chromosomal abnormalities, heat shock and anoxic hydration provide the greatest protection against oxygen-dependent damage in plants following irradiation.

2.5.1.2 Biological factors

The cell's nucleus is the principal site for radiation injury therefore, it is prudent to understand the biological factors that underpin radiosensitivity in the nuclei of different species. The manual on Mutation Breeding by IAEA (1977) meticulously describes the relation between the nucleus volume of a species and its corresponding radiosensitivity. It is known that DNA content,

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number of chromosomes and number of chromosome arms are not responsible for differences in radiosensitivity among species (Leonard *et al.,* 1983; Bakri *et al.,* 2005). Yet, there is an affiliation between cell sensitivity to radiation and interphase nuclear volume (INV). Consequentially, Datta (2014) concludes that the higher the number of chromosomes, the higher a cell's resistance to radiation because the chromosomes' parts may recompense for the mutations especially, in polyploid species (Datta, 2014). The organization of chromosomes, the position and number of centromeres together with the size of chromosomes are also linked with radiosensitivity. Thus, small chromosomal size is less radiosensitive than large chromosome size.

2.5.2 Determination of LD_{50} and RD_{50}

The effects of mutagenic agents depend on the dose of application. However, the dose-effect is non-linear. An increase in mutagenic dose will increase its influence on a plant's growth and reproduction. Generally, lower doses can have stimulatory effect (hormesis) on pollen viability, seed germination, seedling growth and invitro cultures. Determining the effective dose is the most important step in initiating a mutagenic treatment. In the case of lack of detailed data, a small-scale test may be used to estimate the LD_{50} or RD_{50} in the M_1 population. The LD_{50} is basically the dose which results in a 50 % reduction in germination of the M_0 seeds when inducing mutations in plants.

The RD_{50} is equivalent to the dose at which the growth and seed production in M_0 population reduces by 50 percent in induced plants. Measuring seedling height and root length are frequently used in determining the effectiveness of the mutagen. After a radio-sensitivity assay, a graphical

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plot of the measured attribute (shoot or root length) against the relevant doses of the mutagen is displayed. The reductions are often shown as a percentage of the control sample. The indices, LD50 and RD50, as well as any kind of percent reduction, are derived from the gradient of the graph. A basic linear regression model is used to determine the LD50 by using the straight-line equation y=mx+c, where y is the outcome variable (percent germination) and x is the predictor variable (dose of irradiation). Additionally, m and c represent the slope and constant, respectively.

2.6 Cowpea genetic diversity and analysis

Plant breeding programmes are totally dependent on genetic variations in the population. Both biotic and factors have contributed to the decline cowpea genetic variations. Also, artificially selecting genotypes of better performance while neglecting those deemed 'poor-performing' may lead to gene erosion and a narrow gene base. The lack of complementary pre-breeding programmes restricts genetic variation within specific breeding programmes (Gbaguidi *et al.*, 2013). Genetic diversity within cowpea populations develops slowly under natural selection primarily where self-fertilization is the predominant mode of reproduction. Gbaguidi *et al*., (2013) reports that generic variation in cowpea is fast declining between 28 and 60% in some African agro-ecological zones.

Well-designed crosses help to incorporate cowpea traits of economic importance into germplasm. Molecular biological tools in the form of DNA markers like *simple sequence repeat* (SSR), *amplified fragment length polymorphism* (AFLP), *single nucleotide polymorphism* (SNP) and *randomly amplified polymorphic DNA* (RAPD) are available for use in analyzing genetic

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variations. In finding genetic diversity, DNA-based molecular markers have become robust and dependable techniques. Numerous crop plants have been successfully analyzed for genetic diversity using these methods (Adetiloye et al., 2013). Frequently, agromorphological or phenotypic markers are utilized to assess genetic diversity. In cowpea breeding, phenotypic characteristics for quantitative and qualitative qualities are widely utilized to characterize, categorize, and select germplasm (Molosiwa *et al*., 2016).

The most direct measurement of phenotypes is phenotypic genotype characterisation. They are commonly available, fairly inexpensive, and need minimal equipment; hence, they are widely employed. Phenotypic markers, however, are susceptible to environmental stimuli that limit the significant genetic variations between genotypes. Synchronizing the usage of molecular and phenotypic markers may allow for a more precise and efficient assessment of genetic diversity. In recent times, there is advocacy for effective field-based high-throughput phenotyping platforms (HTPPs) for improved selection efficiency during plant breeding exercises (Araus & Cairns, 2014).

2.7 Cowpea Breeding

Various research programmes into breeding for high performing cowpea are being undertaken by international research institutions such as IITA (Dugje *et al*., 2009) in collaboration with national institutions like Crop Research Institute (CRI) and Savannah Agricultural Research Institute (SARI) of the Council for Scientific and Industrial Research (CSIR) of Ghana. These improved cowpea cultivars may be disease and pest resistant, early maturing and or high yielding. To harness cowpea genetic diversity for breeding,

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majority of such breeding programmes use molecular and conventional breeding tools.

Furthermore, the member states of IAEA assist each other in genetically improving crops of interest, including cowpea, through resource sharing in inducing mutagenesis (Mba *et al*.,2010). Hence, improved cowpea genotypes have been developed and released for use worldwide (Viswanatha *et al*., 2011; Reddy *et al*., 2013). More so, the numerous breeding programmes have expanded cowpea's genetic base. This has facilitated cowpea's adaptation to various agroecologies and cropping systems, as well as the creation of consumer-preferred cultivars with improved quality in nutrition. Variation among genotypes is an indispensable prerequisite in breeding for disease resistant varieties with desired morphological traits.

2.7.1 Cowpea breeding methods

In cowpea development initiatives, the breeding techniques below have been utilized extensively:

2.7.1.1 Pure-line selection

Selection based on pure line depends on the performance of individuals (and/or relatives) in a population (Wei *et al*., 1991). The method is best for crop species such as barley, cowpea, sorghum and wheat that highly self-fertilize. After crosses or forced mutagenesis, individuals with promising potential in the assessed traits are systematically selected from segregating populations. The chosen individuals are collected separately and constantly selfed in order to generate and release cultivars with the desirable features. Pure Line Selection is often combined with line (breed or strain) crossing (Wei *et al*, 1991).

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2.7.1.2 Pedigree breeding

Pedigree selection was first practiced by Svalöf of Sweden in 1891. It occurs when seeds from a single plant among existing cultivars or landraces. Seeds from the selected plant can be arrayed in the field in plots of plant rows, head rows or spike rows (Newman, 1912). The French company Vilmorin independently improved this method and it also became known as the 'Vilmorin method of selection' (Fehr *et al.*, 1987). In contrast to pure-line breeding, breeding by pedigree keeps a thorough record to connect seeds of the selected plant and the progenies. This allows any generation of progeny to be linked directly to the F2 parent from where they originated.

In self-pollinated crops, good plants are often selected from segregating populations through pedigree selection. When a well-established variety is lacking a certain characteristic, this strategy can be quite helpful. New recombinant individuals with superior qualities are selected by pedigree. The selected individuals may show qualities in plant height, disease resistance or maturity. Selection by pedigree is a valuable technique in transgressive breeding.

2.7.1.3 Backcross breeding

Backcross as breeding technique was proposed in 1922. It is used primarily to introduce genes of interest into an established cultivar of a self- or cross-fertilizing crop. Backcrossing improves homozygosity and allows for the selection of desirable genotypes of homozygous genetic backgrounds.

2.7.1.4 Single seed descent selection method

The single-seeded pedigree is a method of advancing generations of an inbred stock rapidly before the individual lines are evaluated, often in conjunction with the pedigree selection method. This concept was developed

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by Goulden in 1941 who proposed that a breeding programme could be divided into two: developing pure lines from segregating populations and selecting the desired pure lines from the resulting pure lines. Modifications of the single-seed pedigree method are the multi-seed and single-mountain programmes. These are aimed at rapidly obtaining generation progression. In mutagenesis, the purity and genetic stability of the target material(s) used become important. This is because mutagens are capable of causing variations among plant materials and if plant parts are not uniform the variations observed after exposure to mutagens can be misleading. These methods are adapted for use in greenhouses and winter nurseries where genotypes behave differently from their acclimatization zones.

2.7.1.5 Bulk population breeding

The process of breeding large numbers of animals at once is also known as mass picking or population breeding. The population is allowed to grow with or without selection from F_1 to F_5 generation. A portion of the majority of seed is utilized to establish the subsequent generation, and seed collecting from individual plants begins at the F6 generation or later. Positive mass selection is used to improve the chances of observable characters in plants using bulk selection. It is suitable for studies on the gene repeatability in genotypes in the population, and it is better at isolating transgressive segregates than the pedigree method.

2.7.2 Genotype by environment interaction

The genotype by environment interaction (G E) is the differential in the response of genotype between plants grown in distinct settings (Yan & Hunt, 2001; Annicchiarico, 2002). Multi-environmental trials (METs) are

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necessary for determining the extent of genotype x environment interaction and recommending genotypes with restricted or broad adaptation (Ramburan et al., 2012). G x E trials are useful for cultivars selection trials and the last phases of selecting top breeding material (Annicchiarico, 2002). Agronomists, crop ecologists, and plant breeders may benefit from data generated through G x E interaction studies that establish ecotypes, environmental areas, and megaenvironments (Annicchiarico et al., 2011).

There are two forms of genotype by environment interaction (GEI): cross-over and non-crossover (Annicchiarico & Iannucci, 2008). Cross-pollination interaction is not as important for cultivar growth as non-cross-pollination interaction. This is owing to the fact that cross-over interaction impedes the selection of high-yielding genotypes due to the unpredictability of genotype performance across different locations (Annicchiarico et al., 2010). Conversely, non-cross-over interaction occurs when the performance of the test crops changes. GEI is advantageous for crop development that aims for broad adaptability, but it also indicates opportunities for genetic development in particular places (Annicchiarico et al., 2010). These interactions limit agricultural improvement because they can contribute to the temporal and spatial variability of crop yields (Annicchiarico, 2002).

There are different methods of analyzing and interpreting genotype-environment interactions. These include comparisons (Yan & Hunt, 1998), additive main effect and multiplicative interaction (AMMI), linear regressions (Fleischmann et al., 2016), and principal component analysis (PCA). Biplot of genotype plus genotype-environment interaction (GGE) is the best method for studying G E data. This biplot is employed in mega-environment analysis

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(Yan & Rajcan, 2002), genotype and test environment evaluation (Yan & Rajcan, 2002), and heterotic pattern analysis. The GGE biplot is generated by plotting the two principal components (PC1 & PC2) gained from the singular-value decomposition (SVD) of cantered environmental data (GGE matrix) such that three-component matrices are produced: the singular value matrix (array), the genotype eigenvector matrix, and the environment eigenvector matrix. By selecting superior genotypes from each of the megaenvironments, it is possible to utilize both genotype and genotype environment interactions (Yan & Rajcan, 2002). Static or biological stability and dynamic or agronomic stability are the two stability ideas that have been offered (Kang, 1998). With the static paradigm, a genotype's performance stability does not alter while changing environmental conditions occur. In accordance with the dynamic idea, a genotype is stable when its productivity (yield) is comparable to the test environment's potential.

2.7.3 Contributions of mutagenesis to food security

The genetic variability caused by various mutagens' induced mutation has aided modern plant breeding. During the last five decades, mutagenesis has been significant in developing superior lines with improved qualities such as early maturity, improved nutritional quality, high yield and lodging resistance and disease and pest tolerance/resistance (Kharkwal & Shu, 2009). Ahloowalia *et al.,* (2004) reviewed the influence of developed and released mutants of commercial crops all over the world. Two major outcomes have been achieved through mutation breeding: new genetic stocks with improved characters or better-combining traits and improved varieties that are directly used for commercial cultivation (Roychowdhury & Tah, 2013).

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Though developing new cultivars has been the primary objective of the majority of mutation breeding exercises, the genetic stocks generated can be utilized in a variety of plant breeding contexts, including as a parent in hybrid breeding programmes or as a donor parent in traditional breeding programmes. The procedure of identifying a gene by eliminating phenotypic expressions through artificial mutagenesis is now an important constituent of molecular genetics and genomics research.

2.8 Targets for mutation breeding

Recently, there have been substantial interests in the possibilities of achieving disease tolerance/resistance through induced mutations. Understanding the inheritance behaviour of genes and diseases are vital in mutation breeding for disease resistance. In regard to this, Rao (2012) subjected okra seeds to varying dosages of gamma rays and chemical mutagens, and the resulting mutants were cataloged according to their disease resistance. These catalogued disease resistances included a dwarf and early mutant, a mutant with induced fruit, a mutant with cup-shaped leaves, fat seeds and a mutant with small fruits and bristles and a tall mutant with long stout fruits. Indirectly, the use of ionizing radiation has been successful a few times in transferring genes of disease resistance from some species or genera to others. Also, ionizing radiation has shown for severe linkage and karyotype reconstruction and other ways of enhancing genetic recombination. There have been reports of over 32 distinct molecular mutations, the most of which were caused by chemical mutagens, one by natural causes, and five by radiation (Mejlhede *et al.*, 2006).

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2.8.1 Fungal diseases

Notable fungal diseases like *fusarium wilt*, *cercospora leaf spot*, *anthracnose*, *Pythium*, and *leaf blights* are the most destructive to cowpea. Fungal diseases may cause rot in roots and or stem and also leaf blight. Mbeyagala *et al*., (2014) reported that fungal diseases can cause losses between 20 to 100% in yield. Many African states have victims of yield losses as a result of fungal infections. In countries like Nigeria, regions of the Sudanese savannah, and Sahel have recorded severe epidemics (Marley *et al.* 2001).

2.8.2 Viral diseases

Thottappilly and Rossel (1992) reported about eight viral strains of cowpea that hinder cowpea production in Africa. This was confirmed by Adejumo *et al*., (2001). Cowpea viruses spread through insects such as aphids, beetles, and others that parasite on the cowpea crop. Common cowpea viruses spread by beetles include spotted virus, southern bean mosaic soy virus and yellow mosaic comet virus. Aphids are also responsible for the transmission and spread of cucumber mosaic virus and cowpea mosaic virus. Whiteflies are known to spread cowpea mild mottle virus and cowpea golden mosaic virus. Red mosaic virus affects rhizobia growth and development, leading to about 20-45% reduction in root nodule formation. Introduction of new cowpea variants into new growth habitats may cause viral epidemics (Mbeyagala *et al*., 2014). Several new cowpea varieties developed to be resistant to viral strains include NE15, NE43, and WC18, WC32 and WC35B (Taiwo *et al*., 2014).

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2.8.3 Bacterial diseases

In cowpea literature, the most devastating bacterial diseases are bacterial wilt and bacterial pustules caused by *Xanthomonas campestris pv. vignicola* and *Xanthomonas campestris pv. Vignaeuguiculatae* respectively (Viswanatha *et al*., 2011). In India, these viruses can cause output losses of up to 68 percent in seed, 71 percent in pods, and 53 percent in forage for types that are susceptible (Viswanatha *et al*., 2011). When cowpea is moderately infected, the bacteria cause leaf yellowing with progressively developing irregular to circular spots. Premature leaf drops and senescence result from bacterial infections in cowpea. Numerous biological control agents are effective in controlling cowpea bacterial wilt.

2.8.4 Root-knot nematodes

Root-knot nematodes reduce cowpea production by impeding nutrient and water uptake in the soil. According to Gheysen and Mitchum (2011), nematodes disrupt plant cell differentiation pathways and restrict auxin transport thereby negatively impacting cowpea growth and development. *Meloidogyne incognita* and *M. javanica* are root-knot nematode species that frequently invade cowpea fields (Pretorius., 2017). Transgenic cowpea varieties like CE-01, CE-28, CE-31, CE-237, CE-315 and Frade Preto show significant resistance to nematode infestation (Oliveira *et al*., 2012). Nematode infestations in cowpea can be curbed culturally by crop rotation and post-harvest removal of infected crop residues from the field (Gheysen & Mitchum, 2011).

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2.8.5 Parasitic weeds

The two most parasitic weeds hampering cowpea production in sub-Saharan Africa are *Striga gesnerioides* (Willd.) *Vatke and Alectra vogelii (Benth)* (Horn *et al*., 2015). These weeds survive by developing and connecting their roots to the host plant's root surfaces, where they take water and nutrients. The seeds of these parasite plants can remain latent in the soil for more than twenty years, rendering traditional measures of management useless (Kabambe *et al*., 2013). To reduce the seed stock of Striga and Alectra, the plants need to be destroyed before they flower and set seeds. Using traditional breeding methods, there has been progress in breeding works on cowpeas resistance to Striga and Alectra (Timko *et al*., 2007; Kabambe *et al*., 2013).

2.8.6 Insect pests

Insect pests of cowpea attacked both in storage and in the field (Boukar & Fatokun, 2009). Cowpea field insect pests include *Aphis craccivora* (Koch), leaf beetles, bruchids (*Callosobruchus maculatus* (*Fabricius*)), beetles (*Ootheca mutabilis*), maruca and leafhoppers. (Dugje *et al*., 2009). These pests act as virus vectors may be present throughout the asexual growth stage of the plant, feeding on the leaves. In Namibia, yield losses due to major pests have been reported, leaf beetles (53.2 percent), pod borers (60 percent), aphids (77.8 percent) and cyprinid (100 percent) (Horn *et al*., 2015). The main pest affecting stored grains of cowpea in SSA was cyprinid (Figure 1.2). Cyprinid infests cowpea grains in storage, causing losses up to 100% (Gbaguidi *et al*., 2013; Horn *et al*., 2015). Effective ways of controlling insect pest damage to cowpea include the use of Actellic powder to treat seeds (Swella *et al*., (2007).

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A report by Ilesanmi and Gungula (2011) also indicated that black pepper powder treatment of cowpea grains significantly reduced the proportion of seeds damaged by insect pests. Black pepper powder and coconut oil can also protect cowpea from cyanobacterial damage. In a study on the efficacy of neem (*Azadirachta indica (A. Juss))* and moringa (*Moringa oleifera*) seed oils, it was discovered that cyprinids did not infest seeds treated with different concentrations of the oils (Ilesanmi & Gungula, 2011).

2.8.7 Abiotic constraints

The foremost abiotic conditions that limit cowpea production and productivity in Ghana and other countries of SSA are drought and heat stress, together with poor soil fertility. Since 1968, the negative impacts of heat and drought stress have been reported. Many landraces of crops such as beans, pearl millet and sorghum have been lost due to abiotic stresses (Hall, 2004). Cowpea is susceptible to severe drought, particularly during the phases of podding and filling of grains (Hall, 2004). Heat stress causes between 4 and 14 % losses in pod and grain yield when temperatures exceed a threshold of 16°C. Another major limitation to cowpea production in the sub-region is low soil fertility. Most cowpea farmers in Ghana do not use fertilizers and cultivation takes place in soils with low fertility resulting in the low productivity (Horn *et al*., 2015).

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CHAPTER THREE

MATERIAL AND METHODS

3.1 Introduction

This chapter looked at the materials and methods used in the development of putative mutants. It covers radio-sensitivity test, mass irradiation, selections from M_1 to M_3 and evaluation at M_4 generation.

3.2 Study Areas

The mutagenesis was carried out on the farm plots of Biotechnology and Nuclear Agriculture Research Institute (BNARI) under Nuclear Agricultural Research Centre (NARC) in Ghana Atomic Energy Commission (GAEC). Seed multiplication and some laboratory analyses were ran at Alex Carson Teaching and Research Farm, College of Agriculture, University of Cape Coast, Ghana.

3.2.1 Study Area at GAEC

The GAEC is located at the North-Western part of University of Ghana and about 24 km from Accra central. The area is bounded by latitudes 5.67^0 N - 5.69^0 N and longitude 0.21^0 W - 0.26^0 W at an elevation of 64 m above sea level. The site is characterized by a dry season from November to March with about 32 mm per month while the rainy season starts from April to June with an average of 125 mm of rainfall per month. There is a minor dry season between July and August after which there is another rainy season. The mean annual rainfall is about 830 mm (Essel *et al.*, 2016). Mean monthly temperature is highest (30°C) between March and April while it is lowest 26°C in August. The highest average monthly relative humidity does not exceed 75 % and the lowest is about 60 % (Dickson & Benneh 2004).

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3.2.2 Study Area at University of Cape Coast

The experiment was done at the Alex Carson Teaching and Research Farm of the University of Cape Coast's School of Agriculture in Ghana. The place has a bimodal rainfall trend between May and June and August and October. The average annual precipitation spans from 750 to 1000 millimeters, and the average temperature is 27.6 degrees Celsius. The soil type is Acrisol and the location falls within the coastal savannah zone of Ghana, within latitudes 05°- 03' and 05°-5' north and longitudes 01°-13' and 01°-13' west (Armah, 2011).

3.3 Sources of planting materials

Five cowpea genotypes were selected for the research. The genotypes included Hansadua, an improved cowpea variety released in Ghana by Crop Research Institute (CRI), Fumesua of Council for Scientific and Industrial Research (CSIR), with white seed coat colour and black eye. It has relatively large seeds with a potential yield of about 3500 $kgha^{-1}$. It has an indeterminate growth habit, a kidney-shaped seed, with a maturity period of between 65 and 67 days after sowing. They were known to be tolerant/resistant to insect pests such as thrips and diseases such as Cercospora leaf spot and anthracnose.

WC-10 and WC-36 were landraces and ACC122WxWC-10, an inbred line all from Uganda with brown seed coat and brown eye, they have small seed sizes and have yield potential of up to 3 Mt per hectare and IT97K-819, inbred line from IITA, Ibadan-Nigeria with brown seed coat and brown eye. The seed size is relatively big but with few numbers of seeds per pod and pods per plant accounting for a very low yield. On Average they have a maturity period of 90 to 120 days.

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Out of the five cowpea genotypes used in the preliminary radio-sensitivity test, four were dropped at the M_2 stage since they failed to produce desired characters of interest. Hansadua (Marfo Tuya x Nhyira) was the remaining one which produced many putative mutants of interest for selection. The experiment started with single seed decent, seed multiplication, radiosensitivity testing, M_1, M_2, M_3, M_4 to M_5.

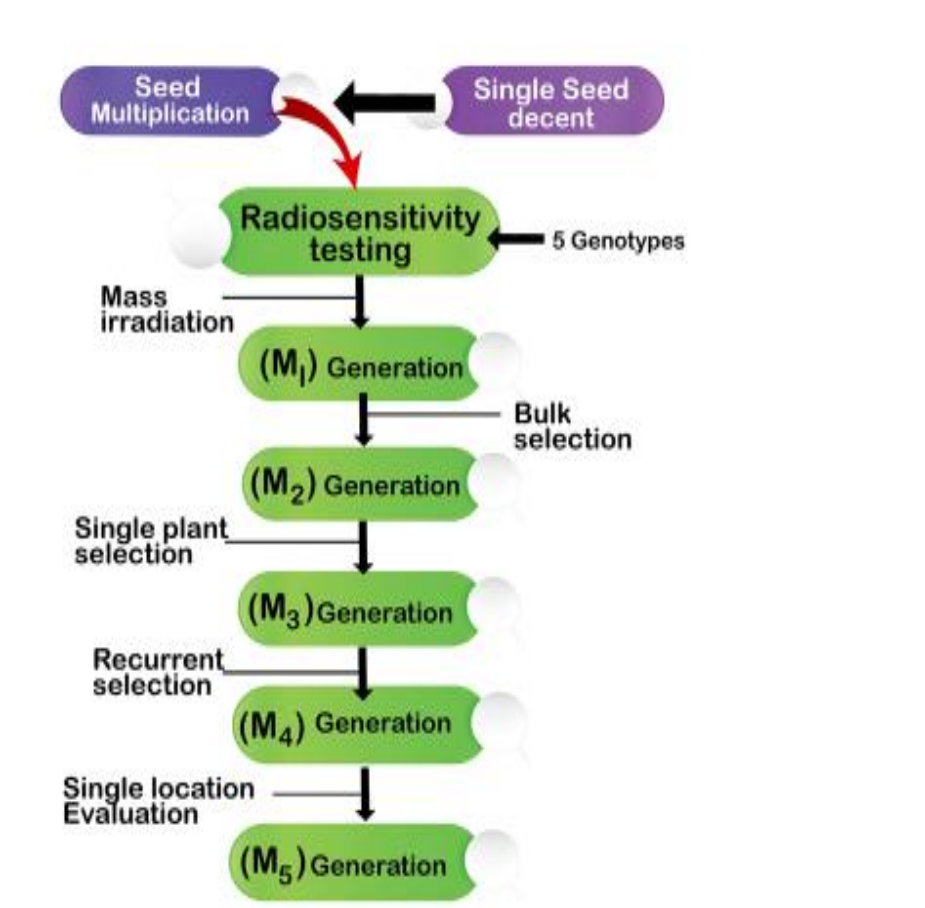

Figure 1: Framework for cowpea mutagenesis

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3.4 Seed multiplication

To ensure seed uniformity and purity, each genotype was multiplied using a single seed descent method at Alex Carson Teaching and Research Farm, School of Agriculture, University of Cape Coast and to generate enough seeds for the experiment. The seeds were sown in a block divided into five with two meters in between each block to avoid crossing over especially with the creeping ones. Two seeds per stand were maintained at 60 cm x 20 cm. Weeds were manually controlled in the second and fifth weeks after sowing. Pods from each block were harvested and bulked to have enough planting materials representative of each genotype prior to radio-sensitivity testing and mass irradiation.

3.5 Radio-sensitivity test

Following the protocol of Mba *et al.*, (2010) and Tshilenge-Lukanda *et al.*, (2012), the seeds of the five cowpea genotypes were gamma irradiated using ^{60}Co source with 12 doses of irradiation from 100 Gy to 1200 Gy at intervals of 100 Gy and planted immediately after irradiation (to prevent back mutation) together with an additional 10 seeds of each genotype serving as control (un-radiated seeds). There were three replications for each genotype per radiation dose, resulting in 30 seeds for a genotype per radiation dose. The seeds were irradiated using gamma irradiation facility category (IV) at Ghana Atomic Energy Commission with ^{60}Co source at dose rate of 303 Gy/hr.

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3.5.2 Sowing of irradiated seeds

The irradiation seeds were sown in polybags measuring 13 cm wide by 16 cm deep and filled with well-mixed loamy soil using a Completely Randomized Design with three replications (Figure 2). Prior to seeding, the irradiation seeds were stored for only seven days. One seed was planted in each polybag. To prevent strain animals and humans from interfering with the experiment, the area surrounding the experimental site was surrounded by net mesh. Twice per week, the seedlings were irrigated to maintain appropriate moisture.

Figure 2: CRD with three replications for radio-sensitivity testing of cowpea genotypes

3.5.3 Determination of LD_{50} and RD_{50}

Data were collected on seed germination, seedling height, fresh whole plant weight, fresh root weight, root length and fresh shoot weight. Germination counts were done on the 3, 5, 8, 13 and 18 days after sowing. The data on seedling height, fresh whole plant weight, fresh root weight, root length and fresh shoot weight were collected on 21st day after sowing. The percent seed germination was determined on average germination count across

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the three replications for the 8th day after sowing. The LD_{50} was estimated using a simple linear regression model by using the straight-line equation y = mx+c, where y represents the response variable (% germination), x represents the independent variable (dosage of irradiation), and m and c indicate the slope and constant, respectively. The value of "x" at half the value of the control percent germination was determined. This value is known as LD_{50}.

To determine RD_{50} the emergence reduction (ER) was determined using the formula:

Emergence reduction [(ER) %] =

$$ER = 100 - \frac{(Average\ emergence\ of\ induce \times 100)}{Average\ emergence\ of\ control} \quad (1)$$

The average reduction in emergence of genotypes (y) (control and induced plant) is displayed vs dosages administered (x). 50% is extrapolated to yield the RD_{50} Emergence reduction of the value for the control genotypes (y).

3.5 Seedling survival (% SS)

The seedling survival determination followed the formula**:**

$$\%\ SS = \frac{Number\ of\ survived\ seedlings}{Number\ of\ germinated\ seeds} \times 100 \quad (2)$$

3.6 Mass radiation at LD_{50}

One thousand seeds each of the cowpea genotypes were acutely radiated at LD_{50} dose determined for each genotype to generate the M_1 seeds which were sown in the research field at NARC/BNARI at GAEC, using Randomized Complete Block Design with three replications. The seeds were sown under field conditions with supplementary irrigation during dry spells. There were 300 seeds sown per plot of genotype in a block. The control for each genotype was planted alongside for easy comparison. Weeds were

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controlled manually. Insects were controlled using Global 4000 insecticide (Thiamethoxam 350G/L SL + DL inert) at the rate of 20ml/12L of water throughout the entire field experiment. The induced plants were closely observed throughout their growth periods to note any phenotypic variations. All physical variations of any kind (earliness, lateness, fresh pod colour, pod size, flower colour etc.) exhibited by each plant in each genotype during growth period were tagged, harvested using bulk selection method to constitute the M_2 planting population. The M_2 seeds of each genotype were basically a combination of the various phenotypic observations based on experimental objectives during the growth period. Morphological and physiological differences observed were recorded. There was deliberate insect control to maintain the necessary plant population for selection. No fertilizer or any other chemical was applied throughout the growth period.

3.6.1 Bulk selection at M_2

Seeds harvested from the M1 population formed M_2 planting materials. These M_2 seeds were sown in the field at BNARI Research farms as M_2 population within the period of 21/10/2019 to 18/02/2020. The sowing was done in progeny lines for easy observation of the individual plants. The respective controls for each genotype were planted alongside for easy comparison at 30 cm within stands and 60 cm between rows. This was to make it easier to observe each individual plant. Standard agronomic practices were observed. The crops were closely observed throughout the growth and developmental stages. The individual plants that exhibited earliness, high yield and other characteristics from the controls were tagged and harvested to form M_3 planting materials. The bulking of the planting materials at this stage were

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done in specific groups for earliness, high yield and large seed sizes (based on seed length, width and thickness). The selection was also based on putative mutant plants that were physically clean of any disease symptoms. Genotypes WC-36, IT97K-819, WC-10 and ACC122WxWC-10 after M_2 were excluded due to their poor growth and development.

3.6.2 Pure line selection at M_3

Pure line selection method was used to select induced plants on the bases of early maturing, high yielding, large seed sizes and resistance to Cercospora leaf spot, Anthracnose and Fusarium wilt and Pythium stem rot diseases. The seeds harvested at M_2 stage constituted the M_3 planting materials. The M_3 seeds were sown on the field at BNARI from the period of 30/04/2020 to 20/07/2020. Pure line selection method led to a reduction in induced population.

3.6.3 Single Seed Decent

Single seed from each of the five cowpea genotypes were selected and grown under field condition. This was to ensure homogeneity, purity and true –to- type for the planting materials chosen for the experiment. This was done following the protocol by Knott & Kumar (1975)

3.7 Selection procedure of elite lines

Twenty elite putative mutant lines were selected on the bases of earliness, high yield, large seed sizes, and resistance to pests and diseases from M_3 to M_4 stages. On the basis of days to 50% maturity, 100 seed weight and seed size, seed coat color, and quantity of seeds per plant, the best six genotypes were selected from among these elite lines. The background for the selection was informed by the findings of Afful (2020) on “Stakeholders

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preference and adoption of improved cowpea varieties in the Northern Region of Ghana". Egbadzor *et al.*, 2013, Quaye *et al.*, 2011, and Langyintuo *et al.*, 2005 reported that cowpea consumers and farmers in Ghana prefer cowpea grains that are large, cream to white seed coat with black eye, short cooking time, high yielding and pests and diseases resistance. A total of twelve elite lines were selected for evaluation and phenotyping as M_4.

3.7.1 Cultivation of elite lines

Twelve elite mutant lines were evaluated at M_4 at BNARI-GAEC Research farms. The field was ploughed and harrowed one week prior to sowing to avoid any seed contamination from the previous harvest since the same field was used. Sowing was done in randomized blocks with three replications. The seeds were sown at 30 cm within stands and 60 cm between rows with one plant per stand to allow for easy observation and data collection. Insecticides were applied following manufacturer's guidelines to effectively control aphids and other insects on the field. No fungicides or other chemicals were applied throughout the experiments. Weed control was done manually.

3.8 Soil and Climatic conditions

Soil samples from the field before M_4 evaluation were collected and analysed to evaluate the essential nutrients such as Nitrogen, Phosphorus, Potassium, salinity and pH levels. The soil analysis was done at the research laboratory of BNARI-GAEC (Appendix 1). Climatic data were also collected at BNARI-GAEC, where the fieldwork took place (Appendix 2).

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3.9 Data Collection

3.9.1 Quantitative Data

Data were collected on morphological characteristics such as germination percentage, average plant height, survival percentage, days to first flowering, 50 percent flowering, maturity, 90 percent maturity, number of seeds per pod, number of pods per peduncle, number of peduncles per plant, pod length, the number of seeds per plant, seed shape, number of main branches, and plant vigor in accordance with IPGRI's morphological characterization standards (Darwin, Brungart, & Simpson, 2003).

Proximate analyses were done to determine the percentage levels of protein, ash, crude fat/oil, and crude fibre across the elite lines selected following the protocols of Association of Official Analytical Chemists (AOAC) (2008) and Motsara & Roy (2008).

3.9.2 Data Analysis

GenStat Release 10.3DE, Discovery Edition 4, 2016 (VSN International Limited, Rothamsted Experimental Station, Hemel Hempstead, UK) and Microsoft Excel were used to analyze the data. PCA and correlation analyses were also performed using IBM SPSS Statistics for Windows, Version 20.0.

Means for germination parameters, elemental analysis and proximate analysis were calculated in Microsoft Excel 2010 and ANOVA was performed in Genstat to test for statistical significance. The treatment factors for the two-way ANOVA radiation dose and the interaction of genotype and dose. Differences between treatments were compared by least significant difference (*lsd*) at P = 0.01.

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Electronic scales were used to determine whole seedling weight and root weight, and genotype yield was used to determine yield.

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CHAPTER FOUR

RESULTS

4.1 Seed multiplication

Enough seeds of each of the five genotypes were generated through single seed decent. One-kilogram (1 Kg) seeds of each genotype was weighed and used for radio-sensitivity test and the remaining for mass radiation.

4.2 Radio-sensitivity test

4.2.1 LD_{50} test

Figures 3, 4, 5, 6 and 7 described the radio-sensitivity curves for Hansadua, ACC122WxWC-10, WC-36, IT97K-819 and WC-10 respectively. In decreasing values, LD_{50} of 903, 858.7, 762, 705 and 531 Gy were estimated for ACC122WxWC-10, WC-36, IT97K-819, WC-10 and Hansadua, Generally, it was observed that the higher the doses applied the lower the percentage germination of the five genotypes. It was also observed that from the control, germination peaked at a point and fell as gamma ray doses increased. Figure 3 showed the radio-sensitivity curve for Hansadua. The curve showed that the induced seed germination peaked at 200 Gy and reached a lethal survival limit at 550 Gy. Hansadua had LD_{50} at 531 Gy which was the lowest LD_{50} value recorded as a sign of the most sensitive.

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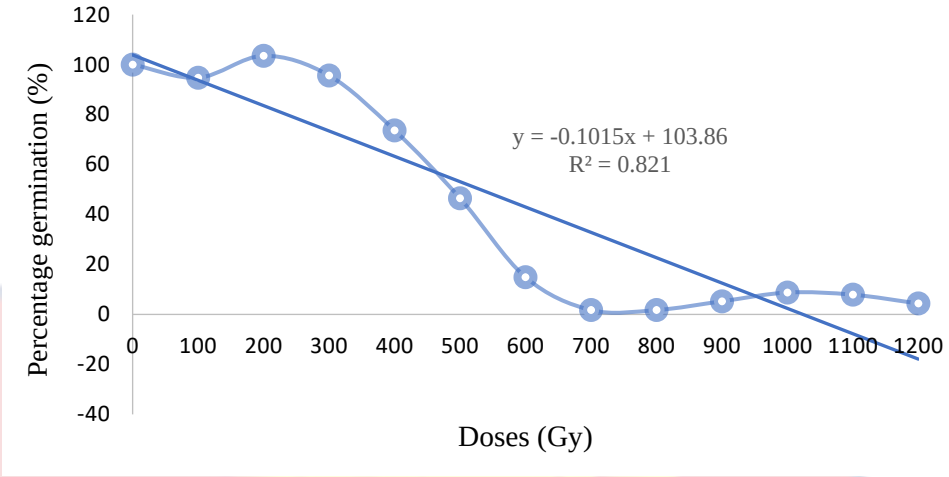

Figure 3: Radio-sensitivity curve of Hansadua

The radio-sensitivity curve for ACC122WxWC-10 showed that the induced seed germination reached its maximum for germination at 700 Gy and lethal survival limit at 950 Gy. ACC122WxWC-10 had LD_{50} at 903 Gy. It appeared this genotype had the highest tolerance level for the gamma rays applied. The effect of the gamma rays can be observed to have taken place from 200 Gy (Figure 4).

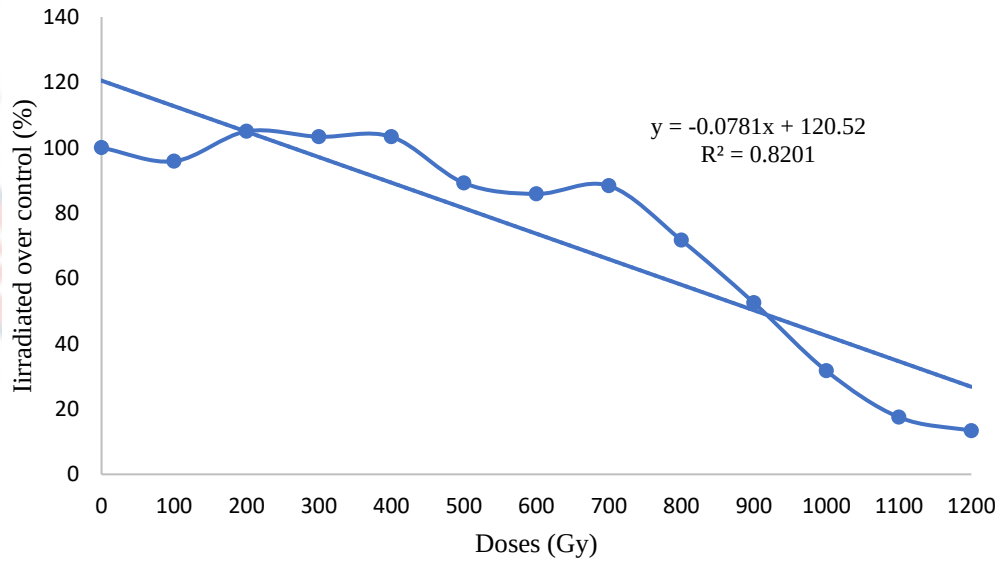

Figure 4: Radio-sensitivity curve for ACC122WxWC-10

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Figure 5 represents the radio-sensitivity curve for WC-36 which got to its maximum for germination at 300 Gy and lethal survival limit at 900 Gy. WC-36 had LD_{50} at 858.7 Gy.

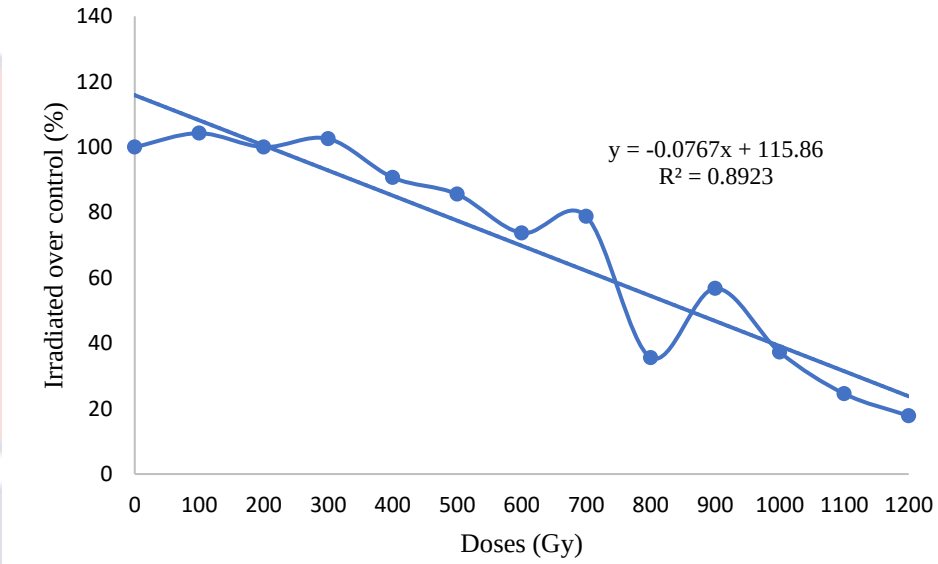

Figure 5:Radio-sensitivity curve for WC-36

Figure 6 showed the radio-sensitivity curve for IT97K-819. The curve indicated the highest germination effect at 400 Gy and lethal survival limit at 800 Gy. IT97K-819 had LD_{50} at 762 Gy.

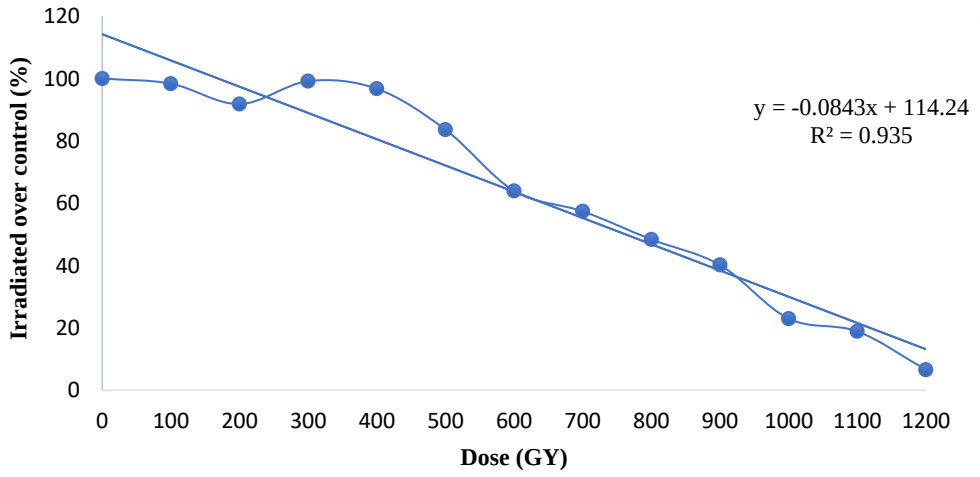

Figure 6: Radio-sensitivity curve for IT97K-819

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Figure 7 also shows the radio-sensitivity curve for WC-10. The curve showed maximum germination effect at 300 Gy and lethal survival limit at 720 Gy. WC-10 had LD_{50} at 705 Gy.

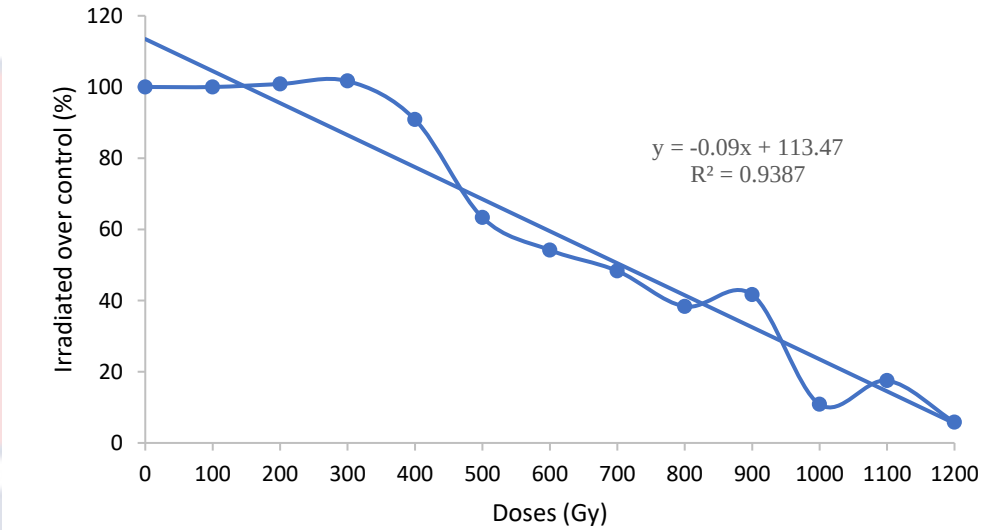

Figure 7: Radio-sensitivity curve of WC-10

4.2.2 Emergence Reduction Test

The figure 8 represents emergence reduction curve of seedling heights for Hansadua, ACC122WxWC-10, WC-36, IT97K-819 and WC-10. The dose rate of induced plants that corresponds with 50% reduction of the seedlings height of parental control is called the emergence reduction. The RD_{50} values were 694.0, 662.0, 591,591and 452 Gy for ACC122WxWC-10, WC-36, IT97K-819, WC-10 and Hansadua, respectively. It was observed that the higher the doses applied the higher the suppression or reduction of plants' height of induced plants relative to their respective controls. It was also observed that the sensitive genotypes responded earlier for height reduction upon gamma ray application than the more tolerant ones.

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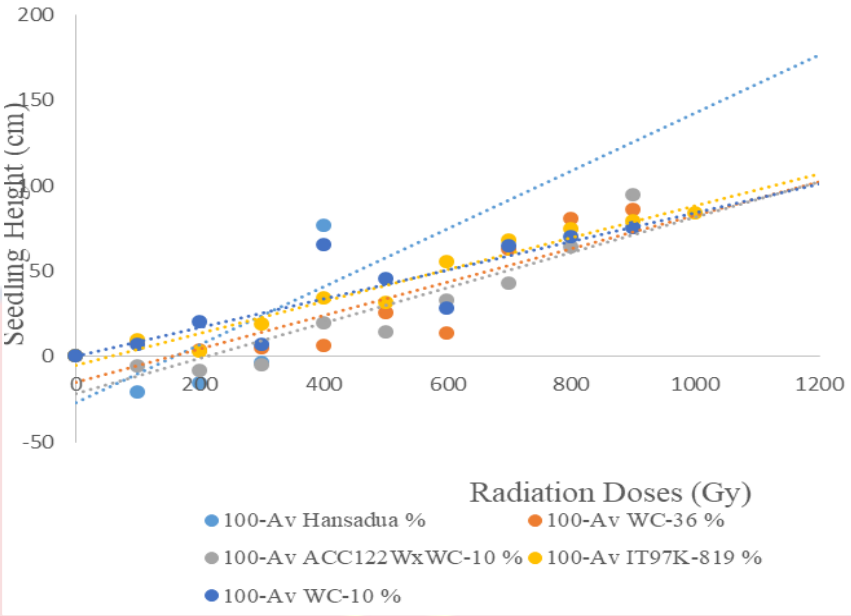

Figure 8: Emergence reduction of five cowpea genotypes

4.2.3 Sensitivity of Genotypes to Radiation (LD_{50} and RD_{50} values)

In decreasing values, LD_{50} of 903, 858.7, 762, 705 and 531 Gy were estimated for ACC122WxWC-10, WC-36, IT97K-819, WC-10 and Hansadua, respectively (Table 2). The LD_{50} values observed were based on the seedling population during germination count, which was later reduced during plant establishment which has affected the overall LD_{50} values estimated. In a similar pattern, the RD_{50} values were 694.0, 662.0, 591,591and 452 Gy for ACC122WxWC-10, WC-36, IT97K-819, WC-10 and Hansadua, respectively. A genotype with the least LD_{50} value is more sensitive to gamma radiation than those with higher values. Hansadua was therefore most sensitive to the radiation applied since it had the least LD_{50} value. On the other hand, ACC122WxWC-10 was the most impervious to gamma rays with the highest LD_{50} value. Consequently, RD_{50} or GR_{50} values followed the same trend of values but with reduction of values from 209 Gy to 79 Gy which may be significant to cause crop damage.

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Table 2: Estimated LD50 and RD50 of five cowpea genotypes

Genotype	Optimum dose	
	LD_{50} (Gy)	RD_{50} (Gy)
Hansadua	531.0	452.0
WC-36	858.7	662.0
ACC122WxWC-10	903.0	694.0
IT97K-819	762.0	590.5
WC-10	705.0	591.0

Source: Field data, Lumorh (2021)

4.2.4 Effects of gamma rays on mean germination, plant height, root length and shoot weight

Table 3 compared the radiation dose effects of gamma rays on mean germination, plant height, root length and shoot weight. It was observed among the five cowpea genotypes that the threshold of radiation dose above which the control plant could be mutated depends only on germination data measured. The threshold for germination percentage was different from the threshold for plant height, root length, shoot weight and plant weight (g). The threshold for plant height, root length, shoot weight and plant weight (g) shows how induced plants suffer growth reduction as a result of irradiation and environmental effects on growth parameters. Therefore, the minimum dose that could cause mutation in cowpea genotypes when seed germination was used was 300 Gy and either 200 Gy when data for plant height was used and 100 Gy for root length and shoot weight respectively. It can be observed also that radiation doses from 200 to 400 Gy may have similar effects on germination, plant height, root length, shoot weight and plant weight. Similar trend was also observed for doses between 400 to 500 Gy. At 600 Gy the

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effects were also seen to be similar beyond which the traits measured responded differently to the gamma ray doses applied.

Table 3 : Effects of gamma rays on mean germination, plant height, root length and shoot weight

Doses (Gy)	Germination	Mean Plant Height (cm)	Root length (cm)	Shoot weight (g)	Plant weight (g)
0	8.0 [a]	20.0 [a]	21.5 [a]	16.5 [a]	18.0 [a]
100	7.8 [a]	19.8 [a]	20.3 [a]	15.9 [a]	16.4 [a]
200	7.8 [a]	19.6 [a]	17.7 [b]	13.6 [b]	14.4 [b]
300	7.8 [a]	19.0 [b]	16.5 [b]	10.9 [b]	12.6 [b]
400	7.2 [b]	12.4 [c]	12.0 [c]	7.60 [c]	7.90 [c]
500	6.0 [c]	12.2 [c]	10.9 [c]	6.90 [c]	7.40 [c]
600	4.6 [d]	11.2 [d]	9.40 [d]	3.20 [d]	4.80 [d]
700	4.4 [d]	6.80 [e]	6.60 [e]	2.30 [d]	2.90 [d]
800	3.2 [e]	4.60 [f]	5.00 [e]	1.70 [e]	2.10 [e]
900	2.8 [e]	2.80 [g]	1.90 [f]	0.90 [e]	1.70 [e]
1000	2.0 [f]	0.80 [h]	0.40 [f]	0.00 [e]	0.00 [e]
Mean	5.6	11.7	11.1	7.7	7.6
STDEV	2.3	7.2	7.3	6.2	6.4
CV%	40.7	61.6	65.4	79.8	84

Source: Field data, Lumorh (2021)

4.2.5 Genetic diversity owing to inheritable morphological variations

4.2.5.1 Variation in leaf shapes

The leaf shape of Hansadua (control) was sub-hastate and has changed to different forms as ovate, lanceolate, elliptical, deltoid and globose because of the application of gamma rays (Figure 9).

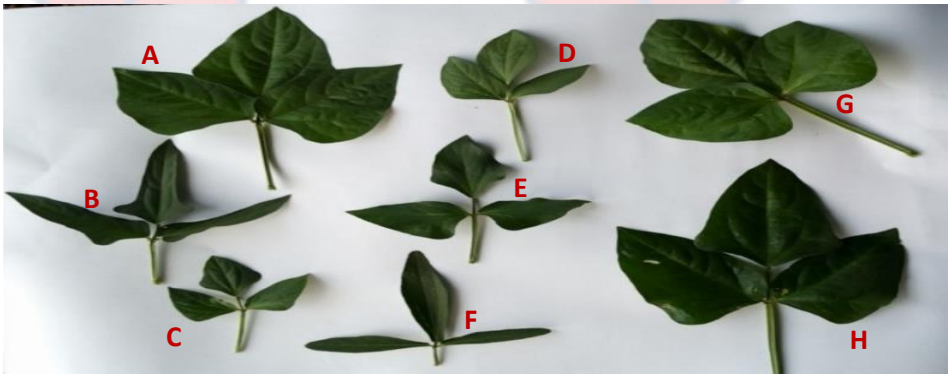

Figure 9: Changes in Leaf Shape

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A-Deltoid, **B**-Hastate, **C**-Deltoid, **D**-Elliptical, **E**-Sub-hastate (control), **F**-Lanceolate, **G**-Ovate, **H**-Deltoid

4.2.5.2 Variations in flower colour.

The flower colour of the parent Hansadua was white standard with white keel as labled 'C' in Figure 10, which changed to mauve-pink and light pink keel with pale pink to white standards in the mutant genotypes as seen in 'A', 'B', 'D', 'E' and 'F when grown in the same environment and conditions.

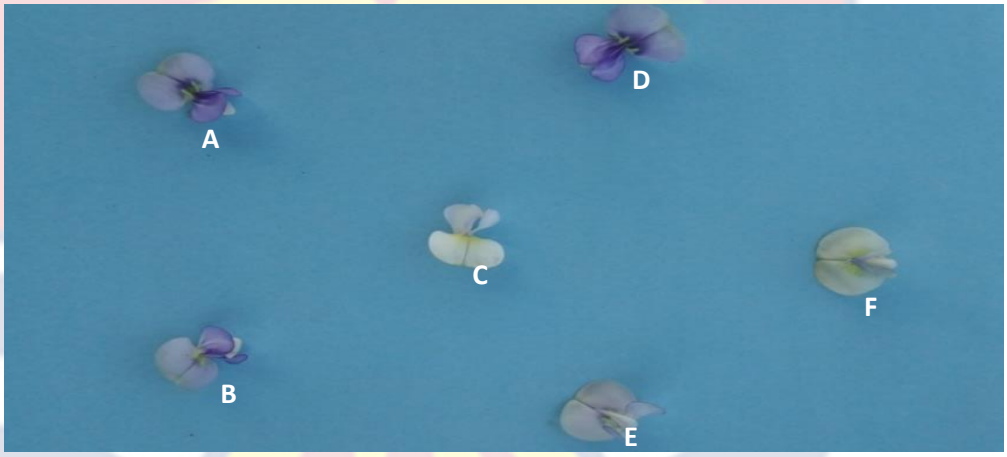

Figure 10: Variations in flower color

4.2.5.3 Deformities in leaf

Deformities were observed during the experimentation because of application of gamma rays. Some leaves were observed to be white, yellow, some extraordinarily bigger with architecture not characteristic of the typical trifoliate leaf of cowpea (Figure 11).

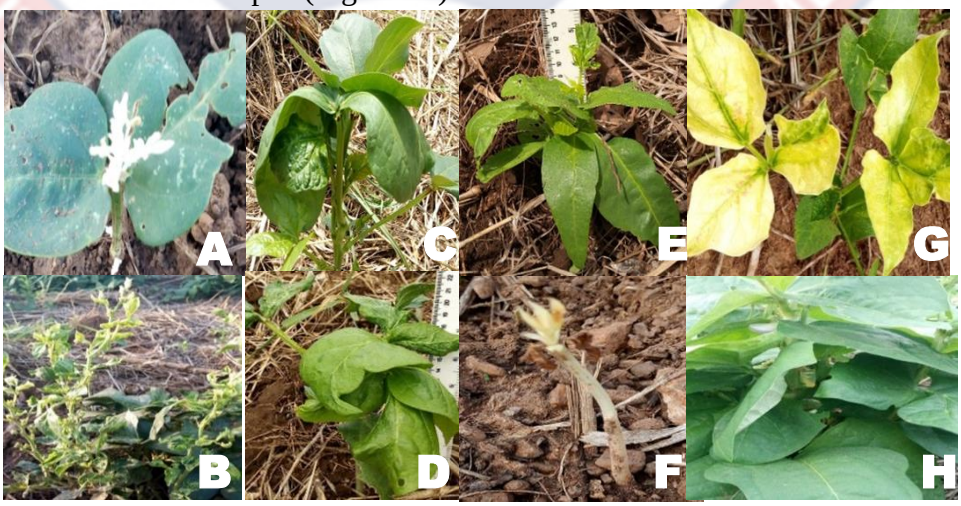

Figure 11: Leaf deformities observed

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4.2.5.4 Variations in plant architecture

Plant architectural changes were observed from M_1 and M_3 generations. In some mutants, the entire plant morphology has changed from the parental line. The architecture has changed from semi-erect to acute erect, climbing and spreading types. Some were looking like soya bean leaves as in 'A', and 'D'

looked like dandelion leaf and 'E' looked like a leaf of *commelina* plant (Figure 12).

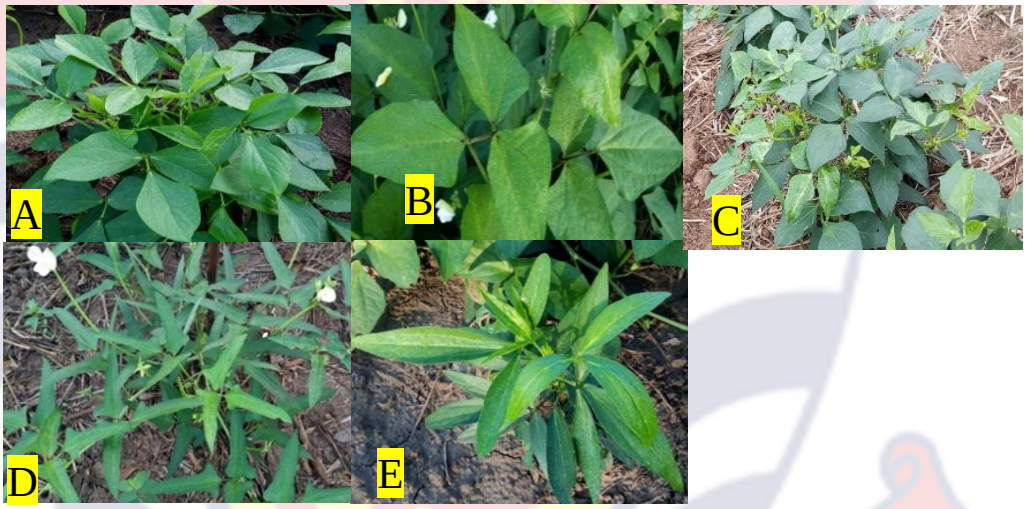

Figure 12: Changes in plant architecture

4.2.6 Diseases, Pests and Pollinators

4.2.6.1 Disease symptoms

Some disease symptoms were observed on the field during experimentation. 'A' is bacteria blight, 'B' and 'D' is golden mosaic, 'C' is lamb's tail pod rot, 'F' and 'H' is *sclerocium* and 'G' is *cercospora* leaf spot (Figure 13).

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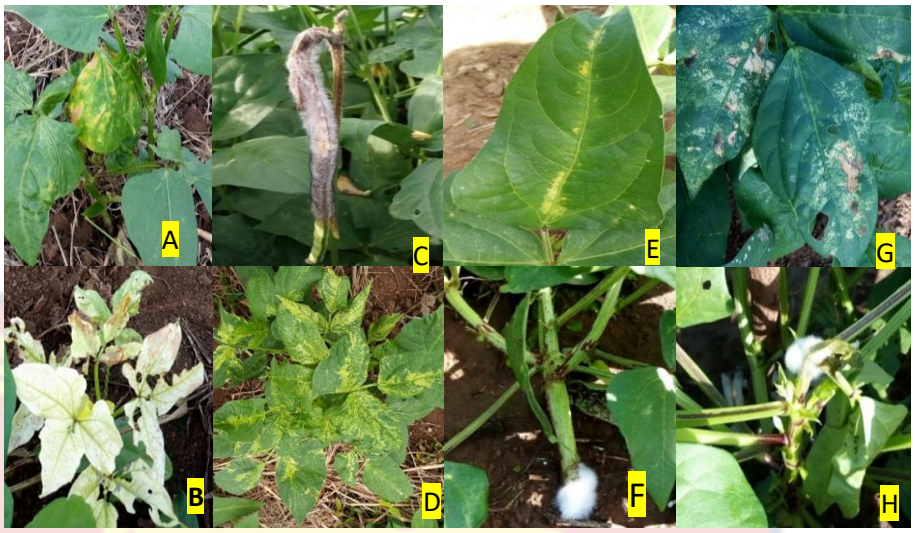

Figure 13: On field disease conditions observed

A *&* ***E****= Bacterial blight,* ***B, C*** *= Lamb's tail pod rot* ***D*** *&* ***B****= Golden mosaic,* ***G*** *= Web blight* ***F*** *&* ***H****= Sclerotium stem rot*

4.2.6.2 Common pests

Some common pests of cowpea observed during field experimentation include cotton stainer, leaf miner, blister beetle, moths and *Riptortus serripes* (Figure 14). These organisms were observed at different growth stages of the mutants.

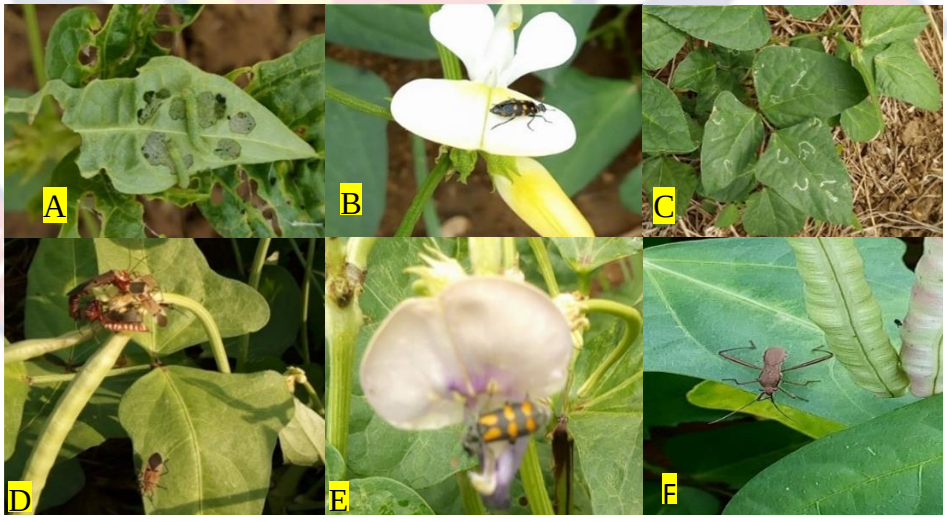

Figure 14: Common pests observed

A*: Moths,* ***B****: Blister beetle,* ***C****: Leaf miner,* ***D****: Cotton stainer,* ***E****: Blister beetle and* ***F****: Riptortus serripes.*

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4.2.6.3 Insect pollinators

Some common insect pollinators observed on field were beetles and butterflies (Figure 15).

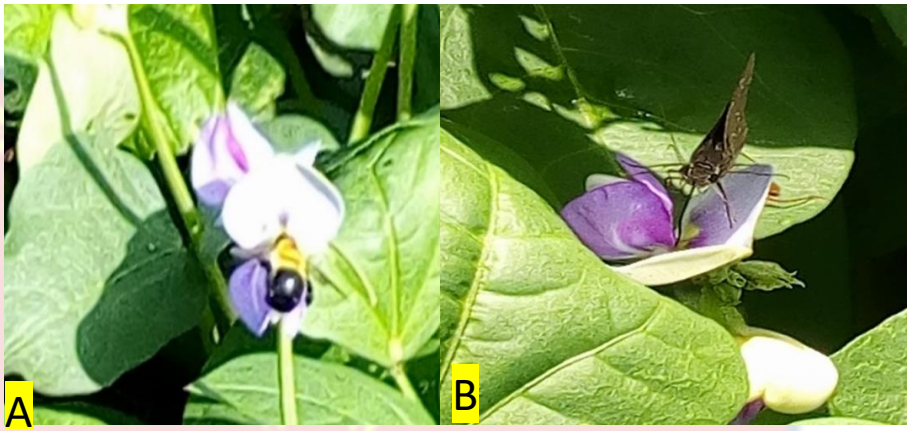

Figure 15: Common pollinators observed

A = Beetle **B** = Butterfly

4.2.7 Variations in flower sizes

Figure 16 shows changes in the flower sizes. The sizes of standard and keel varied and in most cases. Some are smaller or bigger than parents.

Figure 16: Variations in flower sizes

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4.2.8 Variations in pod architecture

Figure 17 shows the pod architecture varied from curved in control to same, straight, and coiled at M_1 generation. The pod of the dried pod also varied from tan-brown to straw.

Figure 17: Variation in pod architecture

4.2.9 Variations in seed coat and eye colour

There were variations in seed coat colour and eye colour among the mutants developed from Hansadua. The control was having cream seed coat with black eye and has changed to brown seed coat with black and brown eye, black seed coat with black and brown eye, mottled seed coat with black and brown eye. (Figure 18).

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Figure 18: Variations in seed coat and eye color

4.3 Evaluation of M_4 Population at a single location

Twelve promising putative cowpea mutant lines and control were evaluated at M_4 for further selection and confirmation. They consist of the top six from high yielding types and six from the early maturing types. Data were collected on morphological characteristics such as germination percentage, average plant height, survival percentage days to first flowering, 50% flowering, maturity, 90% maturity, number of seeds per pod, number of pods per peduncle, number of peduncles per plant, the number of seeds per plant, seed shape, pod length, number of main branches, plant vigour and others.

4.3.1 Germination and seedling survival at M_4

The result of germination of M_4 seeds and survival of induced plants were displayed in Table 4. The highest germination percentage was found in $HanM_4$(41)(HY31) at 94%, followed by $HanM_4$(12)(3) at 93% while the lowest of 43% was recorded by Hansadua. $HanM_4$(12)(3) had the highest

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survival percentage of 99%, followed by HanM4(44) (2B) at 98% and the lowest found in HanM4(17) (1W) at 93%.

Table 4: Germination and survival at M_4 generation

Genotype	% Germination	% Survival
HanM4(02)(02)	92	95
HanM4(12(25)	72	94
HanM4(12)(3)	93	99
HanM4(12)(5)	91	96
HanM4(17) (1W)	89	93
HanM4(33) (HY42)	69	96
HanM4(33) (HY43)	76	96
HanM4(41)(2)	91	96
HanM4(41) (HY30)	83	95
HanM4(41) (HY31)	94	94
HanM4(44) (2B)	79	98
HanM4(52) (HY)	89	95
Hansadua	43	94

Source: Field data, Lumorh (2021)

4.3.2 Characterisation based on growth habit, plant pigmentation, terminal leaf shape and immature pod pigmentation

Results from Table 5 indicated six out of the thirteen mutants and controls exhibited intermediate growth habit, three were semi-erect, one erect, one semi-prostrate and one acute erect. Plant pigmentation was further looked at about stem, branch, petiole and peduncle. Different variations were observed for each genotype for the features mentioned above. Seven genotypes showed very slight stem pigmentation, followed by four moderate stem pigmentations and two had no stem pigmentation. Nine of the genotypes showed no branch pigmentation, followed by three with very slight branch pigmentation and one with moderate branch pigmentation. Ten of the

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genotypes showed moderate plant pigmentation for petiole, two intermediate and one very slight petiole pigmentation. Eight of the genotypes showed no peduncle pigmentation, four very slight and one moderate. The results also showed differences in terminal leaf shape, of which seven of the genotypes were sub-hastate and the remaining six were sub-globose. The result showed that twelve of the genotypes showed no immature pod pigmentation, with only one with pigmented sutures.

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Table 5: Growth habit, plant pigmentation, terminal leaf shape and immature pod pigmentation of 12 mutant lines and parent

		Plant pigmentation					
Mutants	Growth Habit	Stem	Branch	petiole	Peduncle	Terminal Leaf shape	Immature Pod pigmentation
HanM4(41)(2)	Intermediate	Moderate	None	Moderate	None	Sub-hastate	None
HanM4(41)HY31	Intermediate	V. slight	None	Moderate	None	Sub-hastate	None
HanM4(02)(02)	Erect	V. slight	V. slight	Moderate	V. Slight	Sub-globose	None
HanM4(33)HY43	Intermediate	V. slight	V. slight	Moderate	Moderate	Sub-globose	None
HanM4(12)(5)	Semi-erect	V. slight	None	Intermediate	None	Sub-globose	None
HanM4(52)HY	Semi-prostrate	V. slight	None	Moderate	V. Slight	Sub-globose	None
HanM4(12)HY25	Intermediate	None	None	V. Slight	None	Sub-hastate	None
HanM4(17)(1W)	Acute erect	V. slight	V. slight	Moderate	V. Slight	Sub-hastate	None
HanM4(33)HY42	Intermediate	Moderate	None	Moderate	None	Sub-globose	None
HanM4(44)(2B)	Semi-erect	None	Moderate	Moderate	None	Sub-globose	None
Hansadua	Intermediate	V. slight	None	Intermediate	None	Globose	None
HanM4(12)(3)	Intermediate	Moderate	None	Moderate	V. Slight	Sub-hastate	Pigmented sutures
HanM4(41)HY30	Semi-erect	Moderate	None	Moderate	None	Sub-hastate	None

Source: Field data, Lumorh (2021)

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4.3.3 Characterisation based on leaf marking, leaf colour, leaf texture and flower colour

The result from Table 6 indicated that all twelve cowpea mutants including parent had V-marking present on their leaves surface. Eight of the mutant genotypes exhibited intermediate green leaf colour, two were pale green coloured and two mutants and parent had deep green leaf colour. Seven mutant genotypes and parent had coriaceous leaf texture while the remaining five mutants exhibited membranous leaf texture. Five mutants and parent had white flower colour, four mauve-pink and three had violet leaf colour.

Table 6: Characterization based on leaf marking, leaf colour, leaf texture and flower colour

Mutants	Leaf marking	Leaf colour	Leaf Texture	Flower Colour
HanM4(41)(2)	Present	Pale green	Membranous	Violet
HanM4(41)HY31	Present	Intermediate green	Membranous	Mauvepink
HanM4(02)(02)	Present	Deep green	Coriaceous	Mauvepink
HanM4(33)HY43	Present	Intermediate green	Membranous	White
HanM4(12)(5)	Present	Pale green	Membranous	White
HanM4(52)HY	Present	Intermediate green	Coriaceous	Violet
HanM4(12)HY25	Present	Deep green	Coriaceous	White
HanM4(17)(1W)	Present	Intermediate green	Coriaceous	White
HanM4(33)HY42	Present	Intermediate green	Coriaceous	Mauvepink
HanM4(44)(2B)	Present	Intermediate green	Membranous	Mauvepink
Hansadua	Present	Deep green	Coriaceous	White
HanM4(12)(3)	Present	Intermediate green	Coriaceous	White
HanM4(41)HY30	Present	Intermediate green	Coriaceous	Violet

Source: Field data, Lumorh (2021)

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4.3.4 Characterisation based on colour of dry pod, eye colour, seed coat colour, seed shape, pod wall thickness and pod attachment to peduncle

From table 7 all thirteen genotypes, including Hansadua (parent), had the colour of their dry pod being straw. Six of the genotypes showed blue to black eye colour, three exhibited tan-brown, two had blue to black spots, one showed mottled and one also indicated brown splash for eye colour. Seed coat colours were largely mottled and cream each had six genotypes and one had chocolate brown seed coat colour. Eight genotypes had kidney seed shapes, two rhomboids, two globose and one ovoid. Seven of the genotypes had intermediate pod wall thickness, four thick and two had thin pod wall thickness. With pod attachment to peduncle, ten genotypes showed pendant type, 30-90° were two and only one was erect.

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Table 7: Characterisation based on colour of dry pod, eye colour, seed coat colour, seed shape, pod wall thickness and pod attachment to peduncle

Mutants	Colour			Seed Shape	Pod wall thickness	Pod attachment to peduncle
	Dry Pod	Eye	Seed Coat			
HanM4(41)(2)	Straw	Mottled	Mottled	Kidney	Intermediate	Pendant
HanM4(41)HY31	Straw	Blue to Black	Mottled	Kidney	Thin	Pendant
HanM4(02)(02)	Straw	Blue to Black	Mottled	Rhomboid	Thin	30-90°
HanM4(33)HY43	Straw	Tan Brown	Cream	Kidney	Intermediate	Pendant
HanM4(12)(5)	Straw	Blue to Black	Cream	Globose	Thick	Pendant
HanM4(52)HY	Straw	Blue to Black	Mottled	Kidney	Thick	Pendant
HanM4(12)HY25	Straw	Blue to Black	Cream	Ovoid	Thick	Pendant
HanM4(17)(1W)	Straw	Brown splash or gray	Cream	Globose	Intermediate	30-90°
HanM4(33)HY42	Straw	Blue to Black	Mottled	Rhomboid	Thick	Pendant
HanM4(44)(2B)	Straw	Blue to Black spots	Mottled	Kidney	Intermediate	Pendant
Hansadua	Straw	Blue to Black spots	Cream	Kidney	Intermediate	Erect
HanM4(12)(3)	Straw	Tan Brown	Cream	Kidney	Intermediate	Pendant
HanM4(41)HY30	Straw	Tan Brown	Chocolate brown	Kidney	Intermediate	Pendant

Source: Field data, Lumorh (2021)

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4.3.5 Classifications based on growth pattern, twinning tendency, seed crowding, splitting of testa and attachment of testa

Growth pattern of the genotypes were assessed. Eleven genotypes showed determinate growth patterns and two indeterminate. The twinning tendency was not found in nine of the genotypes. Three were showing slight tendency and one intermediate. Eleven of the genotypes did not show seed crowding, with only two which exhibited semi-crowding of seed. Testa splitting ability was absent in all the thirteen genotypes and all genotypes showed the tendency of firm attachment of testa to the seed (Table 8).

Table 8: Classifications based on growth pattern, twinning tendency, seed crowding splitting of testa and attachments of testa

Mutants	Growth Pattern	Twinning tendency	Seed crowding	Splitting of testa	Attachment of testa
HanM4(41)(2)	Determinate	None	Not crowded	Absent	Firmly attached
HanM4(41)HY31	Determinate	None	Not crowded	Absent	Firmly attached
HanM4(02)(02)	Determinate	None	Semi-crowded	Absent	Firmly attached
HanM4(33)HY43	Determinate	Slight	Not crowded	Absent	Firmly attached
HanM4(12)(5)	Determinate	None	Not crowded	Absent	Firmly attached
HanM4(52)HY	Indeterminate	Intermediate	Not crowded	Absent	Firmly attached
HanM4(12)HY25	Determinate	Slight	Not crowded	Absent	Firmly attached
HanM4(17)(1W)	Indeterminate	None	Semi-crowded	Absent	Firmly attached
HanM4(33)HY42	Determinate	None	Not crowded	Absent	Firmly attached
HanM4(44)(2B)	Determinate	None	Not crowded	Absent	Firmly attached
Hansadua	Determinate	None	Not crowded	Absent	Firmly attached
HanM4(12)(3)	Determinate	Slight	Not crowded	Absent	Firmly attached
HanM4(41)HY30	Determinate	None	Not crowded	Absent	Firmly attached

Source: Field data, Lumorh (2021)

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4.4 Effect of radiation on the induced plants

4.4.1 Effect of radiation on germination

The mean germination for Hansadua was significantly different (P<0.001) from HanM4(33)(HY42), HanM4(02)(02), HanM4(12)(3) and HanM4(41)(HY31) (Figure 19). HanM4(33)(HY42) was also significantly different from Hansadua, HanM4(02)(02), HanM4(12)(3) and HanM4(41)(HY31) but not different from the remaining mutants. However, HanM4(02)(02), HanM4(12)(3) and HanM4(41)(HY31) were significantly different from Hansadua and HanM4(33)(HY42) but not different from the remaining mutants.

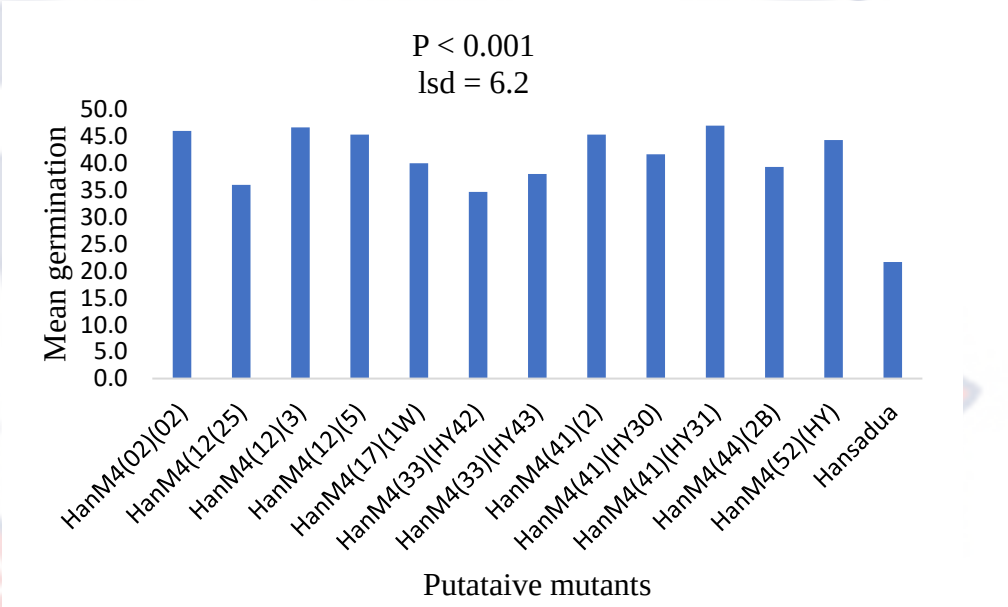

Figure 19: Effects of radiation on mean germination of mutants and control

4.4.2 Effect of radiation on Plant vigour

Seedling vigour for Hansadua was significantly different from HanM$_4$(44) (2B) and HanM$_4$(52) (HY) (P>0.001) but not from the remaining mutants. HanM$_4$(52) (HY) was significantly different from Hansadua and the remaining mutants except for HanM$_4$(44) (2B) (Figure 20).

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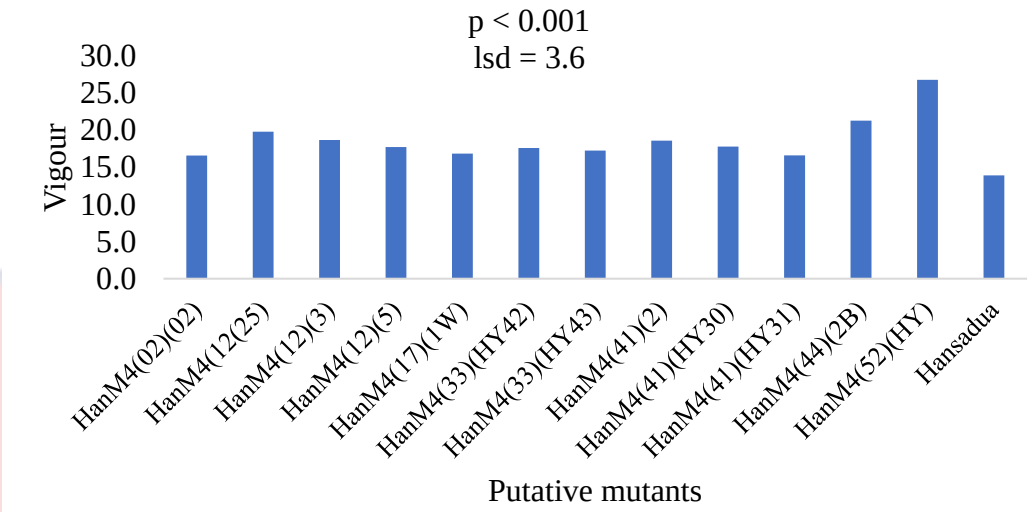

Figure 20: Effects of radiation on vigour of mutants

4.4.3 Effect of radiation on days to first flowering, 50% flowering and 50% pod maturity

Hansadua had four days between the first days of flowering to 50% flowering and took sixteen days from 50% flowering to reach 50% pod maturity. There were three days between the first day of flowering in HanM4(12)(25) to 50 percent flowering and twenty-two days to 50% pod maturity. HanM4(17)(1W) had three days between the day to first flowering and 50% flowering and took only eleven days to 50 % pod maturity. Each mutant had a unique growth cycle (Figure 21).

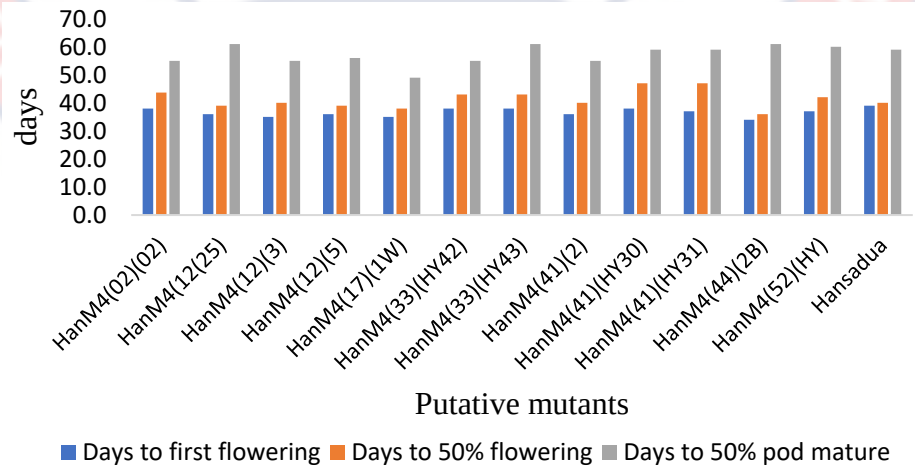

Figure 21: Effects of radiation on days to first flowering, 50% flowering and 50% pod maturity

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4.4.4 Effect of radiation on seeds and haulm weights ratio

The relationship between weight of seeds of dry pods and the weight of haulm of dry pods varied among the mutant genotypes selected. The ratios were found to be 3-4: 1 for dry seeds and corresponding haulms (Figure 22).

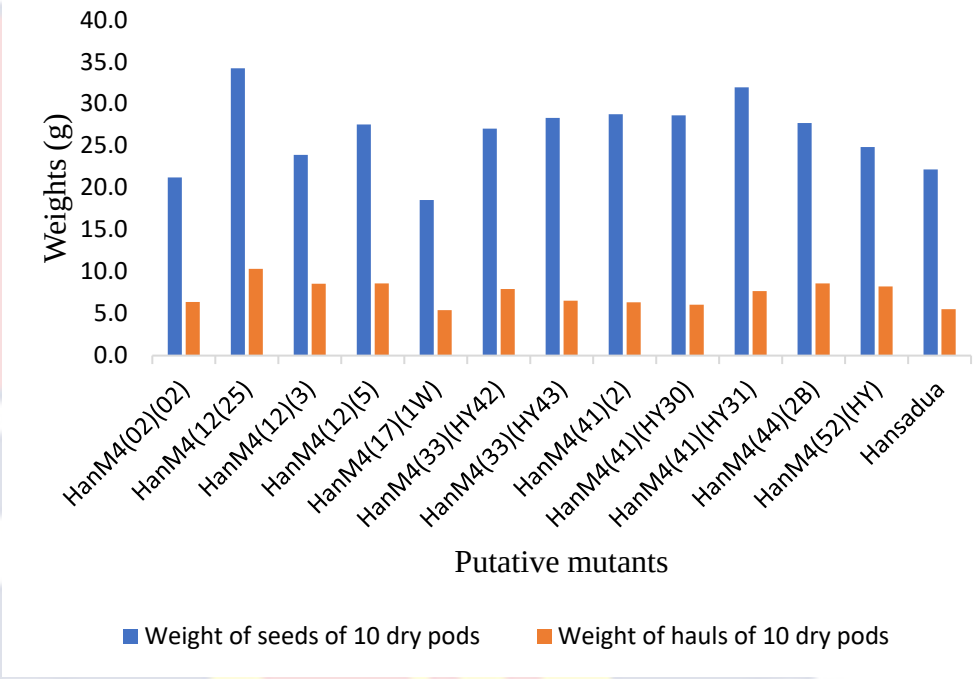

Figure 22: Effects of radiation on weight of seeds 10 dry pods and corresponding haulm

4.4.5 Effect of Radiation on grain yield

HanM4(12)(25) had the highest yield (5800 $kgha^{-1}$) compared with Hansadua, which had 3672 $kgha^{-1}$. The second highest was HanM4(33)(HY43) with about 5200 $kgha^{-1}$ and followed by 4040 $kgha^{-1}$ for HanM4(41)(HY30). The mutant genotype with the lowest total grain yield was HanM4(17)(1W) with 2140 $kgha^{-1}$ (Figure 23).

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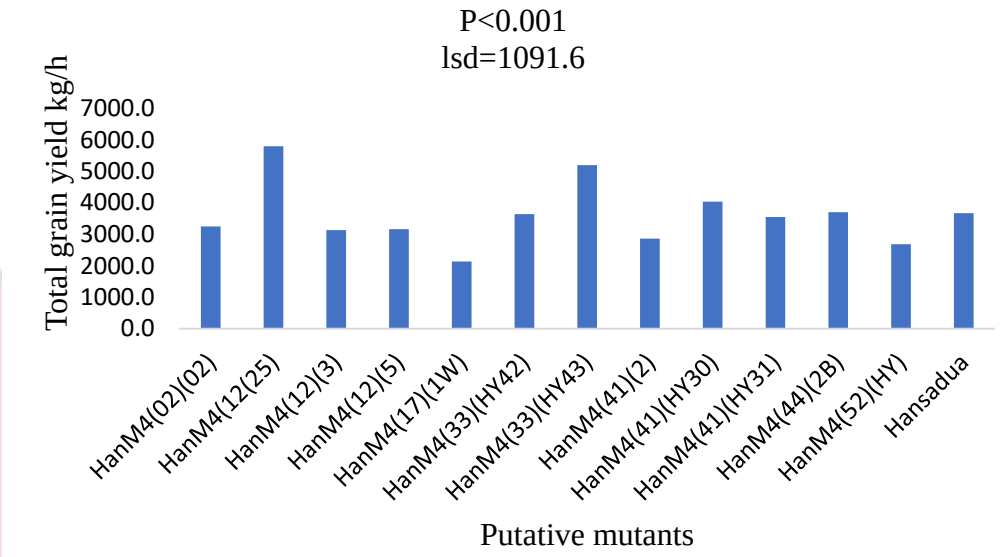

Figure 22: Effects of radiation on total grain yield of putative mutants

4.4.6 Effect of irradiation on number of nodes on main stem, number of main branches, standard flower length, seed length, seed width and seed thickness

The analysis revealed significant differences (P<0.001) among the traits measured (Table 9). The number of nodes on main stem of HanM4(17)(1W) was significantly different from Hansadua, HanM4(52)(HY), HanM4(12)(25) and the remaining mutant genotypes. No significant differences were observed among HanM4(52)(HY), HanM4(41)(HY30), HanM4(44)(2B) and HanM4(12)(5). For the number of main branches, significant differences were observed among HanM4(17)(1W), HanM4(44)(2B) and HanM4(12)(25), HanM4(12)(3) and HanM4(12)(5).

No significant difference were observed for standard length between HanM4(02)(02) and HanM4(41)(HY30) but were different from HanM4(17)(1W), HanM4(12)(25) and HanM4(52)(HY). For seed length, HanM4(02)(02) was not significantly different from HanM4(17)(1W) but

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different from the remaining mutant genotypes. HanM4(12)(25) was significantly different from Hansadua and the remaining mutants. HanM4(17)(1W) had seed width which was substantially different from HanM4(12)(25), HanM4(41)(HY30), HanM4(52)(HY) and the remaining mutant genotypes except for Hansadua. The seed thickness of HanM4(12)(25) was significantly different from Hansadua and the remaining mutant genotypes.

Table 9: Effect of radiation on number of nodes on main stem, number of main branches, standard flower length, seed length, seed width and seed thickness

Genotype	Number of nodes on main stem	Number of main branches	Standard flower length (mm)	Seed Length (mm)	Seed Width (mm)	Seed Thickness (mm)
HanM4(02)(02)	17.9	5.0	26.6	6.1	5.7	4.8
HanM4(12(25)	20.1	4.7	29.0	9.1	6.7	5.9
HanM4(12)(3)	18.5	5.3	28.3	7.7	6.1	4.9
HanM4(12)(5)	17.1	6.0	29.3	8.4	6.8	5.9
HanM4(17)(1W)	13.2	4.0	28.8	6.2	5.1	4.4
HanM4(33)(HY42)	19.1	4.4	28.8	8.2	6.5	5.2
HanM4(33)(HY43)	17.2	4.4	29.0	8.7	6.2	5.3
HanM4(41)(2)	17.4	4.5	28.3	8.7	6.4	5.5
HanM4(41)(HY30)	16.1	5.4	27.5	8.7	6.2	5.0
HanM4(41)(HY31)	17.5	4.8	28.7	8.7	6.3	5.3
HanM4(44)(2B)	16.6	3.5	29.8	7.9	6.1	5.0
HanM4(52)(HY)	16.0	4.0	29.8	8.2	5.8	4.6
Hansadua	14.4	4.3	28.2	7.8	5.4	4.8
Lsd	0.6	0.8	0.6	0.4	0.2	0.4
Se	0.4	0.5	0.4	0.2	0.1	0.2
%cv	2.2	10.1	1.3	2.6	2.1	4.1

Source: Field data, Lumorh (2021)

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4.4.7 Effect of radiation on pod width, pod length, peduncle length, number of pods/peduncles, number of pods/plants, number of peduncles/plants and weight of ten dry pods, one hundred seed weight, total grain yield

The analysis showed significant differences ($P<0.001$) existed among the mutant genotypes for pod width for Hansadua, HanM4(52)(HY) and HanM4(12)(25) (Table 10). No differences were observed in HanM4(02)(02), HanM4(44)(2B) and HanM4(41)(2). For pod length, no differences were seen between HanM4(02)(02) and Hansadua and between HanM4(41)(HY30) and HanM4(44)(2B). HanM4(12)(25) was not different from HanM4(33)(HY42) and differs from the remaining mutant genotypes.

Among the peduncle length, HanM4(12)(2) was significantly different from Hansadua, HanM4(12)(25), HanM4(02)(02), HanM4(33)(HY43) but differed from the remaining genotypes. No significant differences were observed among HanM4(41)(HY31), HanM4(44)(2B) and HanM4(33)(HY42) and HanM4(02)(02). No significant differences were observed for number of pods/peduncles among twelve mutant genotypes except HanM4(33)(HY43) and HanM4(02)(02).

HanM4(33)(HY43) significantly differed from the other genotypes in terms of the quantity of pods/plants, although HanM4(17)(1W), HanM4(52)(HY), and HanM4(41) showed no differences (HY31). Between HanM4(41)(2), HanM4(02)(02), HanM4(12)(25), HanM4(12)(3), and Hansadua, there were no differences.

HanM4(17)(1W) for number of peduncles/plants deferred from all remaining genotypes but no significant differences were seen for HanM4(41)(HY31), HanM4(52)(HY) and HanM4(41)(2). Again, no

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observable differences were made between HanM4(12)(25) and Hansadua. HanM4(02)(02) differed significantly from the other twelve mutant genotypes.

Weight of 10 dry pods, HanM4(17)(1W) was significantly different from the remaining types but no differences were observed for HanM4(02)(02) and Hansadua and likewise for HanM4(44)(2B) and HanM4(12)(5). HanM4(12)(25) was significantly different from the rest of the genotypes

The pod width had a direct positive relationship with 100 seed weight and total grain yield as seen in HanM4(12)(25). All things been equal the bigger the pod width which is well filled with grain the higher the grain yield of cowpea.

The number of seeds per pod does not necessarily lead to more cowpea production. The higher the number of seeds per pod, the smaller the seed size and lower the yield since 100 seed weight and total grain yield as main criterial for estimating yields are highly dependent on seed weight. This was clear in HanM4(02)(02) with an average of 17.5 and HanM4(17)(1W) with an average of 17.7 had the lowest yield of 3.3 t/ha and 2.1 t/ha respectively.

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Table 10: Effect of radiation on quantitative traits

Genotype	Pod width (cm)	Pod Length (cm)	Peduncle Length (cm)	Number of pods/peduncles	Number of pods/plants	Number of peduncles per plant	Weight of 10 dry pods(g)	Weight of 100 seeds(g)	Number of seeds/pods	Total grain Yield ($kgha^{-1}$)
HanM4(02)(02)	8.2	16.1	34.6	2.3	79.3	36.7	27.4	9.8	17.5	3248.0
HanM4(12(25)	10.2	20.0	38.2	2.0	110.0	45.4	44.6	23.2	13.7	5796.0
HanM4(12)(3)	8.8	19.0	33.4	2.0	108.7	47.5	32.5	16.4	14.0	3134.0
HanM4(12)(5)	7.8	18.5	28.7	1.3	102.0	40.4	36.5	15.8	13.2	3163.0
HanM4(17)(1W)	6.6	16.6	30.6	2.0	72.0	27.4	24.0	9.7	17.7	2136.0
HanM4(33)(HY42)	8.8	20.1	34.4	2.0	104.0	41.6	35.0	17.5	13.4	3640.0
HanM4(33)(HY43)	8.7	21.0	42.0	1.0	56.0	42.0	34.9	18.7	13.9	5194.0
HanM4(41)(2)	8.7	21.3	33.0	2.0	77.0	35.0	35.1	20.7	12.4	2863.0
HanM4(41)(HY30)	8.6	21.8	37.0	2.0	90.0	42.6	34.7	19.9	14.2	4035.0
HanM4(41)(HY31)	9.8	22.4	32.0	2.0	73.0	34.0	39.6	21.5	14.2	3551.0
HanM4(44)(2B)	8.4	21.9	32.2	2.0	68.0	28.8	36.3	15.9	16.7	3699.0
HanM4(52)(HY)	9.1	20.3	43.4	1.5	72.0	34.6	33.1	15.1	14.6	2685.0
Hansadua	7.7	16.3	62.0	2.0	107.0	46.0	27.7	16.1	13.8	3672.0
Lsd	0.6	0.1	0.5	0.6	2.2	0.6	0.2	0.5	0.6	1091.6
Se	0.3	0.1	0.3	0.4	1.3	0.3	0.1	0.3	0.4	647.8
%cv	4.0	0.4	0.8	18.9	1.5	0.9	0.4	1.8	2.5	18.0

Source: Field data, Lumorh (2021)

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4.5 Cluster Analysis

Using Ward's hierarchical technique based on the minimal variance linking method and Euclidean distance as the similarity measure, a clustering analysis was conducted (Figure 24).

Figure 24 showed a dendrogram of groups of mutants with genetic similarity. The similarities were observed among mean germination, number of pods from each plant, days to 50 percent flowering, total grain yield, standard flower colour, seed width and seed thickness. All mutant genotypes and controls were put into one cluster for quantitative, qualitative and combined quantitative and qualitative measured traits.

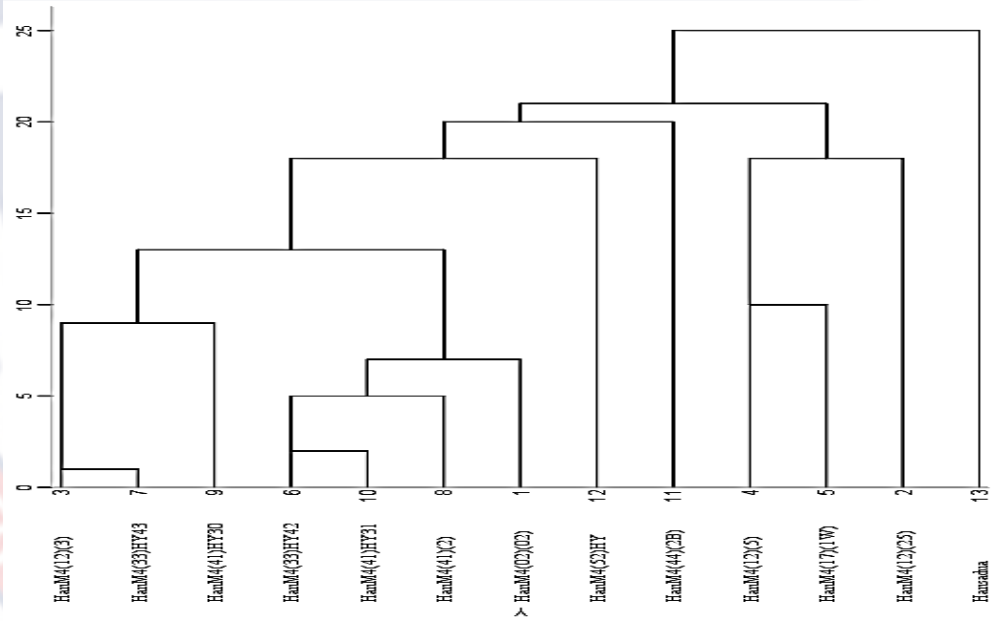

Figure 23: Dendrogram for qualitative traits

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4.6 Principal Component Analysis

Table 11 displays Varimax with Kaiser Normalization PCA results. For assessed traits, a Kaiser-Meyer-Olkin measure of sampling adequacy of 0.668 was found. Principal Component Analysis provides a roadmap for reducing a complex data set to a lower dimension to reveal the sometimes hidden, simplified dynamics that often underlie it. This could result in a smaller data set for cluster analysis. Six different principal components (PCs) were identified in this study based on Eigenvalues (>1) and factor loadings (0.3) that explained 90.3% of the total variance (Table 11). The first PC (PC 1) accounted for 40.3% of all observed changes. It can be explained by the weight of seeds in 10 dry pods, the weight of 10 dry pods, the weight of 100 seeds, the seed length, the seed width, the pod width, the seed thickness, the pod length, the total grain yield, the number of nodes on the main stem, the number of days until 50% of the pods are mature, and the number of seeds per pod (Table 11). The second principal component (PC 2) added 17.1% to the total variations observed through the number of pods/plant, number of peduncles/plant and number of main branches. On the other hand, principal component three (PC 3) accounted for 12.3% of the overall variance observed because of peduncle length and mean germination. The principal component four (PC 4) also contributed 9.4% to the total variation observed among the traits measured. This is accounted for by days to 50% flowering and days to first flowering (Table 11). Principal component five (PC 5) also added 6.5% to the total variation observed through vigour and weight of haulm of 10 dry pods. Principal component six (PC 6) represented 4.7% of the total variation observed for the measured trait.

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Table 11: Principal Component Analysis (PCA) of traits measured

Trait	PC 1	PC 2	PC 3	PC 4	PC5	PC6	Communalities
Weight of seeds of 10 dry pods	**0.986**	0.01	-0.078	0.004	0.065	0.043	0.985
Weight of 10 dry pods	**0.947**	0.108	-0.117	-0.126	0.206	0.034	0.982
Weight of 100 seeds	**0.939**	0.065	0.111	0.089	-0.065	0.091	0.919
Seed Length	**0.885**	0.071	0.112	0.048	0.047	0.385	0.953
Seed Width	**0.831**	0.346	-0.309	-0.058	0.074	0.163	0.941
Pod width	**0.806**	0.084	-0.006	0.213	0.461	-0.172	0.945
Seed Thickness	**0.789**	0.396	-0.176	-0.176	-0.159	0.171	0.896
Pod Length	**0.784**	-0.409	-0.217	0.09	0.162	0.163	0.89
Total grain Yield	**0.71**	0.165	0.412	0.07	0.041	-0.085	0.715
Number of nodes on main stem	**0.65**	0.453	-0.233	0.043	0.296	-0.276	0.848
Days to 50% pod mature	**0.644**	-0.121	0.451	0.141	0.399	0.149	0.834
Number of seeds/pods	**-0.609**	-0.421	-0.105	-0.115	0.132	-0.463	0.805
Number of pods/plants	0.088	**0.877**	0.18	-0.155	-0.067	-0.191	0.873
Number of peduncles/plants	0.27	**0.794**	0.367	0.269	-0.001	0.085	0.917
Number of main branches	0.118	**0.711**	-0.45	0.341	-0.204	0.158	0.905
Peduncle Length	-0.122	0.092	**0.925**	0.223	0.032	0.135	0.947
Mean germination	0.033	-0.095	**-0.922**	0.161	0.264	0.034	0.956
Days to 50% flowering	0.212	-0.051	-0.114	**0.931**	-0.017	-0.021	0.929
Days to first flowering	-0.01	0.176	0.482	**0.771**	-0.138	0.104	0.887
standard flower length	0.257	-0.277	0.078	-0.639	0.336	0.499	0.918
Vigor	0.094	-0.238	-0.154	-0.21	**0.847**	0.213	0.896
Weight of haulm of 10 dry pods	0.541	0.335	-0.178	-0.438	**0.576**	-0.057	0.964
Number of pods/peduncles	-0.145	0.046	-0.046	0.022	-0.123	**-0.903**	0.857
Eigen values	9.268	3.923	2.834	2.157	1.504	1.075	
Percentage of total variance	40.294	17.059	12.321	9.379	6.541	4.673	
Cumulative percentage of variance	40.294	57.353	69.674	79.053	85.593	90.266	

Source: Field data, Lumorh (2021)

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4.6.1 PCA loading plot of 13 genotypes compared on the bases of 23 variables

Figure 25 showed 10 out of the 13 cowpea genotypes are positively correlated with high magnitude. One genotype was negatively correlated with high magnitude and two were positively correlated with high magnitude. There was negative correlation between Hansadua and 10 other genotypes opposite. Most of the putative mutants were influenced by total grain yield. Hansadua was largely influenced by number of pods/plant and number of nodes on main stem. HanM$_4$(17) (1W) and HanM$_4$(02)(02) were influenced by the remaining 20 traits measured.

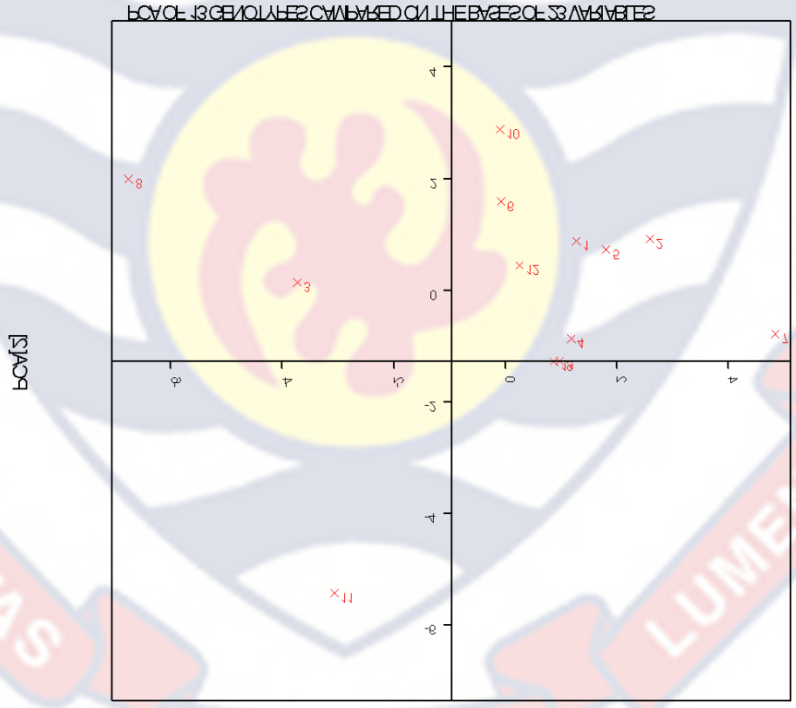

Figure 24: PCA loading plot of 13 genotypes on the bases of 23 variables

1=HanM4(41)(2), 2=HanM4(41)(HY31, 3=HanM4(02)(02), 4=HanM4(33)(HY43), 5=HanM4(12)(5), 6=HanM4(52)(HY), 7=HanM4(12)(25), 8=HanM4(17)(1W), 9=HanM4(33)(HY42), 10=HanM4(44)(2B), 11=Hansadua, 12=HanM4(12)(3), 13=HanM4(41)(HY30)

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4.7 Correlations among traits measured at M_4

There was a positive association of r = 0.61 between days to first flowering and number of pods per peduncle, with a significance level of 0.01 (**). (Table 12). At r = 0.74, there was a positive correlation between days to first flowering and days to 50% flowering. At the r = 0.61 correlation level, there was a positive association between the quantity of seeds per pod and days to 50% pod maturity. Positive correlations of r = 0.59, r = 0.41, and r = 0.78 exist between the number of nodes on the main stem and the number of major branches, the number of pods per plant, and the weight of 100 seeds, respectively. There was a high positive association of r = 0.80 between the number of main branches and the number of pods per plant and r = 0.47 between the weight of 100 seeds. Positive correlations of r = 0.63, r = 0.53, r = 0.59, and r = 0.41 were seen between the number of nodes on the main stem and the number of seeds per pod, peduncle length, number of main branches, and number of pods per plant, respectively. A correlation coefficient of r = 0.65 was observed between the conventional blossom length and seed width. There were observed correlation coefficients of r = 0.75, r = 0.80, r = 0.72, and r = 0.78 for pod width and pod length, 100-seed weight, peduncle length, and number of seeds per pod, respectively. Also discovered were correlation coefficients of r = 0.47, r = 0.74, and r = 0.69 between pod length and weight of 100 seeds, number of seeds per pod, and peduncle length, respectively. Positive correlations of r = 0.50 and r = 0.49 were observed between the number of pods per plant and the weight of 100 seeds and total grain production, respectively. r = 0.46 indicated a positive association between 100 seed weight and number of seeds per pod. At r = 0.59, a negative correlation

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was detected between the quantity of seeds per pod and the overall grain yield. r = 0.83 indicated a positive correlation between the quantity of seeds per pod and peduncle length. At r = 0.46, there was a considerable negative correlation between total grain yield and peduncle length.

At a correlation significance level of 0.05 (*), there was a positive association between days to first blooming, days to 50% mature pod, and number of pods per plant of r = 0.35 and r = 0.39, respectively. The relationship between days to 50% flowering and the quantity of pods per plant was r = 0.39. r = 0.38 indicates a positive association between the number of nodes on the main stem and the number of peduncles per plant. The relationship between the number of major branches and the number of peduncles per plant was r = 0.36. A correlation coefficient of 0.34 was established between the number of peduncles per plant and the weight of 100 seeds.

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Table 12: Correlations among traits measured during M_4 evaluation

	DFF	D50F	D50MP	NNMS	NMB	SFL	PW	PL	NPo/Pe	NPo/Pl	NPe/Pl	W100S	NS/Po	TGY	PeL
DFF															
D50F	0.74**														
D50MP	0.35*	0.27													
NNMS	0.17	0.3	0.38*												
NMB	0.56**	0.68**	0.22	0.59**											
SFL	-0.37*	-0.42**	0.13	-0.09	-0.50**										
PW	0.37*	0.49**	0.62**	0.79**	0.68**	0									
PL	0.12	0.45**	0.58**	0.53**	0.34*	0.14	0.75**								
NPo/Pe	0.61**	0.06	0.44**	-0.22	0.02	-0.06	0.03	-0.23							
NPo/Pl	0.39*	0.39*	0.09	0.41**	0.80**	-0.37*	0.59**	0.28	0.01						
NPe/Pl	0.15	-0.05	-0.09	0.38*	0.36*	-0.17	0.18	-0.27	0.19	0.27					
W100S	-0.05	0.05	0.44**	0.78**	0.47**	0.16	0.80**	0.47**	-0.2	0.50**	0.34*				
NS/Po	0.26	0.35*	0.61**	0.63**	0.37*	0.09	0.78**	0.74**	0.08	0.18	0.21	0.46**			
TGY	-0.09	-0.01	-0.19	-0.21	0.18	-0.2	-0.12	-0.18	-0.21	0.49**	-0.27	0.09	-0.59**		
PeL	0.31	0.34*	0.60**	0.53**	0.36*	-0.15	0.72**	0.69**	0.12	0.2	0.12	0.46**	0.83**	-0.46**	

Source: Field data, Lumorh (2021) * *Correlation is significant at the **0.05** level (2-tailed).** Correlation is significant at the **0.01** level (2-tailed). **DFF** = Days to first flowering, **D50F** = Days to 50% flowering, **D50MP** = Days to 50% pod mature, **NNMS** = Number of nodes on main stem, **NMB** =Number of main branches, **SFL** = Standard flower length, **PW** = Pod width, **PL**=Pod length, **NPo/Pe** = Number of pods/peduncle, **NPo**/Pl = Number of pods/plant, **NPe/Pl** = Number of peduncles/plants, **W100S** = Weight of 100 seeds, **NS/Po** = Number of seeds/pod, **TGY** = Total grain weight, **PeL** = Peduncle length*

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4.8 Disease incidence at M_4 evaluation

There was a deliberate effort from M_1 to M_4 generations to select promising mutant lines against any form of disease incidence to suit the objective of the study. Number of plants affected by any form of disease(s) were counted and recorded for each genotype and the percentages determined based on each plant population. The result showed HanM4(02)(02) had 1.4% bacteria blight and 0.7% sclerotium infection of its population (Table 13). HanM4(12)(25), HanM4(12)(5), HanM4(33)(HY43), HanM4(41)(2), HanM4(41)(30) and HanM4(41)(31) had 7.4%, 1.5%, 4.4%, 1.5%, 0.8% and 1.4% of lamb's tail pod rot respectively. Brown rust was identified with HanM4(17)(1W), HanM4(33)(HY43) and Hansadua of 21.7%, 1.8% and 4.6% respectively. Symptoms of cowpea golden mosaic disease was exhibited by HanM4(41)(30), HanM4(41)(31) and Hansadua of 7.2%, 10.6% and 6.2% respectively. Fusarium wilt was shown by HanM4(41)(31), HanM4(44)(2B) and HanM4(52)HY of percentages of 2.1, 0.8 and 0.8 respectively. Incidence of Sclerotium was also observed on HanM4(41)(30), HanM4(41)(31), HanM4(44)(2B) and Hansadua with percentages of 2.4, 3.5, 1.7 and 3.1 respectively. Mutant genotypes HanM4(12)(3) and HanM4(33)(42) did not show any symptoms of diseases at M_4 generation (Table 14).

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Table 13: Incidence of Diseases at M_4 generation

Genotype	Plants sown	Fusarium wilt	Cowpea golden mosaic	Bacteria blight	Brown rust	Lamb's tail pod rot	*Sclerotium*
HanM4(02)(02)	138	-	-	2 (1.4%)	-	-	1 (0.7%)
HanM4(12(25)	108	-	-	-	-	8 (7.4%)	-
HanM4(12)(3)	140	-	-	-	-	-	-
HanM4(12)(5)	136	-	-	-	-	2 (1.5%)	-
HanM4(17)(1W)	120	-	-	-	26(21.7%)	-	-
HanM4(33)(HY42)	104	-	-	-	-	-	-
HanM4(33)(HY43)	114	-	-	-	2 (1.8%)	5 (4.4%)	-
HanM4(41)(2)	136	-	-	-	-	2 (1.5%)	-
HanM4(41)(HY30)	125	-	9 (7.2%)	-	-	1(0.8%)	3 (2.4%)
HanM4(41)(HY31)	141	3 (2.1%)	15 (10.6%)	-	-	2 (1.4%)	5 (3.5%)
HanM4(44)(2B)	118	1 (0.8%)	-	-	-	-	2 (1.7%)
HanM4(52)(HY)	133	1 (0.8%)	-	-	-	-	-
Hansadua	65	-	4 (6.2%)	-	3 (4.6%)	-	2 (3.1%)

Source: Field data, Lumorh (2021)

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4.9 Proximate Components of cowpea genotypes

Results of analysis of proximate composition are given in Table 14. All proximate components were significantly dissimilar (P 0.001). The parent (Hansadua) was observed to be significantly different but similar to HanM4(41)(HY31) as having the lowest percentage ash content. HanM4(41)(HY31) was not significantly different from HanM4(12)(5), HanM4(41)(HY30), HanM4(12)(HY25), HanM4(41)(2) and HanM4(02)(02) with moderate as content but differs from HanM4(44)(2B), HanM4(52)HY, HanM4(33)(HY42), HanM4(33)(HY43), HanM4(12)(3) and HanM4(17)(1W), having the highest percentage ash content.

The energy level of the putative mutant HanM4(12)(3) was substantially lower than that of the other genotypes and showed a significant difference. HanM4(12)(5) was observed to be significantly different from the remaining genotypes with the highest energy level among the genotypes. Genotypes Hansadua, HanM4(41)(HY30), HanM4(17)(1W), HanM4(41)(HY31) and HanM4(02)(02) were found to be similar but different from HanM4(41)(2), HanM4(33)(HY42), HanM4(44)(2B), HanM4(52)HY and HanM4(12)(HY25) constituted the genotypes with moderate CHO content.

Genotypes HanM4(17)(1W) and HanM4(52)HY were similar but not significantly different from HanM4(12)(3) and Hansadua as the categories of cowpeas with lowest dry matter content. There were no significant differences observed for Hansadua, HanM4(02)(02), HanM4(44)(2B), HanM4(33)(HY43) as the genotypes with moderate dry matter content. Genotypes HanM4(12)(HY25), HanM4(41)(HY31), HanM4(12)(5), HanM4(41)(2),

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HanM4(41)(HY30) and HanM4(33)(HY42), even though were similar showed significant difference among from the rest of the genotypes as possessing the highest dry matter.

HanM4(12)(HY25) exhibited a high significant difference among the rest of the genotypes as having the highest fat/oil content. HanM4(41)(2), HanM4(33)(HY42) and HanM4(33)(HY43) were observed to be similar but significantly different from all the remaining genotypes and have fat/oil in moderate quantities. The remaining genotypes were similar but significantly different from HanM4(12)(HY25), HanM4(41)(2), HanM4(33)(HY42) and HanM4(33)(HY43) as having the lowest fat/oil content (Table 14).

There were also significant differences observed at $P<0.001$ for percentage fibre content. Genotype HanM4(44)(2B) was observed to be significantly different from all the remaining genotypes. It has the highest fibre percentage. HanM4(12)(HY25) and HanM4(17)(1W) were similar but significantly different from the other genotypes as they had a fibre percentage in moderate quantity. Genotypes, HanM4(41)(HY30), HanM4(52)HY and HanM4(41)(HY31) were very similar for fibre content but significantly different from the others. They contain the smallest amount of fibre.

HanM4(17)(1W) and HanM4(52)HY were similar for moisture content but significantly different from the rest as they had the highest moisture percentage. HanM4(12)(HY25), HanM4(41)(HY31), HanM4(12)(5) and HanM4(41)(2) were observed to be similar but significantly different from the remaining as having the smallest moisture content while the remaining seven were moderate. Percentage protein was equally observed to be significant at $P<0.001$. HanM4(12)(3) was observed to be significantly different from the

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rest with the highest crude protein content. HanM4(52)HY was highly significant from the remaining ones with the second highest for percentage protein. HanM4(12)(5) and HanM4(17)(1W) were observed to be similar but significantly different from the rest as having the lowest crude protein as the remaining genotypes were moderate (Table 14).

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Table 14: Proximate Composition of the putative mutants

Genotypes	% Ash	% Carbohydrate	% Dry Matter	% Oil/Fat	% Fibre	% Moisture	% Protein
HanM4(12)(HY25)	3.26	63.28	90.42	2.12	7.62	9.58	23.72
HanM4(33)(HY42)	3.45	66.11	90.13	1.53	6.61	9.87	22.3
HanM4(41)(HY31)	2.93	68.19	90.41	0.99	6.31	9.59	21.59
HanM4(41)(2)	3.30	66.48	90.34	1.55	7.07	9.66	21.6
Hansadua	2.76	68.71	89.58	0.99	7.04	10.42	20.5
HanM4(33)(HY43)	3.52	64.92	89.86	1.41	6.61	10.14	23.53
HanM4(44)(2B)	3.33	65.34	89.8	1.01	8.15	10.2	22.16
HanM4(52)HY	3.37	63.84	89.15	1.14	6.28	10.85	25.37
HanM4(12)(3)	3.54	58.27	89.27	1.13	6.52	10.73	30.53
HanM4(17)(1W)	3.56	68.34	89.15	1.04	7.52	10.85	19.54
HanM4(41)(HY30)	3.22	68.65	90.16	1.02	5.91	9.84	21.21
HanM4(02)(02)	3.31	67.54	89.62	1.12	6.57	10.38	21.46
HanM4(12)(5)	3.21	69.73	90.39	1.04	6.93	9.61	19.09
LSD	0.2225	0.4827	0.2496	0.1389	0.2565	0.2496	0.292
SE	0.1326	0.2876	0.1487	0.0827	0.1528	0.1487	0.174
%CV	4	0.4	0.2	6.7	2.2	1.5	0.8

Source: Field data, Lumorh (2021)

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4.9.1 Elemental analysis

Elemental analysis showed variation in nitrogen, calcium, sodium, potassium, magnesium and iron (Table 15). The percentage of nitrogen ranged from 3.05% (HanM4(12)(5)) to 4.88% (HanM4(12)(3)), calcium from 0.001% (HanM4(17)(1W)) to 0.008% (HanM4(HanM4(02)(02)), sodium from 0.22% (HanM4(12)(5)) to 1.7005% (HanM4(44)(2B)), potassium from 4.25% (HanM4(12)(5)) to 41.43% (HanM4(17)(1W)), magnesium from 0.002% (HanM4(44)(2B)) to 0.19% (HanM4(12)(3)) and iron from 0.06% (HanM4(12)(5)) to 0.20% (HanM4(52)HY) (Table 16).

Table 15: Elemental analysis of twelve putative cowpea mutants and Hansadua

Genotypes	%N	%Ca	%Na	%K	%Mg	%Fe
HanM4(12)(HY25)	3.80	0.007	0.35	6.67	0.022	0.10
HanM4(33)(HY42)	3.57	0.001	0.64	27.67	0.021	0.11
HanM4(41)(HY31)	3.45	0.003	0.54	15.63	0.021	0.18
HanM4(41)(2)	3.46	0.002	0.86	41.31	0.003	0.17
Hansadua	3.28	0.002	0.55	21.58	0.021	0.17
HanM4(33)(HY43)	3.76	0.003	0.61	24.44	0.022	0.13
HanM4(44)(2B)	3.55	0.003	1.70	41.25	0.002	0.18
HanM4(52)HY	4.06	0.004	0.53	13.50	0.021	0.20
HanM4(12)(3)	4.88	0.001	0.41	10.09	0.190	0.09
HanM4(17)(1W)	3.13	0.001	0.69	41.43	0.012	0.17
HanM4(41)(HY30)	3.39	0.002	0.62	27.50	0.021	0.11
HanM4(02)(02)	3.43	0.008	0.40	6.98	0.020	0.15
HanM4(12)(5)	3.05	0.004	0.22	4.25	0.020	0.06

Source: Field data, Lumorh (2021)

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CHAPTER FIVE

DISCUSSION

5.0 Introduction

The radio-sensitivity of plant genera, species, and, to a lesser extent, genotypes and cultivars varies. Genetic, physiological, morphological, and other biological elements, such as ontology, can explain these discrepancies. Together with environmental parameters such as oxygen and water content, these have a significant impact on the response of seeds and other plant propagules to ionizing radiation and chemical mutagens.

5.1 Seed multiplication

The single-seed genetic method was used to propagate representative seeds of each genotype to obtain homogeneous and pure strains for use in radiosensitivity tests and large-scale radiation of M_1 and also to have back-up in the event of total crop failure. The concept was developed by Goulden in 1941 to fast-track generations of inbred populations before starting to evaluate individual strains, and it is often used in conjunction with the pedigree method.

5.2 Radio-Sensitivity Test

Radio-sensitivity determines the relative reaction of organisms, organs, tissues, or cells to the damaging effects of ionizing radiation. Prominent among the methods for determining the effective dose is the determination of LD_{50} and RD_{50}. The LD_{50} is the dose of irradiated seeds that leads to a 50 percent death of the target population compared with parental control but the RD_{50} measures the dose of induced plants that results in a 50% reduction of

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growth or yield of the control plants. The curves for the determination of the LD_{50} and RD_{50} estimates were illustrated in Figures 3 to 7 and 8 respectively.

A radio-sensitivity testing was a prerequisite for mass irradiation of the actual samples to be mutated. It gives an accurate idea of the amount of gamma estimated dose to be applied to induce mutations in crops and other organisms. The study compared the responses of five cowpea genotypes to twelve doses of gamma radiation and the control (unirradiated) to establish the LD_{50} and RD_{50}. It was revealed through the study that the LD_{50} values of the Hansadua, WC-10, IT97K-819, WC-36 and ACC122WxWC-10 genotypes were achieved at 531.0 705.0, 762.0, 858.7 and 903.0 Gy, respectively (Table 2). The most sensitive genotype was Hansadua and the most tolerant was ACC122WxWC-10 since they had the least and highest estimated values. There was a negative relationship between the gamma dose rates and the responses of the cowpea genotypes for germination and other plant parts. The higher the dose rate, the more negative the effects on the genotypes for germination, height and other plant parts. Hansadua had the severest negative effect of gamma rays at the M_1 and consequently produced the largest number of putative mutants at M_2. Among the genotypes studied Hansadua had the lowest LD_{50} and RD_{50} values accounting for its level of highest sensitivity among the five genotypes which resulted in its susceptibility to mutation as it produced the highest number of macroscopic putative mutants. On the other hand, ACC122WxWC-10 had the highest values and therefore more resistant to mutagen mutation. Some studies have demonstrated that high doses of gamma radiation limit mitotic activity, whereas modest doses of gamma irradiation may be employed as safer and more stimulating instruments to

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improve variations. Mutagen mutation of crops can be verified physically (macroscopic types) or through molecular means (Khalil *et al*., 1986; Kumari & Singh 1996). Also, Roy (2016) in a review article reported that the low doses have stimulatory effects on plant growth.

Subsequently, RD_{50} values were achieved at 452.0, 591.0 590.5, 662.0 and 694.0 Gy for Hansadua, WC-10, IT97K-819, WC-36 and ACC122WxWC-10, respectively (Table 2). The emergence reduction curve showed a positive correlation between dose rates and seedling height. The higher the dose rate, the greater the reduction in plant height and other plant propagules which could be as a result of disruption of mitotic structures in the cowpea plants. Most seedlings could not survive a dose higher than 1000 Gy at 21 days after sowing, regardless of the five genotypes, because at this level there was stunted growth and high mortality was recorded and these could be because the mutagens disrupted the cell division and photosynthetic processes. Some genotypes showed some initial germination above 1000 Gy but died completely after three days of germination. This observation corroborates with a review by Roy (2016) which concluded that the higher the doses, the higher the inhibitory activities of the mutagen while lower ones were reported to be stimulatory for both angiosperms and gymnosperms. Higher dosage of gamma radiation reduced germination percentage, number of survival plants and plant height (Minisi *et al*., 2013). Marcu *et al*., (2013) also reported similar effects on application of gamma rays in mutagenesis.

It was evident from the studies that Hansadua was the first to respond to the minimum radiation dose and that ACC122WxWC-10 had a minor effect on plant height, implying that Hansadua had the lowest LD_{50} and RD_{50} values

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making it more vulnerable for mutagen destruction than the remaining genotypes used for the experiment (Table 2). The irradiation had mutagenic effects on seed characteristics such texture, coat colour and size. Olasupo *et al.*, (2016) reported that cowpea varieties with a rough seed surface and thin tests were found to be more radiosensitive to gamma irradiation. Hansadua has a relatively smooth seed surface and lighter seeds, which may explain its sensitivity to gamma rays. Therefore, the sensitivity of Hansadua to gamma rays could be due to its small size coupled with light testa compared with the rest of the genotypes used in the experiment. The remaining genotypes which were more resistant to gamma ray doses were relatively bigger seed sized with thicker and smooth testa compared with Hansadua which could have accounted for higher LD_{50} and RD_{50} values and invariably resulted in stimulatory effects for bushy or vegetative growth. They may be more resistant to mutation when exposed to mutagens. Olasupo *et al*., (2016) who in their study concluded that cowpea accessions with a smooth testa (seed coat) surface have recorded higher values of seedling growth (SG) and LD_{50} and seedling survival (SS) than the accessions with rough seed coat.

The germination rate for the control group (0 Gy) was determined to be 99.5%. The germination rate peaked at 300 Gy, 100.9% higher than the control and gradually decreased to 51.23% at 800 Gy as the gamma dose application increased. The increase in germination over the control could be caused by the stimulatory effects of gamma ray application up to a maximum of 300 Gy. Germination rate increased exponentially to 64.31% at 900 Gy and decreased to 23.94% at 1200 Gy (Figure 7). The increase in germination percentage from 51.23% at 800 Gy to 64.31% at 900 Gy could be due to

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reorganization of genes and hormones responsible for germination between 800 and 900 Gy, Majeed *et al*., (2018) reported of similar finding on cowpea mutagenesis. For cowpea, germination at 1200 Gy was possible but not survival and development. The response of cowpea genotypes to gamma dose was variable and individual radiation sensitivity testing of cowpea genotypes should be conducted prior to large-scale irradiation. The effect of gamma radiation on the mean germination rate of the five cowpea genotypes peaked at 300 Gy, suggesting that cowpea can mutate at 300 Gy. This observation is in agreement with Girija and Dhanavel (2009) that 300 Gy effectively produces the most putative mutants in cowpea, with the frequency of chlorina and xanthan mutants being higher than that of albino and viridis-type mutants.

Low doses of mutagen stimulate plant growth, a phenomenon called homesis while high doses retard plant growth (Table 3). A dose of 0-200 Gy of gamma radiation had a similar effect on germination, plant height, root length, shoot weight and whole plant weight. The mean germination had similar effect from 100 – 300 Gy, which could suggest that in simple germination test of cowpea using mutagens there may not be the need to apply beyond 300 Gy when using gamma rays. Between 200 – 300 Gy, root length, shoot weight and whole plant weight had similar reaction to gamma ray which implied these range could be enough to cause visible changes in these traits. The similarities in the influence of gamma rays on traits measured stretched until 600 - 700 Gy and became diversified beyond. Songsri *et al.,* 2011 reported a significant reduction in plant height in *Jatropha curcas* at 200 Gy and 400 Gy. Gamma rays below 100 Gy did not significantly alter root length, shoot weight or whole plant weight of cowpea. This research supported that

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different gamma radiation doses had different effects on various plant features such as germination, plant height, root length, shoot weight and whole plant weight. The gamma doses estimated from the experiment can be used as a general therapeutic dose to induce large-scale mutagenesis in cowpea.

All genotypes experienced a gradual decrease in seed germination rate, plant height, root length, shoot weight, and whole plant weight as the radiation dose increased. This could be attributed to disruptions in gibberellin activity, metabolic disturbances, and weakened and disrupted growth processes that are regulated during the initial growth period. As the fall in germination rate coincided with an increase in chromosomal abnormalities, this might possibly be explained by cell toxicity resulting to genetic or chromosomal alterations. Manju and Gopimoni (2009) reported that the decrease in plant survival was indicative of post-germination mortality due to irradiation-induced cytological and physiological abnormalities. Similar trials with rice varietals in Sierra Leone similarly revealed decreases in plant height and development (Harding et al., 2012). When the radiation dose was increased to 600 Gy, the scientists observed that the survival of sprouting plants in laboratory circumstances reduced dramatically over 8-14 days. According to Sparrow and Evans (1961), the reduction in ectoderm and hypocotyl length may be attributable to the breakdown of the plant growth hormone auxin as well as genetic loss as a result of chromosomal abnormalities caused by ionizing radiation. Mudibu et al. (2012) also reported that high doses of radiation treatments were harmful and resulted to unfavorable alterations, such as chromosomal abnormalities, mortality, damage, and sterility. Reduced germination rates, survival rates, plant development, and fertility, as well as an increased incidence of

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chromosomal aberrations and chlorophyll-deficient chimaeras, were used to quantify these abnormalities.

The relationship between the dose of gamma radiation and the germination rate of cowpea is inversely proportional. In this study it was observed that as the dose of gamma radiation increased, germination and survival rates decreased. This agreed with Bashir *et al*., (2013) who reported that germination and survival rates of fenugreek decreased with increasing mutagen dose/concentration. They concluded that lower mutagen treatments were less damaging to the organisms and suitable for inducing desirable mutations. Similar results were obtained when higher doses of gamma decreased germination and survival of *Moluccella laevis* (Minisi *et al.*,2013). Spencer-Lopes *et al.*, (2018) reported that irradiation levels used for mutant generation in crop improvement programmes should be within ±20% of the experimentally determined optimal dose. Furthermore, Mba *et al.*, (2010) and Owoseni *et al.*, (2007) revealed that in crop improvement programmes, irradiation levels for mutant generation should be within 5 units of the experimentally determined optimal dose. It is therefore incumbent upon breeders to consider other factors, such as the survival rate of M_1 plants producing viable seed at maturity, to estimate the range of doses that will produce useful putative mutants to determine the appropriate percentage units to apply.

5.3 Mass radiation and effects of gamma ray on cowpea genotypes

Observations from M_1 to M_3 showed that the putative mutants had dramatic morphological alterations compared to the controls. The changes observed were in leaf shape, flower colour, fresh pod colour, malformation, pod

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structure and vegetative structure, seed coat/eye colour and plant architecture. (Figure 8-12). The parent strain (Hansadua) has a globular leaf shape which has changed to several other shapes such as deltoid, hastate, oval, lanceolate and ovate (Figure 8). The change in leaf shapes and size in cowpea may be due to the application of gamma rays, which may alter the plant hormones, transcriptional regulators and mechanical properties of the tissues responsible for leaf initiation, growth, expansion, maturation and polarity. Tsukaya (2002) reported that events of leaf development have been categorized into three namely initiation of leaf primordium, the establishment of dorsiventrality, and the development of a marginal meristem. This result confirms that of Kumar *et al.*, (2009) who exposed dried healthy seeds of cowpea variety Co4 to EMS and observed viable large mutants in the M_2 generation such as dwarf mutants, spreading mutants, late mutants, early mutants, semi-sterile, mono- and trifoliolate mutants, basal parthenogenic, multifoliolate mutants, white-flowered mutants and chimeric mutants.

Morphological variation was also observed in flower colour. In the mutant genotypes there were pale pink, light pink and white keels, while Hansadua has white standards and white keels (Figure 9). Meaning the hormone florigen which was the first to cause change in flower colours was mutated to generate several colours observed.

There were also variations in the colour of the fresh pods, which are light green in Hansadua, while the putative mutant has dark green, pink and pink spots (Figure 16). Gamma radiation can cause changes in gibberellins and auxins at the apical part of cowpea plants that determine the colour of the pods. Rahimi and Bahrani (2011) concluded in a study on Effect of gamma

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irradiation on qualitative and quantitative characteristics of rapeseed (*Brassica napus* L.) flowers that gamma radiation can irradiate various plant components such as seeds, flowers, anthers, pollen grains and single cells. Gamma irradiation affects the growth of plants mainly through cytology, biochemistry, physiology and morphogenesis of the cells, causing changes in yield.

The deformities observed were in the leaves and plant structures. Some putative mutants had beige, white and yellow-coloured leaves and some leaves were also unusually large and not of the typical shape of cowpea capillaries. In some cases, the plant structure was completely altered, changing from semi-erect in the parents to prostrate and curled types (Figures 11 and 12). Some putative mutants with completely abnormal leaf colour and shape became sterile and failed to produce any pods after flowering. These changes occur because of the application of gamma rays, which can cause changes in hormones, leading to a myriad of phenotypic expressions observed. The results of this study confirm the findings of Nair and Mehta (2014) who concluded that gamma radiation can cause abnormal and structural changes in cowpea from M_1 to M_3 generations. Badr *et al.,* (2014) reported in a similar study that gamma radiation can cause cytological effects on growth and yield in M_1 and M_2 strains of cowpea varieties.

For the purposes of the study, no disease control measures were implemented. Selection in the field was made for diseased and abnormal plants, while high yielding, early maturing and other useful characteristics were considered. This was done to maintain a clean material for further disease assessment towards establishing a resistance or tolerant varieties. Some of the disease conditions observed on the field during trials from M_1 to M_4, were bacterial wilt, golden

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mosaic, lamb's tail pod rot, sclerotium and cercospora leaf spot, as shown in Figure 12. Mutagenesis can produce crop mutants that can be resistant/tolerant as well as susceptible to diseases. Gamma irradiation has been shown to interfere with plant regulators such as ethylene, jasmonic acid, and salicylic acid (SA), which play an important role in the regulation of plant immune responses. In addition, interference may also occur with other plant hormones such as auxin, abscisic acid (ABA), cytokinins, gibberellins, and brassinosteroids, which have been reported to contribute to plant immunity, leading to phenomena such as those observed during the experiment. This observation agrees with the report of Panstruga *et al*., (2009).

Some common pests of cowpea observed in field trials included cotton stainer, leaf miner, blister beetle and moth (Figure 13). Pollinators seen flying from one flower to another were beetles and butterflies (Figure 14.) Hordzi (2011) reported on same common insects and pollinators of cowpea, confirmed by this finding.

The application of gamma rays has also caused variations in flower size. The standard and keeled flowers differed in size and were in most cases larger than those of the parent. Experimental results confirmed that mutagens can change the size of cowpea flowers compared to parents (Figure 15). Meaning the florigen hormone which is responsible for flower initiation and development in plants may have been activated by the gamma rays. Gamma rays were able to induce changes in the structure of cowpea pods. In M_1 and M_2, some pods were very straight, coiled and curved, whereas the parents were only observed to have curved pods (Figure 16). In their study on "Genomic Regions, Cellular Components, and Gene Regulatory Basis

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Underlying Variation in Cowpea Pod Length," Xu et al. (2017) concluded that durable cell propagation rather than cell elongation or expansion is the main reason for longer pods, and transcriptome analysis suggests that regulation of pod length is mediated by sugar, the Transcriptome analysis also confirms the implication of sugar, gibberellins, and nutrient signalling in the regulation of pod length. Variations were also observed for seed coat colour and eye colour. The colour of the seed coat of the parents, which was creamy white, changed to black, blue-black, red, pink, mottled, creamy white and brown. Eye colour also changed from black to brown, pink, black and miscellaneous (Figure 17). In a similar review on morphological, agronomic and molecular characterization of irradiated cowpea using gamma rays, Ezzat *et al.,* (2019) observed morphological changes associated with changes in leaf shape, colour, fresh pod colour, flower colour, plant height and other. Similar results have been reported by Gaafar *et al.,* (2016) for the application of gamma radiation induction in cowpea.

From M_2 to M_4, there was an enhancement of survival rate of the mutant seeds as were higher than that of the parent. Germination and survival rates were generally higher than 90% in plant populations of all mutant genotypes (Table 4). This could be due to increased auxin levels and enhanced auxin synthesis, reduced chromosomal aberrations and increased assimilation mechanisms, in contrast to M_1. The results of this study confirm that of Kumari *et al.,* (2016) who reported on the effects of mutagenesis on germination, growth and fertility in sesame. Ariraman *et al.,* (2014) and Aparna *et al.,* (2013) reported similar results for pigeonpea and groundnut, respectively.

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This result indicated that six of the 12 putative mutants and controls had an intermediate growth habit, three semi-erect, one erect, one semi-prostrate and one acutely erect (Table 5). This may be due to a limitation or enhancement of the function of auxin, cytokinin, gibberellin and ethylene, which are primarily responsible for plant structure. Lu *et al*., (2015) reported similar results for rice.

Phytochrome pigmentation of stems, branches, petioles and pedicels was studied. For each genotype, different variations in the above traits were observed. Seven genotypes showed very weak stem pigmentation, four genotypes had moderate stem pigmentation and two genotypes had no stem pigmentation. Nine genotypes had no branch pigmentation, followed by three with very weak branch pigmentation and one with moderate branch pigmentation. Ten genotypes showed moderate, eight genotypes showed no pedicel pigmentation, four showed very weak pigmentation and one showed moderate pigmentation. The differences in pigmentation may be because gamma rays can affect chlorophyll, the main determining pigment in plants. Strid *et al*., (1990) stated that gamma radiation can destroy pigmentation with a concomitant loss of photosynthetic capacity.

The results also showed differences in the shape of the terminal leaf, with seven genotypes being sub-hastate and the remaining six being sub-globose. The results showed no pigmentation in the immature pods of 12 genotypes and only one genotype had pigmentation in the slits. These results confirm the findings of Olasupo *et al.*, (2016) on the mutagenic effects of gamma radiation on eight cowpea varieties. Kusmiyati *et al.*, (2018) had similar results on the mutagenic effects of gamma radiation on soybean

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(*Glycine max* L.) germination and seedlings. Ashraf *et al.,* (2004) applied to Basmati rice of the M_1 generation, similar results were obtained after gamma radiation.

The V-shaped markings on cowpea leaves may be a common feature of cowpea genotypes, as all putative mutants, including the parents, exhibit the same characteristics, even after the application of gamma rays. Gamma rays changed the colour of the leaves from dark green to intermediate green and light green. The texture of the leaves also changed from leathery to membranous. The colour of the flowers changed from the white of the parents to the pale pink and purple of the putative mutant genotype. These results are consistent with those of Adekola and Oluleye (2007) on the induction of genetic variation in cowpea by gamma irradiation. Setia *et al.,* (2020) and Dai and Magnusson (2012) reported the induction of new inflorescence traits and similar results for morphological changes in Buddleia induced by gamma irradiation.

The results showed that gamma radiation could cause changes in eye colour, seed coat colour, seed shape, pod wall thickness and pod-stem adhesion. All twelve putative mutant lines, including the parent, maintained the straw colour of dry pods. Eye colour changed from blue to black spots in the parents to tan, blue-black, brown splash or grey and miscellaneous colours. Seed coat colour varied from cream to miscellaneous and chocolate and seed shape varies from reni form to rhombic, spherical and ovoid. Pod wall thickness varied from intermediate to thick and thin and pod attachment to the inflorescence varied from erect to pendulous and 30-90°. Gaafar *et al.,* (2016) made similar findings in cowpea experiments on variation in seed coat colour,

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weight and eye shape in the M_2 mutant strain. Horn (2016) reported on the effects of gamma radiation on cowpea to improve yield and breeding for related traits.

Gamma rays can change the growth pattern of cowpea from determinate (parental) to non-determinate. In a similar study, Haleem (2012) reported changes in growth parameters due to application of gamma rays in cowpea in the experiment “Pre-exposure to gamma rays mitigates deleterious effects of salinity on cowpea plants". Changes in genetic composition due to gamma ray application altered the propensity for twinning in cowpea from none in the parents to slight and moderate in the mutant system. Gaafar *et al.*, (2016) made similar findings for changes in seed coat colour, weight and eye shape in the M_2 mutant strain and other changes in cowpea. There were similar changes in the degree of seed crowding from no crowding in the parents to semi-crowding in some mutant strains. It was observed that the putative mutant strains showing seed crowding had the smallest seed size. It appears that the degree of crowding of cowpea seeds within the pod is influenced by seed size, with the smaller the seed size, the greater the likelihood of crowding. This observation confirms the conclusion of Boukar *et al.*, (2015) that seeds tend to be kidney-shaped when they have sufficient space within the pod but when they become crowded, referred to as the 'crowded' type, the seeds gradually become spherical. No effect of gamma radiation on seed coat division was observed in either the putative mutants or the parents. The seed coat was firmly attached to the seeds in all putative mutants and parents.

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5.4 Evaluation of elite putative mutants at M_4

Positive and negative correlations were observed between measured traits (days to first flowering, 50% days to flowering, 50% days to pod maturity, number of main stem nodes, number of main branches, standard flower length, pod width, pod length, number of pods/inflorescence, number of pods/plant, number of inflorescences/plant, weight of 100 seeds, number of seeds/pod, total grain weight, inflorescence length). Meena *et al.*, (2015) conducted a study on cowpea to investigate the relationship between seed yield and its component traits and they concluded that there was a positive correlation between quantitative and qualitative traits of cowpea. Oladejo *et al.*, (2011) also made a similar finding where they found significant correlation between seed yield and other physiological traits in cowpea cultivars.

With an apparently high negative connection, cowpea germination rate did not lead to an increase in pod production per plant. Aliyu et al. (2016) validated this in a study on the phenotypic characterization of seed yield and yield components of cowpea. Uguru (1995) revealed similar findings about the genetic relationship and variability of yield and yield components of vegetable cowpea. This conclusion was supported by Ngalamu et al. (2012) and Malik et al. (2012), who found a strong positive association between the number of days to first flowering and the number of days to 50% flowering (2007). Malik et al. (2007) revealed similar findings in their examination of genetic variability, correlation, and route analysis of yield and its components in soybean. The correlation between the quantity of seeds per pod and the number of days to 50% pod maturity was strong. Ariyo et al. (1987) obtained

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comparable results from an okra study. In a study on the genetic diversity and route analysis of peanuts, Zaman et al. (2011) obtained a similar conclusion. Positive correlations were found between the number of nodes on the main stem, the number of major branches, the number of pods per plant, and the weight of 100 seeds. Srivastava and Singh (2012) came to comparable conclusions about mung beans. Nienhuis and Singh (1986) presented a comparable report for dry beans. There was a substantial link between the number of main branches, the number of pods per plant, and the weight of one hundred seeds. This study validates the findings of Sodavadiya et al. (2009) about the association and path analysis of seed yield and its components in pigeonpea. In a study examining the genetic diversity and interrelationships of a number of agronomic characteristics in chickpea, Malik et al. (2010) got comparable outcomes. A strong association was established between conventional flower length and seed width. This result is congruent with the conclusions reached by Akinyele and Osekita (2006) in their correlation and path coefficient analysis of seed yield characteristics in okra. There were significant positive connections between pod width and pod length, 100 seed weight, peduncle length, and seed length. This conclusion is consistent with Aliyu and Makinde's (2016) findings regarding cowpea seed yield and yield components. Mohammed et al. (2010) found comparable results. We noticed a positive correlation between pod length, hundred seed weight, number of seeds per pod, and peduncle length. There was a favorable correlation between the number of pods per plant and the weight of 100 seeds and overall output. There was a negative connection between overall grain yield and the quantity of seeds per pod. The overall cowpea yield was determined by the weight of

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all seeds gathered in each region, therefore an increase in the number of seeds per pod did not necessarily result in a higher yield. Additionally, there was a link between the quantity of seeds per pod and seed length. Malik et al. (2007) drew same conclusions in their investigation of genetic variability, correlation, and route analysis of soybean yield and its components.

At low correlations, there was a negative correlation between mean germination rate and days to first flowering. The life cycle of a crop depends on its genetic composition and environmental factors, not necessarily its viability, a view confirmed by Ghaderi *et al.,* (1984), who concluded that the relationship between genetic distance and heterosis for yield and morphological traits in dry edible beans and broad beans.

Low positive correlations were observed between the number of main stem nodes and the number of inflorescences per plant, the number of main branches and the number of inflorescences per plant, the number of pods per plant and seed width. These findings were confirmed in the studies of Ajay *et al.,* (2014) and Bisht *et al.,* (1998).

The results showed putative mutants had higher or lower vigour than the parents. Plant vigour may be influenced by the genotype and environment of the crop. Irradiation with gamma rays can induce growth stimulation of cowpea by changing the hormonal signalling network in plant cells or by increasing the anti-oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light and may result in vegetative growth. This result was confirmed by Olasupo *et al.,* (2016) in study on the mutagenic effects of gamma rays on eight cowpea varieties. Similar results were found by Aparna *et al.,* (2013) in a study on peanuts.

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The results show that the periods of first flowering, 50% flowering and 50% pod maturity are largely influenced by the unique genetic make-up of individual crops. Some genotypes took 4 days to go from first flowering to 50% flowering and 16 days to reach 50% pod maturity. This phenomenon varies from genotype to genotype, so it is necessary to study these traits for a particular genotype, especially if breeders intend to cross-pollinate. The study of these traits will also allow breeders to plan the planting season under rain-fed conditions to maximise yields. Under irrigated conditions, farmers can plan how to regulate water availability to reduce wastage. Owusu *et al.*, (2018) confirmed this result in a study on early maturity of some cowpea genotypes under rain-fed conditions in northern Ghana and concluded that selection criteria to improve maturity of cowpea should focus on days to first flowering, days to 50% flowering and days to first pod maturity. Pandey (2007) also reported that days to 50% flowering and maturity could be utilised in future breeding programmes to obtain desirable recombinant varieties with high yields and extra early maturity. It was also observed that genotypes with shorter days between days to first flowering and 50% flowering could be a determinate type since they often have homogenous maturity time. The indeterminate genotypes may have more days between first flowering, 50% flowering and 50% pod maturity. Genotypes with more days between days to 50% flowering and days to 50% pod maturity had larger seed sizes as can be observed for HanM4(12)(25).

In the selected putative mutant genotypes, the relationship between the weight of the seeds of the dry pods and the weight of the stalks of the dry pods differed. The heavier the seeds, the lighter corresponding stalks but in a ratio

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of 3-4:1. This means that, all things being equal, the weight of total seeds of a cowpea pod may be three to four times heavier than corresponding stalks but factors such as planting time, genotype, available temperature, rainfall patterns, spacing, seed rate and soil environment (availability of certain nutrients) can alter this ratio, depending on what is favourable for a particular mutant. This position is supported by Sutar *et al.*, (2019) in study where they found that the interaction of Jeevamrutha and panchagavya had a significant effect on improving grain yield of cowpea, in addition to yield attributes such as number of pods per plant, pod length, pod weight, number of seeds per pod, seed weight per plant and 100 seed weight. Kurubetta (2006) also confirmed that sowing time, spacing and seed rate had an effect on seed yield and fodder quality.

Advancement in the yield and quality of crops has been the primary objective of the crop breeder since the beginning of time. This is achieved through various means such as conventional, molecular and mutagenesis. Mutagenesis is known to modify or create new sets of genes in the existing genotypes through the process of breaking and recombination of the DNA exposed to mutagens.

Mutation induction in cowpea can either increase or decrease the yield from the parents. In the present study, the application of gamma radiation increased the yield potential of Hansadua (the parent) from 3.7Mt/ha to 5.8Mt/ha, an increase of 60%. On the other hand, under the same growth conditions, some putative mutants also showed a 60% yield reduction compared to the parents. The total grain yield recorded were $HanM_4(12)(25)$ = 5.8Mt/ha, $HanM_4(33)(43)$ = 5.2Mt/ha and $HanM_4(41)(30)$ = 4.0M4/ha, these

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putative mutants had yields higher than the control (Hansadua) which yielded 3.7Mt/ha. There is a relationship between weight of haulm, fodder and grain yield which determines the overall yield of cowpea. In most instances they are inversely related. The higher grain yield recorded for these three putative mutants could be because grain yields are higher than the other yield components. The grain yield to the haulm was in the ratio of 3-4: 1 for grain and haulm, respectively. A similar study was conducted by Justin *et al.*, (2012), in which they reported a 13% and 15% increase in grain yield for Vuangi and Kitoko respectively, a 70% increase in seeds per plant for Vuangi, a 15% increase for Kitoko and a 6% increase for TGX814-49D. Similar results were reported by Raina *et al.*, (2020) in study on the use of physiological, biochemical and molecular markers to induce high-yielding cowpea putative mutant lines. $HanM_4$(02)(02) = 3.3Mt/ha, $HanM_4$(12)(3) = 3.1Mt/ha, $HanM_4$(12)(5) = 3.2Mt/ha, $HanM_4$(33)(42) = 3.6Mt/ha, $HanM_4$(41)(HY31) = 3.6Mt/ha, $HanM_4$(44)(2B) = 3.7Mt/ha had grain yields close or similar to the parent. This study recorded grain yields far higher than the proposed estimated yield of 2.5Mt/ha achievable in Ghana (MoFA, 2016). Putative mutants with the minimum grain yield achieved were $HanM_4$(17)(1W) = 2.1Mt/ha, $HanM_4$(41)(2) = 2.9Mt/ha and $HanM_4$(52)(HY) = 2.7Mt/ha. In exception of $HanM_4$(17)(1W), all the putative mutant lines recorded higher grain yields than the estimated yield in Ghana with the outstanding grain yield of 5.8Mt/ha and 5.2Mt/ha for $HanM_4$(12)(25) and $HanM_4$(33)(43) respectively. The skewed selection for grain yield was deliberate to discover genetic materials for higher yields than the existing ones in Ghana to satisfy the goals and objectives of the study. This result is confirmed by Samireddypalle *et al.*,

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(2017) and Singh *et al*., (2003) in separate studies on yields of cowpea and other legumes which implies gamma rays are capable of inducing cowpea seeds for higher yield from the parents.

The results showed significant differences between the measured traits (P>0.001). The number of main stem nodes, number of main branches, standard flower length, seed length, seed width and seed thickness all were significantly different from the parent. The number and size of nodes, main branches and standard flower length were all increased due to the application of gamma rays (Table 9). The morpho-physiological changes in the putative mutant genotypes showed higher seed yield and robustness compared to the parents, or vice versa. In a similar study by Alghamd and Migdadi (2020) on faba beans, results showed various morphological changes occurred because of the application of gamma radiation. Similar conclusions were reached by Raina *et al.*, (2020) in a study using physiological, biochemical and molecular markers on induced high-yielding cowpea putative mutant lines.

Analysis showed that putative mutant genotypes and parents differed significantly (P<0.001) in pod width, pod length, inflorescence length, number of pods/peduncles, number of pods/plants, number of inflorescences/plant , weight of 10 dry pods, weight of 100 seeds and total grain yield. Gamma radiation can alter the morphology of cowpea plants to achieve desirable or undesirable characters. This result was confirmed in a study by Piri *et al.*, (2011) on the use of gamma rays in agriculture. The changes in the morphological features could be because gamma rays have modified the genes responsible for the production of hormones such as auxin, gibberellin, ethylene, cytokinin, kinase, that play key roles in the morphological features

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plants. El-Mashad and Mohamed (2012) and Doebley *et al*., (2006) confirmed that morphological variations observed in crops are brought about by differences in genetic, enzymatic and hormonal compositions.

The clustering analysis revealed a strong genetic linkage between the control and putative mutant lines. This linkage was observed for qualitative traits (Figure 23). Despite the genetic modifications observed in the putative mutants, there was still a high, moderate, and low level of association with the parents. There were closer relationships between the putative mutants and the parent than the others; accounting for generally one cluster for qualitative traits measured. However, Rangel *et al*., (2006) who study putative mutants and isolates of *Metarhizium anisopliae* found diverse relationships between conidial pigmentation and stress tolerance, and they concluded that there may be closer or remote relationships among putative mutants and respective parents due to random nature of mutagen effects on living cells. Mutagenesis does not necessarily change the entire genetic composition of the mutant versus the control but it does modify some aspects to produce the expected changes (Figure 24) and this might be the reason for the results obtained in this study. Similar findings were reported by Campbell and Sederoff (1996) and Hunter *et al.*, (2002) in studies.

Principal components 1-6 weighed cumulatively up to 90.3% variance with a Kaiser-Meyer-Olkin (KMO) measure of >0.5 and thus was sufficient to rely upon to explain most of the variations in induced plants were caused by the gamma radiation (Ho, 2006). The first principal component (PC 1) explained 40.3% of variation in terms of seed weight of 10 dry pods, weight of 10 dry pods, weight of 100 seeds, seed length, seed width, pod width, seed

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thickness, pod length, total grain yield, number of nodes on the main stem, number of days to maturity of 50% of pods and number of seeds/pod. This phenomenon could account for the higher yield of putative mutants and earliness for maturity (Table 9). Shimelis and Shiringani (2010) in study on cowpea confirmed this result in study on the components of variation and heritability of yield and agronomic traits in genotypes. In addition, Henshaw (2008) and Malik *et al.,* (2007) also concluded that seed weight was the most discriminating physical characteristic among the cowpea varieties studied and could be an important criterion for the selection of cowpea varieties.

The second principal component (PC 2) observed a 17.1% increase in total variation through number of pods/plant, number of inflorescences/plant and number of primary branches. Similar results were found by Gerrano *et al.,* (2019) in an agronomic evaluation and identification study of potential cowpea genotypes in South Africa. In addition, Manggoel *et al.,* (2012) had similar findings in study on genetic variability, correlation and path coefficient analysis of some yield components in 10 cowpeas.

On the other hand, principal component three (PC3) accounted for 12.3% of the total variation observed, as inflorescence length and mean germination rate. This result agrees with the findings of Brahmaiah *et al.,* (2014) on genetic variation in yield components and seed quality parameters in cowpea. Other studies by Hegde and Mishra (2009) have similar findings.

Principal component four (PC4) also contributed 9.4% to the overall variance observed in the traits tested. This could be explained by the number of days to 50% flowering and the number of days to first flowering. This result

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was confirmed by separate studies by Adetumbi *et al.*, (2019) and Owusu *et al.*, (2018).

Principal Component Five (PC5) also added 6.5% to the total variation observed through vigour and traction weight of the 10 dry pods. This finding is consistent with the findings of Ismail and Hall (1992) on the relationship between water use efficiency and carbon isotope differentiation in different cowpea genotypes and homozygous lines.

Principal Component Six (PC6) accounted for only 4.7% of the total variation in the traits measured. This was mainly determined by the number of pods/peduncle. The findings of Hall (2004) and Amoatey (1987) confirm the results of this study.

Cowpea putative mutants and parents showed some degree of tolerance and susceptibility to several common cowpea diseases observed on the field. The incidence of diseases at the M_4 generation were reported. Common diseases observed were bacterial blight, sclerotinia, lamb's tail pod rot, brown rust, Sclerotium and Fusarium wilt. Mutations in genes can produce mutants that may be tolerant to the diseases, while others are susceptible to infection, a result confirmed by Jain (2005) in his study of mutation-assisted plant breeding programmes.

Appiah *et al.*, (2011) reported in Asare *et al.*, (2013) that compositional differences in cowpea may be attributed to soil type, cultural practices, environmental and genetic factors. Since cowpea genotypes are cultivated under similar conditions, differences in proximate composition may be mainly genetic. The proximate composition of 13 different genotypes (parents and putative mutants) of cowpea was determined. Data collected

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included: ash%, CHO%, DM%, fat/oil%, fibre%, moisture% and protein content%. The results showed that there were significant differences in nutrient composition between the putative mutant and the parent ($p<0.001$). The ranges for the percentage proximate compositions of the mutants were ash (2.757 - 3.559%), CHO (68.71 - 69.73), DM (89.58 - 90.42), fat/lipid (0.998-2.122%), fibre (7.041 - 8.154), moisture (10.42 - 10.85%) and protein (20.5 - 30.53%). Compared to the parents, some nutrient components were also reduced in the putative mutants.

Crude protein ranged from 19.09 to 30.53%, with HanM$_4$(12)(3) having the highest protein content (30.53%) and was about 11.44% higher than the control. This result differs from that of Appiah *et al.*, (2011) who, in study of the physicochemical and functional properties of bean flour from three Ghanaian cowpea varieties (Nhyira, Tona and Adom), concluded that Nhyira had the highest crude protein content of 29%. In similar studies, Ajeigbe *et al.*, (2008), Fatokun *et al.*, (2000) and Mamiro *et al.*, (2011) also found that most of the IITA improved varieties had protein contents ranging from 20% to 27%. Similarly, the protein content of 12 West African and US cultivars ranged from 22 to 29%, with most accessions having protein content values between 22 and 24% (Hall *et al.*, 2003). However, in a study of 100 cowpea breeding lines collected by ICAR, seed protein content ranged from 23% to 32% of seed weight (Nielson *et al.*, 1993). Again, Adekola and Oluleye (2007) compared the crude protein of a cowpea genotype (IT84S 2246 D) with its putative mutant and concluded that the putative mutant showed the highest protein content (31.06 %).

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The ash content of the samples varied significantly ($P < 0.001$) between 2.93% and 3.559% (Table 14). There were significant differences between the putative mutant varieties and the parents. This finding is in line with Chinma *et al.*, (2011) who observed ash content between 2.72% and 3.73% in four cowpea varieties in Nigeria and Appiah *et al.*, (2011) who reported 2.95%-3.22% in three cowpea varieties in Ghana.

The fat percentage of samples of cowpea ranged from 0.987% to 2.122%. (Table 14). Except for HanM4(41(2), HanM4(33)(HY42, HanM4(33)(HY43, and HanM4(12)(25), there were no significant changes in fat content among the parents and the majority of putative mutant genotypes (P0.001). This research is comparable to that conducted by Chinma et al. (2008) for four Nigerian cowpea cultivars with fat contents ranging from 0.79 to 2.4%. Similarly, Masood and Batool's (2011) research on potential legume protein isolates from Pakistan revealed that cowpea had approximately 1.27 percent fat. Some of the putative mutants have a relatively high fat content and could be employed to enhance the palatability of foods (Appiah et al., 2011), with HanM4(41(2), HanM4(33)(HY42), HanM4(33)(HY43), and HanM4(12)(25) being favoured due to their high fat content.

The moisture levels of the samples varied between 9.58 and 10.85 percent. Moisture content was significantly different between putative cowpea mutants and their parents (P 0.001). There were also statistically significant differences (P 0.001) between the putative mutant lines. These findings align with those of Appiah et al. (2011), Chinma et al. (2008), and Animasaun et al. (2015), with moisture content values ranging from 9.15% to 9.83%, 9.25% to 10.07%, and 8.20 to 10.21, respectively. The variability in ranges of moisture

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content can be ascribed to soil type, cultural practices, environmental and genetic factors.

The sample fibre content ranged from 5.905% to 8.154%. (Table 14). The fibre content of the putative mutants was significantly different from that of the parents (P 0.001). This study found a higher fiber content than the ranges reported by Asare et al., (2013) and Chinma et al., (2008), but was comparable to the range reported by Gondwe et al., (2005). (2019). As proven by Appiah et al., these genotypes may be excellent sources of dietary fiber and particularly effective for adding bulk to food to treat constipation (2011).

The variation in carbohydrate content among cowpea genotypes was statistically significant (P 0.001), ranging from 58.27% to 69.73%. (Table 14). Additionally, there were substantial discrepancies between the putative mutant genotypes and the parents. This result contradicts the findings of Appiah et al. (2011) and Chinma et al. (2008), who found a range of 53.56% to 57.36% for four cowpea varieties in Ghana and Nigeria, respectively. Asare et al. (2013), Owolabi et al. (2012), and Animasaun et al. (2015) recorded ranges of 49.06–65.81%, 55.73-63.63%, and 56.10–59.59%, respectively. The variance in carbohydrate content may be attributable to hereditary factors. They may be an essential energy source for users (Appiah et al., 2011).

The dry matter composition of the samples varied between 89.15 and 90.42 percent (Table 14). There was a substantial difference between the putative mutant genotypes and the parents in terms of dry matter content (P 0.001). This result is similar with the findings of Appiah et al. (2011), who showed dry matter concentrations between 90.17 and 90.85 percent.

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The elemental analysis revealed that the proportions of elements changed between the putative mutants and progenitor of enhanced cowpea. The majority of the improved putative mutant cowpeas have higher elemental concentrations than the parent, resulting in greater nutritious concentrations. Nitrogen varied between 3.05 and 4.88, calcium between 0.00058 and 0.00779, sodium between 0.223 and 1.7005, potassium between 4.2455 and 41.43, magnesium between 0.002065 and 0.19045, and iron between 0.06 and 0.203. (Table 14). Mamiro et al(2011) .'s study entitled "Nutritional quality and usage of local and enhanced cowpea varieties in some regions of Tanzania" yielded comparable results.

Cowpeas that are rich in nitrogen, calcium, potassium, magnesium, and iron should be included in weaning meals for children and some adults, as they can enhance human and animal health and general inadequacies. Additionally, they can be employed as additions in the manufacturing of drugs and other dietary supplements.

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CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Mutagenesis of cowpea is one of the cheapest, fastest and most environmentally friendly means of breeding and transforming crops for sustainable development and food security. The yields of cowpeas in Ghana are generally low and susceptible to some common diseases. This experiment was set out to solve the problems of low yield, early maturing, resistance to cercospora, fusarium wilt, anthracnose and pythium diseases of cowpea. Since the narrow genetic base of cowpea is mainly due to its self-pollinating characteristic, there will be the need for innovative methods of increasing its genetic base to enhance the chances of selecting elite putative mutants. After irradiation and field assessment of the mutant generations, single seed decent was used to achieve genetic homogeneity of raw materials for the experiment. Determination of the treatment doses of gamma rays from ^{60}Co provided a benchmark for the large-scale irradiation to achieve acute and successful mutagenesis. This was done by estimating the LD_{50} or RD_{50} to determine values as markers for consideration as a prerequisite before embarking on mass irradiation of same. Cowpea genotypes used for the experiment responded differently to gamma ray doses applied.

Bulk selection was used as a means of reducing mutant population based on high yield, earliness and against any form of diseases on the field from M_1 to M_3.

The application of gamma rays has altered the flower colour, structure or growth habit, seed coat and eye colour of cowpeas and increased or decreased

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the yield of an already accepted cowpea variety. Germination and morphology diminish with increasing irradiation doses and low radiation doses encourage biotypic stimulatory effects. There were also deformities and sterility largely among mutants that have completely changed from the parents. The overall performance and behaviour of any organism is underpinned by the interaction of genetics, phenotype and environment, so that any manipulation of these factors will induce the organism to behave differently in time or permanently. The results further support the findings that mutagens can produce putative mutants that are useful and enhanced than the parents and some can equally be undesirable.

The yield of the putative mutant increased from 3.5 to 5.8 t/ha, an increase of more than 60% compared to the parents. HanM4(12)(25) was the highest yielding putative mutant. The application of gamma-irradiation has the ability to activate hormones responsible for nutritional components of cowpea. For example, compared to the control, the ash content of HanM4(17)(1W) increased from 2.757-3.559, the CHO content of HanM4(12)(5) was 68.71-69.73, the DM content of HanM4(12)(25) was 89.58-90.42 and the fat/oil content was 0. 998-2.122 and the fat/oil content of HanM4(44)(2B) had a fibre percentage of 7.041-8.154, HanM4(52) HY and HanM4(17)(1W) had a moisture percentage of 10.42-10.85 and HanM4(12)(3) had a protein percentage of 20.5-30.53. Most of the putative mutants outperformed the control in terms of early maturity, yield and nutritional quality.

6.2 Conclusions

The results from the study revealed cowpea genotypes responded differently to gamma ray doses. Several changes were observed for flower

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colour, size, earliness to maturity, plant pigmentation, seed coat and eye colour, structure and seed sizes from the parents due to gamma rays application. Four (ACC122WxWC-10, WC-36, WC-10 and IT97K-819) out of the five parents failed to generate macroscopic and useful mutants even though the estimated gamma ray doses were applied. The modification of hansadua resulted in yield increases from 3.5t/ha to 5.8t/ha of parent to mutants in the experimental field. Proximate and elemental analyses proved enhancement in nutritional contents of the improved putative mutant genotypes as well. Early genotypes were developed and were tolerant to most diseases at a single location. The study confirmed the results of other works done on crops by using gamma rays to induce crops to produce useful and undesirable mutants.

6.3 Recommendations

The mutagenic knowledge and experience gained in this study can be applied to the improvement of any crop in Ghana for high yield, early maturity, resistance/tolerance to biotic and abiotic factors and any other traits of interest.

The government and the Ministry of Agriculture must work hand in hand with mutagen specialists to produce cowpea varieties of interest to meet consumer preferences and adapt to changing soil and climatic conditions.

All other research funding organisations should use mutagenesis expertise to address the food security crisis in Ghana and other regions of the world where cowpea is consumed.

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6.4 Suggestions for further research

1. Multi-site studies in various agro-ecological zones in Ghana to determine the interaction of the genotypes of the hypothetical mutants developed with the environment.
2. Molecular analysis should be carried out to find out the extent of genetic alterations that might have occurred because of the application of gamma 222irradiation.
3. Greenhouse and field disease assessments should be carried out to determine the tolerance/resistance or susceptibility of putative mutants to cowpea diseases in Ghana.
4. Consumer preference tests should be conducted to determine the acceptability of the putative mutant.
5. Cookability tests should be conducted to determine the cooking time of putative mutant varieties.

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APPENDICES

Appendix 1: Soil characteristics of the field taken at varied depths

Soil Depth (cm)	Parameter	Value
20	pH	5.85
	Electrical Conductivity	1285.2 µS/cm
	Potassium	85.11 mg/kg
	Phosphorus	60.9 mg/kg
	Organic carbon	4.10 %
	Organic matter	7.08 %
40	pH	5.95
	Electrical Conductivity	916.2 µS/cm
	Potassium	89.4 mg/kg
	Phosphorus	64.0 mg/kg
	Organic carbon	3.31 %
	Organic matter	5.70 %

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Appendix 2: Weather Data

	DATE	Rainfall (mm)	Temperature (°C)
M_1	Aug-18	31.1	23.7
	Sep-18	103.1	24.4
	Oct-18	194.5	25.2
	Nov-18	122.1	25.4
M_2	May 19	241.6	26.2
	Jun-19	83.6	25.1
	Jul-19	30.2	23.1
	Aug-19	24.7	24.2
M_3	May 20	223.9	27.5
	Jun-20	100.4	24.1
	Jul-20	41.4	22.2
	Aug-20	20.9	24.5
M_4	Sep-20	31.1	25.7
	Oct-20	103.1	23.6
	Nov-20	194.5	26.1
	Dec-20	122.1	26.4

Appendix 3: Summary ANOVA for measured traits on Hansadua mutants

Source of variation	*df*	Mean germination	Vigor	Days to first flowering	Days to 50% flowering	Days to 50% pod mature
		Mean sum of squares				
Genotypes	12	147.53***	28.19***	6.69***	32.86***	37.19***
Residual	24	13.74	4.663	0.141	0.5897	0.141

df = degree of freedom, *** shows significant differences at P<0.001

Appendix 3 continued

Source of variation	*df*	Weight of 10 dry pods	Weight of seeds of 10 dry pods	Weight of hauls of 10 dry pods	Weight of 100 seeds	Total grain Yield
		Mean sum of squares				
Genotypes	12	86.25811***	56.1164***	6.507823***	49.20685***	2907846***
Residual	24	0.01796	0.1095	0.0063	0.09608	419638

df = degree of freedom, *** shows significant differences at P<0.00

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Appendix 3 continued

Source of variation	*df*	No. of nodes on main stem	Main branches	Standard flower length	Seed length	Seed width	Seed thickness
		Mean sum of squares					
Genotypes	12	10.1773***	1.565***	2.4718***	2.55598***	0.72558***	6.13714***
Residual	24	0.141	0.141	0.1009	0.04533	0.01709	0.04358

df = degree of freedom, *** shows significant differences at P<0.001

Appendix 3 continued

Source of variation	*df*	Pod width	Pod Length	Peduncle Length	Number of pods/peduncles	Number of pods/plants	Number of peduncles per plant	Weight of 10 dry pods	Weight of 100 seeds	Number of seeds/pods
		Mean sum of squares								
Genotypes	12	2.5141***	14.370781***	222.87731***	0.0386***	1009.286***	123.9209***	56.1164***	49.20685***	8.3079***
Residual	24	0.1161	0.006842	0.0816	0.1239	1.722	0.1191	0.1095	0.09608	0.134

df = degree of freedom, *** shows significant differences at P<0.001

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