MORPHOLOGICAL AND CYTOGENETIC STUDIES ON PIGEON PEA [*CAJANUS CAJAN* (L.) MILLSPAUGH] TREATED WITH SODIUM AZIDE AND GAMMA RADIATION

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BY

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DEPARTMENT OF BIOLOGICAL SCIENCES,
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OCTOBER, 2014.

DEC RATION

I declare that the work in this thesis entitled **"Morphological and Cytogenetic Studies on Pigeon Pea [*Cajanus cajan* (L.) Millspaugh] Treated with Sodium Azide and Gamma Radiation"** was performed by me in the Department of Biological Sciences under the supervision of Dr. M. A. Adelanwa and Prof S. O. Alonge. The information derived from literature has been duly acknowledged in the text and list of references provided. No part of this work has been presented for another Diploma or Degree at any institution.

Aledare Bolaji MATHEW _____ _____

Signature Date

CERTIFICATION

This thesis entitled **"Morphological and Cytogenetic Studies on Pigeon Pea [*Cajanus cajan* (L.) Millspaugh] Treated with Sodium Azide and Gamma Radiation"** written by **Aledare Bolaji MATHEW** meets the regulations governing the award of the degree of Master of Science (M.Sc) of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my mother Late Mrs. M. F. Aledare

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ABSTRACT

The aim of this study was to determine the morphological and cytogenetic responses of pigeon pea *Cajnus cajan* (L.) Millspaugh to treatments with sodium azide and gamma radiation. This was performed by exposing the seeds of landraces pigeon pea to gamma rays at Centre for Radiotherapy and Oncology Department, ABUTH, Zaria at doses of 0(control), 50, 100, 150 and 200Gy. These seeds were further treated with sodium azide (NaN_3) concentrations at 0.00, 0.01, 0.02, 0.03 and 0.04% SA, giving a total of 25 treatments. The growth parameters were recorded at 4, 8, 12, 16 and 20 Weeks After Planting (WAP). The parameters measured include germination percentage, Leaf and branch number, plant height, root length and the yield parameters which include days to 50% flowering, number of seeds per pod, number of pods per plants, pod weight, 100-seed weight, total grain yield and crude protein percentage. The result of these treatments showed a symmetric reduction in germination percentage with respect to most of the mutagenic treatments. There was a higher leaf number with those that received 100Gy + 0.03% SA (41.93 leaves). Also, the branch number of treated plant showed an increase over the R0A0 (control) treatment; Similarly, the mean comparison of the plant height presented showed that 150Gy + 0.02% SA (190.93 cm), produced the highest plant height and the highest root length due to 100Gy + 0.01% SA (43.13 cm), was significantly higher than those of other treatments. The data on the days to 50% flowering showed that all the mutagenic treatments elicited shorter days to flowering. The mutagenic treatments, 0Gy + 0.03% SA, 50Gy + 0.02% SA and 100Gy + 0.02% SA resulted in the highest number of seeds per pod of 9 seeds. The highest number of pods and pod weight per plant, observed

with 100Gy + 0.01%SA, was significantly higher than those from other treatments. Also, the highest 100 – seed weight, observed at 150Gy + 0.00% SA (15.6 g) and 150Gy + 0.03%SA (15.6 g), were similar to that due to R0A3, R1A0, R1A1, R1A4, R2A0, R2A4 and R4A1 treatments. High total grain yield (TGY) was observed in all the mutagenic treatments. Similarly, the highest crude protein (CP) percentage value was obtained from 150Gy + 0.03%SA (25.1 %), followed by that due to 100Gy + 0.01%SA (24.7 %), 100Gy + 0.04%SA (24.4 %), 100Gy + 0.03%SA (23.7 %) and 150Gy + 0.00%SA (23.6 %). Also, the meiotic stages showed some abnormalities, ranging from stickiness to bridged chromosomes, which results in polyploidy mutants with characteristic advantages over a diploid plant, like earliness in flowering and maturity amongst other traits. A positive correlation was observed between seed yield and many other quantitative traits like number of pods per plants, mean number of seeds per pod, and protein content. It is, therefore, concluded that, the two mutagens affected the pigeon pea plant population morphologically as prominent Tall, High yield, Early maturing and beneficial yield components were observed by exposing pigeon pea to single and combined treatments of gamma ray (50Gy – 150Gy) and Sodium azide (0.01% - 0.04%).

TABLE OF CONTENTS

Contents **Page**

Cover Page.....i

Fly Leaf.....ii

Title Page.....iii

Declaration.....iv

Certification.....v

Dedication.....vi

Acknowledgement.....vii

Abstract.....viii

Table of Contents.....x

List of Tables.....xiv

List of Plates.....xv

CHAPTER ONE

1.0 INTRODUCTION.....1

1.1 Chemical Composition and Uses.....2

1.2 Pigeon Pea Production.....3

1.3 Statement of the Problem.....6

1.4 Justification of the Study.....7

1.5 Aim of the Study.....7

1.6 Objectives of the Study.....7

1.7 Research Hypotheses.....8

CHAPTER TWO

2.0 LITERATURE REVIEW.....9

2.1 Mutagenesis as a Means of Crop Improvement.....9

2.2 Mutagen Types.....10

2.2.1 Physical mutagen.....11

2.2.2 Chemical mutagen.....13

2.2.3 Combined mutagenic treatments.....13

2.3 Effect of Mutagens on Biological Parameters.....15

2.4 Induction of Cytological Abnormalities.....15

2.5 Mutations Affecting Morphology.....19

2.6 Mutagenic Effectiveness and Efficiency.....21

2.7 Induced Variability in Quantitative Traits.....23

2.8 Economic Impact of Mutation Breeding.....26

CHAPTER THREE

3.0 MATERIALS AND METHODS.....28

3.1 Source of Research Materials 28

3.2 Study Area.....28

3.3 Treatments.....29

3.3.1 Seeds radiated with gamma rays.....29

3.3.2 Preparation of sodium azide and buffer solution 29

3.3.3 Seeds treated with sodium azide.....30

3.4 Collection of Soil Samples.....30

3.5 Pot Preparation and Seed Planting.....30

3.6 Cytogenetic Studies.....30

3.6.1 Meiosis.....30

3.7 Data Collection.....33

3.7.1 Growth parameters.....33

3.7.2 Yield parameters.....34

3.8 Statistical Analysis.....37

CHAPTER FOUR

4.0 RESULTS.....39

4.1 Observation on Morphological Traits.....39

4.2 Germination Percentage.....39

4.3 Number of Leaves Per Plant.....41

4.4 Number of Branches Per Plant.....43

4.5 Plant Height.....45

4.6 Root Length.....48

4.7 Yield Parameter.....50

4.7.1 Days to 50% of flowering.....50

4.7.2 Number of seeds per pod.....50

4.7.3 Number of pods per plant 52

4.7.4 Pod weight.....52

4.7.5 Hundred seed weight.....52

4.7.6 Total grain yield.....53

4.7.7 Determination of crude protein.....53

4.8 Correlation Analyses.....54

4.9 Regression Coefficient Analysis.....58

4.10 Observation of Meiotic Division.....61

CHAPTER FIVE

5.0 DISCUSSION.....65

5.1 Discussion.....65

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION.....73

6.1 Summary.....73

6.2 Conclusions.....74

6.3 Recommendations.....75

REFERENCES.....76

APPENDIX.....90

LIST OF TABLES

Table 3.1 Details of Mutagenic Treatment.....31

Table 3.2 Treatments Illustration.....32

Table 4.1 Effect of Gamma and Sodium Azide on Germination Percentage of Pigeon Pea.....40

Table 4.2 Effect of Gamma Rays and Sodium Azide on Leaf Number of Pigeon Pea at Growth Stages.....42

Table 4.3 Effect of Gamma Rays and Sodium Azide on Branch Number of Pigeon Pea at Growth Stages.....44

Table 4.4 Effect of Gamma Rays and Sodium Azide on Plant Height (Cm) of Pigeon Pea at Growth Stages.....46

Table 4.5 Effect of Gamma Rays and Sodium Azide on Root Length (Cm) of Pigeon Pea at Growth Stages.....49

Table 4.6 Effect of Gamma Rays and Sodium Azide on Some Yield Parameters of Pigeon Pea at Growth Stages.....51

Table 4.7 Correlation Analyses of some Growth and Yield Components of Pigeon pea.....55

Table 4.8a Summary of The Regression Estimates for The Two Factors Contribution to The Plant Yield.....59

Table 4.8b Summary of The Regression Estimates for The Two Factors Contribution to The Crude Protein Percentage.....60

LIST OF PLATES

Plate I: Pollen Mother Cells showing normal meiosis (control) 62

Plate II: Chromosomal Abnormalities Induced By Gamma Rays and Sodium Azide.....63

Plate III: Chromosomal Abnormalities Induced By Gamma Rays and Sodium Azide.....64

Plate IV: Pigeon pea Plant on the field at 8 Week After Planting (WAP) 90

LIST OF TABLES

Table 3.1 Details of Mutagenic Treatment.....31

Table 3.2 Treatments Illustration.....32

Table 4.1 Effect of Gamma and Sodium Azide on Germination Percentage of Pigeon Pea.....40

Table 4.2 Effect of Gamma Rays and Sodium Azide on Leaf Number of Pigeon Pea at Growth Stages.....43

Table 4.3 Effect of Gamma Rays and Sodium Azide on Branch Number of Pigeon Pea at Growth Stages.....45

Table 4.4 Effect of Gamma Rays and Sodium Azide on Plant Height (Cm) of Pigeon Pea at Growth Stages.....47

Table 4.5 Effect of Gamma Rays and Sodium Azide on Root Length (Cm) of Pigeon Pea at Growth Stages.....49

Table 4.6 Effect of Gamma Rays and Sodium Azide on Some Yield Parameters of Pigeon Pea at Growth Stages.....53

Table 4.7 Correlation Analyses of some Growth and Yield Components of Pigeon pea.....57

Table 4.8a Summary of The Regression Estimates for The Two Factors Contribution to The Plant Yield.....59

Table 4.8b Summary of The Regression Estimates for The Two Factors Contribution to The Crude Protein Percentage.....60

LIST OF PLATES

Plate I: Pollen Mother Cells showing normal meiosis (control) 62

Plate II: Chromosomal Abnormalities Induced By Gamma Rays and Sodium Azide.....62

Plate III: Chromosomal Abnormalities Induced By Gamma Rays and Sodium Azide.....63

Plate IV: Pigeon pea Plant on the field at 8 Week After Planting (WAP) 89

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

Pigeon pea belongs to Family Fabaceae, sub family Papilionaceae, tribe Phaseoleae, sub-tribe Cajanae, genus *Cajanus*, species *cajan* (L.) Millspaugh, which also contain soybean (*Glycine max* (L) and Field bean *(Phaseolus vulgaris* (L), an often cross-pollinated diploid 2n = 2x = 22, 44 or 66 chromosomes (Varshney *et al*., 2012).

Pigeon pea originated in India, where the largest diversity exists and is one of the major grain legume (pulse) crops cultivated in many countries in the Tropics and subtropics. Grain legumes are cherished for their opulence in protein which makes them indispensable along with cereals in daily human diet. Pigeon pea due, to their high genetic potential to thrive well under varied environmental conditions, capacity of soil fertility restoration and soil ameliorative properties, have become the most important component of sustainable agriculture and an important crop in the semi-arid areas (Van der Maesen, 1990).

Pigeon pea is an erect perennial legume shrub often grown as an annual, reaching 91-366cm in height. Pigeon pea is heat-tolerant, prefers hot moist conditions. It grows in temperature between 18°C and 30°C and the optimum rainfall required for pigeon pea is 600-1000 mm/year. A rain at flowering time has very adverse effect on the seed yield (Edwards, 1981).

Pigeon pea does well in low fertility soils with reasonable water-holding capacity and pH 5-7 is favourable for its growth. Pigeon pea does not tolerate shallow soils or water logging. It tolerates a wide range of soils, from sandy to heavy black clays. It is sensitive to salt spray and high salinity. It is a short day plant, flowering is triggered by short days, whilst with long days plant grows vegetatively and the leaves have three leaflets that are green and pubescent above and silvery greyish – green with longer hairs on the underside. The flowers are yellow with red to reddish-brown lines or red outside (Centre for New Crops and Plants Products, 2002).

1.1 Chemical Composition and Uses

Pigeon pea improves the physical, chemical, and biological properties of the soil; it functions as a mini-nitrogen factory (Okaka *et al*., 2002). The plant is rich in protein and fats, making it an ideal supplement to traditional cereal, banana or tuber-based diets of most Africans which are generally protein deficient. Protein content of commonly grown plant ranges between 18 – 26% (John, 2005). The seed is made up of 85% cotyledon, 14% seed coat, and about 1% embryo and it is a rich source of carbohydrates, minerals and vitamins. It CHO content ranges between of 51.4 – 58.8%, CF ranges between 1.2 – 8.1% and lipid ranges between 0.6 – 3.8% (Singh *et al*., 1990). The plant seed is also a good source of dietary minerals such as Calcium, Phosphorus, Magnesium, Iron, Sulphur and Potassium and water soluble vitamins especially thiamine, riboflavin, niacin. It contains more minerals, ten times more fats, five times more vitamin A and three times more vitamin C than cereal grains. It also provides firewood and income for poor small scale farmers (Saxena, 2000).

1.2 Pigeon Pea Production

Globally, Pigeon pea is cultivated on 4.79 million hectares in 22 countries, but with only a few major producers. India (3.58 million hectares) and Nepal (20,703 hectares) are important pigeon pea producing countries (FAOSTAT, 2008). The Caribbean islands and some South American countries also have reasonable areas under pigeon pea cultivation. India has the pride of being the world's principal grower and producer of pigeon pea, (accounting for three quarters of the world's pigeon pea production), followed by Myanmar, whose production forms about 560,000 hectares, accounted for about 15% of the global production. Malawi's production from 123,000 hectares accounted for 2.6%, while Kenya (196,261 hectares), Uganda (86,000 hectares), Mozambique (85,000 hectares), and Tanzania (68,000hectares) contributed considerable amounts up to 2.5%, 2%, 1.5% and 0.6% respectively of the world's pigeon pea production (FAOSTAT, 2008).

However, cultivation of this plant has also been reported in Nigeria (Aiyeloja and Bello, 2006), Niger, Mali and Benin (Versteeg and Koudokpon, 1993), Ethiopia, Zimbabwe (Kamanga and Shamudzarira, 2001), Zambia (Boehringer and Caldwell, 1989), Botswana (Amarteifo *et al*., 2002), and South Africa (Swart *et al*., 2000). Production trends seem to be increasing since the turn of the Century. The increase in production is largely a result of area expansion rather than increase in yields (Jones *et al*., 2002). Drought poses one of the most important environmental constraints to plant survival and productivity (and hence food security) in the Tropics (Speranza *et al*., 2007). Pigeon pea remains one of the most drought- tolerant legumes (Valenzuela and Smith, 2002).

In Africa, Pigeon pea generally produces low yield, due to limiting effects of biotic and a biotic factors. The abiotic factors include water logging, extremes of temperature, injury and salinity. The biotic factors are insect pests (pod borers) and pod fly and diseases (e.g. *Fusarium* wilt). Lack of quality seeds and low use of improved varieties have left the poor farmers with no option but to grow local landraces that are low yielding and late maturing (Mergeai *et al*., 2001).

The major constraint in the development of improved varieties is the limited genetic variability among existing plant genotypes (Irfag and Nawab, 2003). Mutation, which is described as an error during deoxyribonucleic acid (DNA) replication, that results in a change in the sequence of deoxyribonucleic bases, this is the bases in the DNA are the bases of plant evolution, can either be spontaneous or induced (Kaizer, 2001). The naturally occurring mutation is effective but its rate is too slow to collect naturally originating mutant significantly. This poses a lot of difficulty to natural breeders of crop plants, thus making the use of mutagenic agents to induce genetic variation pertinent (Adamu, 1997). Mutagens may be physical or chemical. The first of these to be discovered and employed as a mutagenic agent was the physical mutagens (ionizing radiation). The research work that put mutation research on a firm bases were the pioneer works of Muller (1927) on *Drosophila*, who devised objective, efficient, and quantitative techniques for its treatment. Later, Stadler (1928) worked on crop plants; this effort opened a new chapter into the field of plant breeding that can supplement the existing germplasm. Initially, ionizing radiation showed great potential for inducing beneficial genetic changes but it was

soon discovered that, such changes were structural and in chromosome distribution only. After the discovery of radiation as a mutagenic agent it was not until after four decades before chemical mutagenesis was discovered. A pioneer in this work was Charlotte Auerbach who discovered the mutagenic action of mustard gas and Nitrogen on *Drosophila* during the course of World War II and afterwards (FAO/IAEA, 1985). A wide range of chemical agents have been shown to be mutagenic in recent years. Manganese chloride, urethane, phenols, ethyl methane sulphonate, sodium azide are some of such chemicals that have been employed for carrying out mutagenesis in both animals and plant materials (Konzak *et al.*, 1984). Mutagens, both chemical and physical, are known to produce abnormal chromosomal aberrations leading to chromosome mal-behaviour during meiosis; they therefore provide a viable additional option to plant breeders for creating useful genetic variability. In most cases the use of mutants either directly or indirectly via crosses with other gene sources in the development of plant cultivars, i.e., mutation breeding, represents a complementary method of modern plant improvement (Lysenkov, 1989).

Mutagenic agents have been employed to induce beneficial mutants for different purposes, such as resistance to pests and diseases, early maturity, improved yield and quality. For any mutation breeding programme, selection of effective and efficient mutagen is very essential to recover higher frequency of desirable mutations (Solanki and Sharma, 2002). A number of chemical and physical mutagens are widely employed to induce genetic variability in plants (Restaino, 2003). In spite of the huge success that has been recorded using mutagenic agents to induce beneficial mutation, the curtain has not been drawn on

this area. Researchers can still explore the use of single or combined mutagenic agents to bring about more desirable mutants useful in achieving plant-breeding goals. It is worthy of note that in spite of numerous works, that have been done on crop plants by induced mutation, in Nigeria such works are inadequate (Odeigah, 1991). Pigeon pea *Cajanus cajan* (L) with all its numerous uses, crop breeders have done very little in this area.

1.3 Statement of The Problem

Due to the geometric increase in world's population, there is an increase in food demand and because pigeon pea is a rich source of dietary vegetable proteins, it could well complement the cereals (Tabo *et al*., 1995). There is too much dependence on beans (*Vigna unguiculata*) which has led to tremendous increase in market price. Pigeon pea research in terms of crop improvement is still at a low level in Nigeria compared to other grain legumes such as Cowpea, and farmers still cultivate the traditional varieties (Akande, 2007). In large parts of Sub-Saharan Africa particularly in Nigeria, small holder agricultural production has remained consistently low and food security is catastrophically poor (Asiegbu *et al.*, 1993). Mutations are known to enhance the genetic variability of crop plants and since spontaneous mutations occur at very low frequency, induced mutations facilitate the development of improved varieties at a swifter rate (Maluszynski *et al*., 2000). Mutation breeding is one of the plant breeding techniques used for creating genetic variability in yield contributing traits and to improve the yield of crops (Ahloowalia *et al*., 2004). The high demand for improved varieties of Pigeon pea that combine quality, high yield and disease resistance can be achieved through induction of beneficial mutation in

traditional landrace pigeon pea *Cajanus cajan* (L.) through the use of radiation and chemical mutagens.

1.4 Justification of The Study

This study will provide information on the effect of gamma irradiation and sodium azide induction of mutation in pigeon pea. The treatments could possibly lead to an increase in beneficial genetic variability in pigeon pea which might be useful to plant breeders. Therefore, the present study needs to be undertaken to investigate the mutagenic effects as a means of increasing the beneficial variability within cultivars and hence improve its productivity.

1.5 Aim of The Study

The study aimed at determining the potential of sodium azide and gamma irradiation in generating genetic variability that may be used in developing an improved pigeon pea with high yield potential.

1.6 Objectives of The Study

The main objectives of the present study were to determine the:

1. Effect of the different doses/concentrations of gamma irradiation and sodium azide on morphological traits of pigeon pea.

2. Effect of application of different doses/concentration of sodium azide and gamma rays on meiotic divisions in pigeon pea.

3. Concentration at which sodium azide and gamma irradiation best induced beneficial mutation in pigeon pea.

4. Effects of the mutagens on the protein composition of the pigeon pea.

1.7 Research Hypotheses

The following hypotheses were postulated:

1. The two mutagens have no significant effect on the morphology of pigeon pea plant.

2. The two mutagens induced no significant chromosomal changes on pigeon pea plant.

3. The two mutagens cannot induce significant beneficial traits in pigeon pea plant.

4. The mutagens have no significant effect on the protein content of pigeon pea plant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mutagenesis As A Means of Crop Improvement

Mutation means a sudden heritable change in genetic material at the gene and chromosome level. Mutations are the tools used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007). Mutation can bring about genetic variability, which is most desirable in self pollinated crops such as pigeon pea. In the past, several researchers have tried to improve the crop by applying the techniques of mutation breeding Mutagenesis alters the genetic makeup of plants by interference and modification of genes. Mutagenesis is an efficient tool to create new desirable genetic variability (Koornneef, 2002). Cytogenetic studies are pivotal for obtaining information pertaining the role and effects of various mutagens and also elucidating the responses of different crop genotypes to a particular mutagen (Khan and Tyagi, 2009). Mutation breeding is an important method used for the improvement of crops through the induction of mutations at loci that control economically important traits and/or by eliminating undesirable genes from elite breeding lines. In several mutation derived varieties, the changed traits have resulted in increasing the yield and quality of the crop, improving the agronomic inputs and consumer acceptance (Ahloowalia *et al*., 2004). Induced mutagenesis has been successfully utilized in several crop plants viz., rice, common beans, artemesia and Chickpea (Wani and Anis, 2008), suggesting the great

potential of this technique for crop improvement. Many agronomical important mutations affecting plant and grain characters have been identified, including alteration of grain colour, stem rust resistance and earliness in wheat (Chopra, 2005). Mutation breeding has been successfully used for turning the non–edible oil from Linseed (*Linum usitatissimum*), into an edible seed oil (Bertagne *et al*., 1996).

Mutagenesis has been successfully employed in rape seed and mustard by plant breeders, to alter the genetic architecture of plant and isolate mutants with desired economic characters such as plant height, number of pods per plant, number of grain per pod, 1000 - grain weight, grain yield, oil content and disease resistance (Javed *et al*., 2000). Mutation breeding has been used to produce many cultivars with improved economic value and study of genetic and plant developmental phenomena (Van *et al*., 1990).

It has been demonstrated that genetic variability for several desired characters can be induced successfully through mutation and its practical value in plant improvement programs has been well established. The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001).

2.2 Mutagen Types

Various mutagenic agents have been used to induce favourable mutations at high frequency such include ionizing radiation and chemical mutagens (Ahloowalia and Maluszynsky, 2001). Physical mutagens like X-rays, gamma rays, fast neutrons, thermal

neutrons, ultraviolet and beta radiations have been frequently used to induce mutagenesis (Yaqoob and Rashid, 2001).

Apart from physical mutagens, several chemical mutagens were also frequently used to induce mutagenesis in crops including Ethyl Methane Sulphonate (EMS), Ethylene imine (EI), Methyl Nitroso Urea (MNU), N-nitroso-N-methyl urea (NMU), Ethyl Nitroso Urea (ENU) and Sodium azide (SA) (Sharma and Chopra, 2000). The ethylated agents such as EMS have been found to be more effective and efficient than physical mutagens in crops like Lentil (Gaikwad and Kothekar, 2004), Pea (Waghmare and Mehra, 2001) and Chickpea (Kharakwal, 1998).

Seed mutagenesis through EMS treatment has been used for induction of male sterility in wheat (Maan and Williams, 1984), herbicide resistance in Soybean (Sebastian *et al*., 1989), early flowering in spring rape (Thurling and Depittayanan, 1992), increased pollen viability and fruit rot resistance in bell pepper (Ashok *et al.,* 1995). EMS has been successfully used to develop fenugreek mutants with the ability to produce early maturing mutants with a determinate growth habit, high seed yield, seed quality and adaptation to a short growing season (Basu *et al.,* 2008).

2.2.1 Physical mutagens

Gamma rays generally influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Gunckel and Sparrow, 1961). There are some reports which showed that higher exposures of gamma rays were usually inhibitory (Kumari and Singh, 1996), whereas, lower

exposures were sometimes stimulatory (Thapa, 1999). Sjodin (1962) reported that the material and energy necessary for initial growth are already available in the seeds, and so the young embryo has no need to form new substances, but only to activate those already stored in the cotyledons. The role of low doses of gamma radiations may be to increase the enzymatic activation and awakening the young embryo, which result in an increasing rate of cell division, which affects not only germination, but also vegetative growth and flowering. Exposing the dry seeds to low **γ**-irradiation doses resulted in increasing yield of some plants such as Sunflower (Abo-Hegazi *et al*., 1988) and *Ammi visnage* (El-Shafie *et al*., 1993). Gustafsson (1947) observed the occurrence of induced, useful mutations in Barley for such characters as height of straw, earliness and lateness, strength of straw, chemical properties, brewing characteristics, protein content, 1000 grain weight and littering capacity. These observations led him to suggest that the radiations could be used as a new tool in plant breeding.

Induced mutation through gamma rays have played a significant role in the alteration of plant architecture and selection of mutants with enhanced yield potential in rapeseed and mustard (Rahman, 1996; Shah *et al.,* 1999).

Uma and Salimath (2001) in Cowpeas, Ashraf *et al*. (2003) in Basmati rice and Khatri *et al.* (2005) in *Brassica juncea* reported early flowering due to gamma irradiation. Kaul and Singh (1972) subjected dormant seeds of *Datura metel* to different doses of gamma rays with an aim to investigate the effect of gamma rays on its seeds, the growth and metabolic activities of the plant and frequency and spectrum of viable mutations. Irradiation with

different doses resulted in the production of chromosomal aberrations including deletions, duplications and translocations both at mitosis as well as meiosis. With increasing doses of radiations increase in seed lethality, seedling injury and production of chimera effects such as morphological freaks were observed.

2.2.2 Chemical mutagens

Sodium azide is known to affect seed germination, shoot length, and root length and also induces high frequency of chlorophyll deficient mutations (Khan and Tyagi, 2009). Reduction in seed germination in mutagenic treatments has been explained due to delay or inhibition in physiological and biological processes necessary for seed germination which include inhibition of mitotic process (Sato and Gaul, 1967), hormonal imbalance (Ananthaswamy *et al*., 1971) and enzyme activity (Chrispeds and Varner, 1967). Molina-cano *et al.* (2003) reported Mildew resistant mutants of *Hordeum vulgare* induced by sodium azide mutagen. Seeds of *Spathoglottis plicata* Blume, a terrestrial orchid were treated with sodium azide which induced strikingly attractive flower colour modification thereby improving its floricultural significance (Roy and Biswas, 2005). Increase in stearic acid content was induced in Sunflower up to 35% when treated with sodium azide mutagen (Scoric *et al.*, 2008).

2.2.3 Combined mutagenic treatments

Rao and Rao (1983) produced higher proportions of albinos in rice by applying Methyl Methane Sulphonate (MMS), N-nitrous-N-methyl Urethane (NMU) and Hydroxyl amine (HA) as mutagens. Reddy and Vishwanathan (1999) induced rust resistance in hexaploid

wheat variety “WH147” by using gamma rays and Ethyl Methane Sulphonate. Joshua *et al*. (1974a) observed that combined treatment of fast neutrons and diethyl sulphate on barley resulted in synergistic effects on both anaphase fragments and bridges. Storage of seeds after neutron irradiation had no significant effect on frequency of fragments and bridges.

Joshua *et al*. (1974b) further studied the effect of fast neutrons and gamma rays on seedling height and chromosome aberrations in barley. In response to the treatments, a synergistic effect in seedling height was noted. Furthermore, the presence of chromosome fragments and anaphase bridges showed an additive effect with combined treatments. Wanjari and Phadnis (1977) subjected the seeds of *Momordica charantia* L. to different concentrations of colchicines to induce polyploidy. All concentrations of colchicines proved to be lethal when sprouted seeds were treated. Subjecting pre-soaked seeds to 0.3% colchicines was found effective in inducing tetraploidy. Increased doses resulted in decreased germination, poor survival and poor plant growth at early stages. Induced tetraploids were devoid of vigorous growth and exhibited delayed flowering. However, their foliar size was bigger than that of diploids. Meiotic studies in tetraploids revealed multivalent formation at metaphase–I and irregularities such as laggards during anaphase. Raut and Thombre (1977) were successful in inducing tetraploidy in *Impatiens balsamia* L., by treating its seeds with 0.05% and 0.1% colchicines. Colchicines treated population plants showed arrested growth, height retardation, abnormal plumules and leaf shape, autotetraploids with bigger flower size and longer blooming period.

2.3 Effects of Mutagens On Biological Parameters

The effects of physical and chemical mutagens and their combination treatments on different biological parameters such as germination, survival, injury and sterility have been studied by many workers (Khan *et al.,* 2005). Chaudhary (1983) reported a symmetric reduction in germination in different varieties of wheat with higher doses of gamma rays. Khanna (1991) reported the effect of seed treatment with different concentration of EMS on germination and growth of seedlings in chickpea. There was a proportionate decrease in germination percentage with the increasing concentrations of EMS. Reduction in seed germination with increase in dose of gamma rays in chickpea was reported by Khanna and Maherchandani (1981). Lal *et al.* (2009) studied mutagenicity of gamma rays, sodium azide and their different combinations in M1 generation of black gram and observed that an increase in sodium azide concentrations resulted in decrease in M1 germination. The plant survival was also affected with different doses of gamma rays and Sodium azide. The combination treatments of gamma rays and Sodium azide had more depressive effect on seedling growth.

2.4 Induction of Cytological Abnormalities

The study of chromosomal behaviour during mitosis and meiosis is considered to be a suitable method for evaluating the effect of mutagens and also helps in determining the radio-sensitivity of plants to both physical and chemical mutagens. Auerbach and Robson (1942) presented the first elaborate report which showed that Mustard gas could induce mutations as well as chromosomal aberrations in *Drosophila*. Gamma rays, Maleic Hydrazide and their combination treatments have been shown to induce disturbed mitotic

behaviour in *Vigna radiata* (Grover and Tejpaul, 1982). The sticky chromosomes, fragments and ring chromosomes at metaphase and the laggards and bridges at anaphase were noticed by these workers. These chromosomal aberrations were found to be significantly correlated with dose and the combined treatment which also affect the meiotic process. The quadrivalents presumably due to translocations, were occasionally encountered on metaphase-I. Grover and Virk (1986) reported induced chromosomal aberrations like unequal separation at anaphase II in mungbean after treatments with gamma rays, N'methyl-N-nitro-N-nitroso guanidine (MNNG), Ethyl methane sulphonate (EMS) and Hydroxyl amine (HA). The maximum frequency of chromosomal aberrations was noticed with gamma rays followed by MNNG, EMS and HA. Variety G-65 was found to be more sensitive with treatments of EMS and HA.

Vandana and Dubey (1996) reported the meiotic anomalies induced by EMS and Diethyl sulphonate (DES) in *Vicia faba*. These anomalies were found to increase with increase in concentrations of mutagens applied. Overall frequency of meiotic anomalies induced by various concentrations of DES was higher than those of EMS. However, EMS treatments induced higher proportion of anormalies in pairing whereas Diethyl sulphonate (DES) induced higher proportion of anormalies during anaphase disjunction. A relative account of cytological and developmental effects of gamma rays, EMS and MMS on meiotic features and pollen fertility in *Vicia faba L*. was provided by Bhat *et al.* (2006).

The various kinds of chromosomal abnormalities and reduction in pollen fertility were found to be dose dependent. The induction of meiotic abnormalities was observed to be

higher under Methyl methane sulphonate (MMS) treatments, followed by gamma rays and Ethyl methane sulphonate (EMS), suggesting that Methyl methane sulphonate (MMS) could be more effective in inducing chromosomal abnormalities followed by gamma rays and EMS.

Khan and Tyagi (2009) reported bridges and laggards in soybean when treated with EMS, gamma rays and their combination. In maize, sticky chromosomes were first reported by Beadle (1932) and are seen as intense chromatin clustering in the pachytene stage. The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense, with the formation of pycnotic nuclei that may involve the entire genome, culminating in chromatin degeneration.

Chromosome stickiness may be caused by genetic or environmental factors. Genetically controlled stickiness has been described in many cultivated plants such as maize (Caetano-Periera *et al.*, 1995), Pearl millet (Rao *et al.*, 1990) and wheat (Zanella *et al*., 1991). Several agents have been reported to cause chromosome stickiness, including x-rays (Stephenson, 1956), gamma rays (Rao and Rao, 1977; Al-Achkar *et al.*, 1989), temperature (Erikisson, 1968), herbicides (Badr and Ibrahim, 1987) and some chemicals present in soil (Caetano-Pereira *et al.*, 1995). However, the primary cause and biochemical basis of chromosome stickiness are still unknown. Jayabalan and Rao (1987) postulated that sticky chromosomes may result from the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to

stickiness is caused by mutations in the structural genes coding for them (hereditary stickiness) or by the action of mutagens on the proteins (induced stickiness).

Katyayani *et al*. (1980) studied the mutagenic effects of Maleic Hydrazide (MH) and Ethyl methane sulphonate (EMS) on germinating seeds of Fenugreek, (*Trigonella foenum-graecum* L.), the results showed that higher concentrations of EMS (0.05-0.1%) and MH (0.1%) exercised retarding effects on seedling growth while low concentrations of both the chemicals, particularly 0.001% EMS and up to 0.05% MH resulted in its promotion. Induction of binucleate, trinucleate and tetranucleate conditions and some chromosomal aberrations including bridges, fragments were noted in 0.01 and 0.001% concentrations of MH and EMS respectively. It was also observed that seed treatment with 0.001 and 0.01% MH and EMS resulted in induction of early flowering.

Sharma and Kumar (2004) treated seeds of two cultivars of *Cicer arietinum* L. viz., CSG-89.62 and KPG-59 with four different concentrations, i.e., 0.1, 0.2, 0.3 and 0.4% of ethyl methane sulphonate. They observed different types of meiotic abnormalities such as stickiness, univalents, multivalent, unorientation of chromosomes, precocious separation of chromosomes at metaphase and bridges, laggards and unequal separation of chromosomes at anaphase. In general, the meiotic abnormalities increased along with the increase in concentration of EMS in both the cultivars. However, cultivar KPG-59 showed more chromosomal abnormalities as compared to cultivar CSG-89.62 at the same treatment.

Goyal and Khan (2009) treated seeds of two varieties, (PU-19 and T-9) of Urdbean (*Vigna mungo* L.) with four concentrations (0.1, 0.2, 0.3 and 0.4%) of EMS and (0.01, 0.02, 0.03

and 0.04%) of Hydrazide. Chromosomal aberrations like univalents, multivalent, laggards, bridges, micronuclei, stickiness, cytomixis and precocious movement were noticed in mutagen treated populations. Chromosomal aberrations were found to be correlated with the concentration of chemical mutagens. The maximum frequency of abnormalities was induced by EMS in both varieties of Urdbean. Kumar and Srivastava (2010) studied the mutagenic potential of gamma rays and laser rays on seeds of Safflower (*Carthamus tinctorius* L.). The results showed that, a wide spectrum of chromosomal aberrations were encountered in both the laser and gamma rays treatments, but the most frequent anomaly that dominated was the stickiness of chromosomes. The percentage of chromosomal aberrations observed in case of gamma rays treated set was higher than laser rays suggesting that gamma rays could be successfully employed for creating additional genetic variability in Safflower.

2.5 Mutations Affecting Morphology

Plant morphology is considered to be an important tool for isolation of desirable mutants. Several induced morphological mutations have been reported in literature showing alterations in the morphology of various plant parts.

Rao and Jana (1976) subjected the seeds of Black gram (*Phaseolus mungo*) to X-rays and EMS treatments with the objective of obtaining some promising mutants. The induced leaf mutants scored comprised of crinkled leaf, waxy leaf, narrow leaf and unifoliate mutants. Plants with crinkled leaf and waxy leaf mutants had normal fertility and vitality whereas

the narrow leaf mutant was partially sterile and the unifoliate–an extreme dwarf mutant was also isolated which was completely sterile.

Chandra and Tewari (1978) observed in bean (*Phaseolus aureus*) varieties S-8 and Pusa Baisakhi that increasing doses of gamma rays and neutrons caused a gradual reduction in germination of seeds and pollen and ovule fertility. Irradiation caused the appearance of leaf abnormalities including unifoliate, bifoliate, trifoliate, tetrafoliate and pentafoliate characters. Under the influence of neutrons both tetrafoliate and pentafoliate leaves were observed on the same plant of cv. S-8 apparently associated with enhanced luxuriance of plants which resulted in enhanced pod formation.

Narsinghani and Kumar (1976) in a mutation breeding programme subjected the seeds of Cowpea (*Vigna sinensis* L.) to EMS and MMS treatments. In M1and M2 generations, reduction in survival percentage, mean pod number, seed yield per plant and average pollen fertility was observed with less in M2 generation. A few long podded mutants, chlorophyll mutations and leaflet modifications were also recorded. Meiotic studies revealed the presence of reciprocal translocations, inversions and other anormalies. In comparison to M1 a decrease in total aberrations was recorded in M2.

Gamma ray induced morphological mutations have also been reported by Morishita (2001) in Buckwheat and by Tah (2006) in Mungbean. Kumar and Singh (2003) reported several viable mutants induced by gamma rays in Lima bean (*Phaseolus lanatus* L.) which included earliness, erect plants, profuse flowering and high yielding mutants. Wani (2011) reported a series of morphological mutants in chickpea isolated in separate and combined

treatments of gamma rays and EMS. The various types of mutants reported included plant height, leaf, pods and seed mutants, combined treatments in general were found more effective and efficient in inducing various types of morphological mutants.

2.6 Mutagenic Effectiveness and Efficiency

The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also on its efficiency. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen, while mutagenic efficiency is the production of desirable changes which are free from associations with undesirable genetic alterations. This is generally measured by the proportion of the mutation frequency in relation to damages associated to mutagenic treatments such as: height reduction, chromosomes breakage, sterility and lethality (Gaul *et al*., 1972).

Studies on effectiveness and efficiency of the physical and chemical mutagens have been carried out in various crops by several workers (Mehraj-ud-din *et al*., 1999; Koli and Ramakrishna, 2002). The ethylated agents like Ethyl methane sulphonate (EMS) have been found to be more effective and efficient than physical mutagens in crops like chick pea (Kharakwal, 1998), Cowpea (Jhon, 1999), *Lathyrus sativus* (Waghmare and Mehra, 2001) and lentil (Gaikwad and Kothekar, 2004).

Deepalakashmi and Kumar (2003) studied the efficiency and effectiveness of physical and chemical mutagens in Urdbean and reported that gamma rays were found to be more effective than EMS in producing chlorophyll and viable mutants. Gamma rays were also found more efficient in causing lethality and sterility. Dhanavel *et al.* (2008) reported

decrease in mutagenic effectiveness with an increase in concentration of EMS, DES and Sodium azide in cowpea, it is obvious that the higher efficiency at lower and intermediate doses of mutagens may be due to the fact that the biological damage (Lethality and sterility) increased with the dose at a rate greater than the frequency of mutations. Shirsat *et al*. (2010) treated two varieties of horse gram, viz., SINA (K-42) and KS-2 with three concentrations of EMS (0.05%, 0.1% and 0.125%), Sodium azide (0.001%, 0.002% and 0.003%) and Nitroso ethyl urea (NEU) (0.001%, 0.003% and 0.005%). In M2 generation, Sodium azide was found more effective followed by Nitroso ethyl urea in both varieties. In variety SINA, the highest effectiveness was seen at 0.001 % Sodium azide treatment and the lowest value at 0.125 % EMS. In case of variety KS-2, the highest effectiveness value was recorded at 0.001% SA concentration and lowest value at 0.10 % EMS. SA was also found to be more efficient than EMS and NEU in both the varieties of horse gram.

Khan *et al.* (2005) exposed seeds of two chickpea (*Cicer arietinum* L.) varieties viz., Avrodhi and BG-256 to EMS, SA and Hydrazide (HZ) for 6 hours. They observed that the mutagenic effectiveness for EMS and SA followed a dose dependent decreasing trend in both varieties. In case of HZ, mutagenic effectiveness exhibited a dose dependent increase but decreased abruptly at the highest dose in both the varieties. The order of mutagenic effectiveness was HZ > SA > EMS.

To determine the efficiency of the mutagens, three criteria viz., seedling injury (Mf/I), pollen sterility (Mf/S) and meiotic abnormalities (Mf/Me) were taken into consideration. Based on seedling injury, the order of efficiency was HZ > EMS > SA whereas on the basis of sterility, it was EMS > HZ > SA. Based on meiotic aberrations induced, EMS was

found to be efficient followed by SA and HZ in var. Avrodhi and HZ proved to be the most efficient mutagen followed by EMS and SA in var. BG-256.

Dhulgande *et al*. (2011) treated seeds of two varieties of pea (*Pisum sativum* L.) namely DDR-53 and DMR-55 with varying doses/concentrations of gamma rays and EMS. Results recorded for mutagenic effectiveness and efficiency revealed that the 0.10% EMS concentration induced the highest values of effectiveness followed by 400Gy treatments in both varieties. It was further observed that the 0.125% EMS dose proved to be most effective than the higher ones in all the mutagenic treatments. The effectiveness values decreased with increasing dose or concentration of the mutagens. For mutagenic efficiency, it was noted that the efficiency decreased for lethality, pollen sterility and mitotic aberrations from EMS concentration to gamma rays in variety DDR-53 while in variety DMR-55 the efficiency for lethality and pollen sterility increased from EMS to gamma rays but efficiency for mitotic aberrations decreased from EMS to gamma rays.

2.7 Induced Variability In Quantitative Traits

Breeding is the most commonly used method for crop improvement and genetic variability is the basis of any breeding program. Genetic variability is also important to adapt a population to the inevitable changes in the environment and to promote the survival of the species. The role of mutation breeding in increasing the genetic variability for quantitative traits in various crop plants have been proved beyond doubt (Vyas and Chauhan, 1994; Khan *et al.,* 1994, 1998, 1999; Khan and Siddiqui, 1995; Das and Chakraborty, 1998;

Jabeen and Mirza, 2002; Kumar and Mishra, 2004; Singh *et al*., 2006; and Khan and Goyal, 2009).

Jabeen and Mirza (2002) treated the seeds of *Capsicum annuum* cv. Longhi with varying concentrations of EMS. Data was recorded on eight different characters in M1 generation including leaf area, number of leaves, number of branches, height of plants, days to flowering, days to fruiting, number of fruits per plant and chlorophyll content. Wani and Khan (2006) treated the seeds of Mungbean var. PDM-11 with EMS (0.1% and 0.2%) and Hydrazine hydrates (HZ) (0.01% and 0.02%) to induce mutations. Results revealed that variability in the treated population was higher than the Control for all the quantitative traits, namely fertile branches per plant, pods per plant, 100 seed weight and seed yield per plant. However, EMS was found to be more effective. Similar increases in the number of pods of some other varieties of urdbean have been reported by Tickoo and Chandra (1999) using EMS, Nitroso methyl urea, Hydroxyl amine and gamma rays.

Mensah *et al*. (2007) exposed seeds of Sesame (*Sesame indicum* L.) to varying concentration of sodium azide and colchicine solutions ranging from 0-0.25%. They observed dose related effects of the mutagenic treatments on quantitative traits resulting in reduction in traits such as germination and survival percentages, plant height, number of fruit per plant, but increase in leaf area, maturity time and fruit size. Low doses of both mutagens (<0.125%) produced early maturing and robust / high yield variants.

Yakoob and Rashid (2001) reported that various quantitative traits can be improved in various genotypes through variable gamma ray doses. Kumar and Rai (2006) studied the

effect of different doses of gamma rays (100Gy to500Gy) in soybean. They have shown that with increasing doses of gamma rays the pollen germination percentage and fertility continuously decreased as compared to control. However, the pollen tube lengths showed an improvement over control up to 100Gy followed by simultaneous decrease at higher doses. Among the morphological parameters, seed setting was found to be adversely affected along with the increasing doses of gamma rays. Number of seeds and number of pods/plant also registered a considerable decrease over the control along with the increasing doses of gamma rays except for 100Gy dose at which number of pods/plant showed a slight enhancement over the control. In some cases reduction in flower size was also noticed.

Kon *et al*. (2007) exposed seeds of long bean (*Vigna sesquipedalis)* to different doses of gamma rays (300, 400, 500, 600 and 800Gy). The study revealed that germination percentage, plant height, survival percentage, root length, root and shoot dry weight decreased with increasing dose of gamma ray. The 800Gy gamma ray in particular had a negative effect on these morphological characteristics probably because of injury it might have caused to the seeds of Long bean. As a result, poor growth and development was noticed. Wani and Anis (2001) treated seeds of two Chickpea varieties viz., Avrodhi-T3 and KPG-59 with gamma rays and EMS, separately as well as in combination. Data on germination percentage, percent survival and pollen sterility were recorded. Seed germination and plant survival decreased. However, pollen sterility increased with an increase in dose/concentration of the mutagens. Combined treatments proved to be more effective in chickpea varieties than the individual treatments.

2.8 Economic Impact of Mutation Breeding

Mutation breeding has significantly enhanced the yield per hectare and uplifted the socio-economic conditions of the farmers, the mutation breeding program is a profitable approach which may enable the mutant crops to withstand particular environmental stresses such us water shortage, extreme temperature and pH better than wild-type crops, or reproduce more quickly. Modern plant breeders and farmers can exploit a wealth of natural biodiversity, which may be widely broadened through the application of mutation induction techniques which translates into a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares (Kharkwal and Shu, 2009).

During the past seventy years, worldwide more than 2250 varieties have been released that have been derived either as direct mutants or from their progenies. Induction of mutations with radiation has been the most frequently used method for directly developed mutant varieties. The prime strategy in mutation-based breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits, which limit their productivity or enhance their quality value. In addition, the economic contribution of the selected mutant varieties of rice, barley, cotton, groundnut, pulses, sunflower, rapeseed and Japanese pear, several mutation-derived varieties, the changed traits have resulted in synergistic effect on increasing the yield and quality of the crop, improving agronomic inputs, crop rotation, and consumer acceptance. In contrast to the currently protected plant varieties or germplasm and increasing restrictions on their use, the induced mutants have been freely

available for plant breeding. Many mutants have made transnational impact on increasing yield and quality of several seed-propagated crops.

According to Ahloowalia, *et al* (2004) Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil, protein, starch quality and enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of the environmentally sustainable agriculture.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Research Materials

The seeds of pigeon pea (*Cajanus cajan* (L.) Millsp) was obtained from local farmers in Ankpa Local Government Area, latitude 7^{o} 38'E and longitude, 7^{o} 22'N (163m elevation above sea level), Kogi state, Nigeria (Garmin eTrex Venture HC Handheld GPS). The Gamma radiation source (Cirus Cobalt 60 Teletherapy) used for seed treatment was located at the Radiology and Oncology Department, Ahmadu Bello University Teaching Hospital Shika, Zaria. The Sodium azide (SA) used for this research work was manufactured by KEM Light Laboratories P.V.T. Ltd.

3.2 Study Area

This research was conducted both in the laboratory and in the Biological Garden of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria (Longitude 07^{o} 39'E and latitude 11^{o} 09'N, 2148 above sea level), Nigeria. (Garmin eTrex Venture HC Handheld GPS). Samaru lies in the Northern Guinea savannah agro-ecological zone of Nigeria with mean annual rainfall of about 1100m. Rainfall in this region is essentially between May and September and dry season between October and April. Hottest months of the year are March and April and the region is with a mean daily temperature of 27^{o}C. The coldest months are November – January (Osuhor *et al.*, 2004).

3.3 Treatments

3.3.1 Exposure of seeds to gamma radiation

Uniform healthy dry seeds of *Cajanus cajan* (L.) Millspaugh were exposed to different doses of gamma rays (control 0, 50, 100, 150 and 200Gy), derived from Cobalt-60 (^{60}CO) source with a measured dose rate of 124.5Gy/min which lasted for 8hrs 52mins at the Oncology Department, Ahmadu Bello University Teaching Hospital, Zaria.

3.3.2 Preparation of sodium azide (SA) and buffer solutions

One percent stock solution of SA was prepared and from this, different concentrations (control 0.00, 0.01, 0.02, 0.03 and 0.04 %) of SA were prepared by using the formula $N_1V_1=N_2V_2$ where,

N_1 =strength of stock solution

N=200, pH =3.0

V_1=Volume of stock

N_2=strength of desired solution

V_2=Volume of desired solution

The specificity of action of chemical mutagen depends upon the particular conditions of treatment, the more important of which are temperature and hydrogen ion concentration. In the course of the present study, Sodium azide solutions were prepared by dissolving appropriate quantity of this chemical in phosphate buffer having a pH of 3.0.

3.3.3 Seeds treated with sodium azide

The radiated healthy and dry (10-12% moisture) seeds of *Cajanus cajan* (L.) Millspaugh were pre- soaked in a phosphate buffer (pH 3.0) to maintain the osmotic content of the cell for six (6) hours and later subjected to the four (4) concentrations (0.01%, 0.02%, 0.03% and 0.04%) of sodium azide [SA] (NaN_3) solutions at room temperature (25°C) for six (6) hours. The seeds were washed thoroughly to remove the residual amount of mutagens and sown immediately (table 3.1).

3.4 Collection of Soil Samples

Top soil was collected from uncultivated land within the Botanical Garden in Ahmadu Bello University, Zaria. A sample of the top soil was air dried and taken to the Department of Soil Science, Institute for Agricultural Research, Zaria for physico-chemical analysis.

3.5 Pot Preparation and Seed Planting

Top soil was used to fill six hundred and fifty (650) polythene bags and four (4) treated seeds were sown inside each polythene bag during rainy season. The polythene bags were arranged in a complete randomized design (CRD) (Table 3.2).

3.6 Cytogenetic Studies

3.6.1 Meiosis

The effect of the mutagens on Meiosis was studied using young flower buds of Pigeon pea plants. This is essentially the study of the cell division, which precedes the formation of pollen grains. The young flower buds was collected between 12 noon – 2 pm and fixed in Carnoy's fluid for 24 hours before being transferred to 70% ethanol for preservation and

Table 3.1: Details of Mutagenic Treatment given to Pigeon pea seeds

Mutagen Used	Dose/Conc.	Duration of Pre-soaking (Hours)	Duration of Treatment (Hours)
Control	0	6.0	-
Gamma Rays (Gy)	50	6.0	46.01 mins
	100	6.0	92.02 mins
	150	6.0	138.04 mins
	200	6.0	184.05 mins
Sodium Azide (%)	0.01	6.0	6.0
	0.02	6.0	6.0
	0.03	6.0	6.0
	0.04	6.0	6.0

Table 3.2: Treatments Illustration

	Treatments	Sodium Azide Concentrations (%)				
		A(0.00)	B(0.01)	C(0.02)	D(0.03)	E(0.04)
Gamma Doses (Gy)	F(0)	FA	FB	FC	FD	FE
	G(50)	GA	GB	GC	GD	GE
	H(100)	HA	HB	HC	HD	HE
	I(150)	IA	IB	IC	ID	IE
	J(200)	JA	JB	JC	JD	JE

stored in refrigerator at 4^{o}C until needed. The flower bud was dissected on a clean glass slide by carefully teasing out the anther with fine pointed forceps and a needle. Then these were squashed in one or two drops of 1% aceto - carmine stain with the aid of a round end glass rod while debris from the anther was removed. Cover slip was then placed in position and gently pressed in between two folds of filter paper with the thumb to remove excess stain. The slide was then passed gently over the spirit flame to ensure maximum absorption of stain by the chromosomes and clarity of dividing cells (Swaminathan *et al.,* 1954). The preparation was then examined under the microscope at x10 and x40 and photographed using digital camera (Samsung PL120, 14.2 megapixels).

3.7 Data Collection

3.7.1 Growth parameters

(a) **Germination percentage**: Germination count was taken after 2 weeks i.e. after (15days) sowing and expressed in percentage as follows:

$$Germination\ (\%) = \frac{Number\ of Seeds\ Germinated}{Number\ of Seeds\ Sown} x\ 100$$

(b) **Number of leaves/plants:** The number of leaves selected on three randomly sampled plants was taken at 4, 8, 12, 16 and 20 weeks after planting (WAP) and recorded.

(c) **Number of branches/plants:** The primary branches borne on the main shoot of three randomly sampled plants were counted and recorded.

(d) **Plant height and root length:** The plant heights were determined by measuring the height of three randomly sampled plants per treatments from soil surface of the polythene bag to the tip of the apex using a metre rule. This measurement was taken at 4, 8, 12, 16 and 20 WAP; the average height was calculated and recorded. Similarly, the root were carefully dug from the soil and washed, root length of the three randomly sampled plants per treatment was determined at 4, 8, 12, 16 and 20 WAP. The average length was calculated and recorded.

3.7.2 Yield parameters

At harvest, the following yield parameters were determined:

(a) **Days to 50% flowering:** Days to 50% flowering were determined as the number of days from the time of sowing to when at least 50% or half of the plants in each treatment have at least one open flower.

(b) **Number of pods:** The pods produced by three randomly selected plants in each polythene bag were counted and recorded.

(c) **Pod weight:** The pod weight of three randomly selected plants in the polythene bags were determined by using the weighing balance (ISO 9001 model).

(d) **Total grain yield:** The plants were harvested when 75 - 80% of the pods were at physiological maturity, that is when pods turn brown and dry .The plants were harvested by cutting the stem at the base. The harvested plants were left in the field for a week for sun drying and then threshed in small plot thresher. The seeds were

collected and weighed using the weighing balance and the weight converted to yield in kg/h^{-1}.

(e) 100 seed weight: One hundred seeds were counted from that obtained under each treatment and their weights were taken with electric weighing balance (ISO9001 model).

(f) Determination of nitrogen and crude protein

The proximate analyses were carried out as recommended by the Association of Official Analytical Chemists (AOAC, 1990) using the kjeldahl method. The analysis of protein content was determination of the amount of reduced Nitrogen present in food substance, i.e., its $-NH_2$ and $=NH_2$.

Principle: These are the major compounds containing Nitrogen (minor nitrogenous ingredients of food include Amino acids, Purines, Ammonium salts and Vitamin B_1). So Nitrogen is used as an index of protein termed 'crude protein' as distinct from true protein.

Procedure: Proteins determination was carried out in three stages, as follows:

(A) **Digestion**: two grams of sample was weighed and placed into a 50ml digestion – flask and the Kjeldahl mixture which acts as a digestion catalyst was added. The flask containing the sample mixture was heated gently at an inclined angle in a Kjeldahl digestion rack until frothing subsided. It was then boiled until the solution became colourless. Heating of the mixture released the Nitrogen in the various samples which was then converted to ammonia with the concentrated Sulphuric acid. It was later allowed to cool. The sample was transferred to a 100ml

volumetric flask and diluted with distilled water to the mark. It was then mixed thoroughly. The mixture was further allowed to cool before distillation. A blank containing only the Sulphuric acid and catalyst was also heated

$$\text{Sample (N)} + \text{Conc. } H_2SO_4 \longrightarrow (NH_4)_2SO_4\,(\text{N – free})$$

$$C \xrightarrow{\text{Oxidation}} CO_2$$

(B) **Distillation**: A known aliquot (10ml) was transferred to the distillation apparatus and then introduced to the sample chamber 10ml of 40% sodium hydroxide was added to the sample addition tunnel and released to the sample chamber at a slow rate. The ammonia was entrapped in a receiving solution containing 10ml 2% boric acid solution into which 4 drops of bromocresol green and 2 drops of methyl red indicator had been put. Distillation was continued until the pink colour turned greenish.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O + 2NH_3$$

$$NH_3 + H_2O \longrightarrow NH_4OH \text{ (ammonium hydroxide)}$$

$$H_3BO_3 + 3NH_4OH \longrightarrow (NH_4)_3BO_3 + H_2O$$

(C) **Titration**: Back titration method was employed, i.e., the ammonia reacts with the Boric acid in the receiving flask and the amount of excess acid is determined by titration with Hydrochloric acid.

$$(NH_4)_3BO_3 + 3HCl \longrightarrow NH_4Cl + H_3BO_3$$

The percentage total Nitrogen was calculated and crude protein was estimated by multiplying the percentage Nitrogen with standard conversion factor 6.25. (i.e. % crude protein (cp) = 6.25 x N

$$\% N = \frac{V_1 - \mathrm{V_0} \text{ x M x } 14 \text{ x } 100 \text{ x } 100}{0.2 \text{ x } 1000 \text{ x } 10 \text{ x } 1}$$

V_o = Vol. of Hydrochloric acid require for blank

V_1 = Vol. of the Hydrochloric acid required for 10ml sample solution

M = Molarity of acid (0.1M)

14 = atomic weight of N

100 = total volume of digest

100 = % conversion

10 = Volume of distillate

0.2 = amount of sample taken in gram

Note: protein contains 16% N_2. This makes the general conversion factor to be 6.25.

3.8 Statistical Analysis

The data obtained from the parameters growth and yield parameters were subjected to statistical analysis to assess the extent of induced variations using the analysis of variance (ANOVA) to establish if there was any significant difference between the means of the doses/concentrations of software the mutagens using Statistical Analysis System (SAS 2004) Version 9.0.

Duncan's Multiple Range Test (DMRT) was used to separate and compare means where significance difference was observed using Statistical Analysis System (SAS 2004) Version 9.0.

Pearson's correlation coefficients were used to determine the relationships between total grain yield and quantitative traits using Statistical Package for Social Sciences (SPSS).

CHAPTER FOUR

4.0 RESULTS

4.1 Observation on Morphological Traits

Observation on the general morphology of the treated plants shows that they were tall and bushy; foliage was dark green with larger pods compared with the Control. The data obtained on the mutagenic effects of gamma rays and sodium azide on seed germination, number of leaves per plants, number of branches per plants, days to 50% flowering, plant height and root length and some yield parameters are presented in the Tables.

4.2 Germination Percentage

The germination percentage was symmetrically reduced in all the mutagenic treatments compared to the R0A0 (Control) treatment as shown in Table 4.1. The highest seed germination recorded for R0A2(0Gy + 0.02% SA) was not significantly higher than that due to R0A0 (control: 0Gy + 0.00 % SA) and R0A1 (0Gy + 0.01% SA); R0A3 (0Gy + 0.03% SA); R0A4 (0Gy + 0.04% SA).This was followed by those that were treated with R1A0 (50Gy + 0.0% SA) and R1A1 (50Gy +0.0%SA).The lowest seed germination observed with R4A3 (200Gy + 0.03% SA) and R4A4 (200Gy + 0.04% SA) was not significantly lower than that due to R3A4 (150Gy + 0.04% SA), R4A0 (200Gy + 0.00% SA), R4A1 (200Gy + 0.01 % SA) and R4A2 (200Gy + 0.02 % SA) treatments.

Table 4.1: Effect of Gamma radiation and Sodium Azide on Germination Percentage of Pigeon pea (*Cajanus cajan*).

Mutagen	Germination Percentage (%)
R0A0	97.2^{ab}
R0A1	97.2^{ab}
R0A2	100.0^{a}
R0A3	91.7^{abc}
R0A4	91.7^{abc}
R1A0	83.3^{abc}
R1A1	83.3^{abc}
R1A2	80.5^{bcd}
R1A3	80.6^{bcd}
R1A4	77.8^{c-f}
R2A0	77.8^{c-f}
R2A1	75.0^{c-f}
R2A2	72.2^{d-g}
R2A3	66.7^{d-h}
R2A4	63.9^{f-i}
R3A0	63.9^{f-i}
R3A1	61.1^{ghi}
R3A2	58.3^{ghi}
R3A3	52.8^{hi}
R3A4	44.5^{jk}
R4A0	41.6^{jk}
R4A1	41.6^{jk}
R4A2	38.9^{jk}
R4A3	36.1^{k}
R4A4	36.1^{k}
SE±	4.30

Means followed by the same letter(s) along column are not significantly different (P > 0.05). Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

4.3 Number of Leaves Per Plant

The lowest leaf number was due to the control treatment at 16 and 20 WAP (Table 4.2). At 4 WAP, with the exception of the significantly higher leaf number observed in plants under R1A3 (50Gy + 0.03 %SA)(22 leaves) and R3A0 (150Gy + 0.00 %SA)(20.3 leaves), other treatments produced leaf number that were not significantly different from the control, R0A0 (20.0 leaves). At 8 WAP, there were no significant differences between the treatments compared with the control ($P \geq 0.05$). At 12 WAP, most of the treatment with 100Gy – 200Gy produced significantly higher leaf number than the other treatments. The highest leaf number in pigeon pea observed with treatment 150Gy + 0.03%SA (R3A3)(35.3 leaves) was comparable some of the other treatments in that category. Other treatments resulted in lower leaf number with the lowest from R1A2 (50Gy + 0.02 % SA)(24.7 leaves) treatment. At 16 WAP, treatment 100Gy + 0.02%SA (R2A2)(62.3 leaves) and 0.03SA (R2A3)(61.7 leaves) produced the highest leaf number per plant. This was followed by that due to most treatments with 100Gy – 200Gy in combination with SA concentrations. Lowest leaf number at 16 WAP was observed with treatments that received 0 – 50Gy in combination with SA concentration with the lowest due to R0A0 (0Gy +0.00%SA)(44.0 leaves) (Table 4.2). Similarly, at 20 WAP, treatments R2A3, (100Gy+ 0.03%SA)(75.3 leaves) had the highest leaf number. The lowest mean leaf number was observed in the control treatment (R0A0)(52.7 leaves).

The combined ANOVA of data on leaf number showed that, the highest leaf number due to R2A3 (100Gy + 0.03%SA)(41.93 leaves) was only comparable with that due to R2A2 (100Gy + 0.02%SA)(40.80 leaves) treatment. This was followed by that due to plants that

Table 4.2: Effect of Gamma Radiation and Sodium Azide on Leaf Number Per Plant of Pigeon Pea at growth stages

Mutagen	Age of plant (WAP) Leaf number per plant					
	4	8	12	16	20	Mean
R0A0	20.0^{abc}	22.0^{a}	28.7^{de}	44.0^{h}	52.7^{f}	33.47^{i}
R0A1	15.3^{bc}	20.3^{a}	27.3 ^{efg}	54.3^{cde}	66.3^{bcd}	36.73^{e-h}
R0A2	18.0^{abc}	21.7^{a}	26.7 ^{efg}	47.7^{gh}	64.7^{bcd}	35.73^{gh}
R0A3	15.0^{c}	23.0^{a}	25.0^{fg}	49.0^{fg}	61.7^{cde}	34.73^{hi}
R0A4	18.3^{abc}	22.0^{a}	25.7 ^{efg}	52.0^{efg}	60.0^{cde}	35.60^{ghi}
R1A0	15.7^{bc}	20.7^{a}	25.3 ^{efg}	54.0^{def}	63.3^{b-e}	35.80^{gh}
R1A1	20.0^{abc}	23.7^{a}	25.7 ^{efg}	48.0^{gh}	56.3^{ef}	34.73^{hi}
R1A2	18.7^{abc}	21.7^{a}	24.7^{g}	50.7^{efg}	61.3^{cde}	35.40^{ghi}
R1A3	22.0^{a}	24.3^{a}	25.7 ^{efg}	54.3^{cde}	59.7^{de}	37.20^{d-g}
R1A4	18.7^{abc}	21.7^{a}	25.3 ^{efg}	58.0^{a-d}	67.0^{bc}	38.13^{c-f}
R2A0	17.0^{abc}	23.0^{a}	27.3^{efg}	55.3^{b-e}	61.3^{cde}	36.80^{e-h}
R2A1	18.3^{abc}	23.0^{a}	28.3^{def}	54.3^{cde}	61.0^{cde}	37.00^{d-h}
R2A2	16.7^{bc}	23.7^{a}	31.3^{cd}	62.3^{a}	70.0^{ab}	40.80^{ab}
R2A3	18.0^{abc}	24.0^{a}	30.7^{cd}	61.7^{a}	75.3^{a}	41.93^{a}
R2A4	19.3^{abc}	23.7^{a}	32.7^{abc}	59.0^{a-d}	62.7^{cde}	39.47^{bc}
R3A0	20.3^{ab}	23.0^{a}	25.3 ^{efg}	54.7^{cde}	60.0^{cde}	36.67^{e-h}
R3A1	19.0^{abc}	24.3^{a}	32.0^{bc}	58.3^{a-d}	64.0^{bcd}	39.53^{bc}
R3A2	17.0^{abc}	22.3^{a}	33.7^{abc}	59.3^{a-d}	63.3^{b-e}	39.13^{bcd}
R3A3	18.0^{abc}	19.3^{a}	35.3^{a}	60.3^{ab}	64.7^{bcd}	39.53^{bc}
R3A4	18.3^{abc}	22.0^{a}	32.3^{abc}	58.0^{a-d}	62.7^{cde}	38.67^{b-e}
R4A0	15.7^{bc}	21.3^{a}	32.0^{bc}	56.0^{b-e}	63.3^{b-e}	37.67^{c-g}
R4A1	16.3^{bc}	22.3^{a}	34.7^{ab}	59.0^{a-d}	63.0^{b-e}	39.07^{bcd}
R4A2	16.0^{bc}	21.7^{a}	27.0 ^{efg}	52.3^{efg}	59.7^{de}	35.33^{ghi}
R4A3	15.3^{bc}	20.0^{a}	33.3^{abc}	59.7^{abc}	64.7^{bcd}	38.60^{b-f}
R4A4	17.3^{abc}	22.0^{a}	30.7^{cd}	51.3^{efg}	60.3^{cde}	36.33^{fgh}
SE±	1.41	1.11	1.00	1.30	2.10	0.14

Means followed by the same letter(s) along column are not significantly different ($P > 0.05$). Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy, A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

received 100Gy in combination with SA concentrations. The other treatments resulted in lower leaf number with the lowest due to the Control (R0A0)(33.47 leaves) treatment (Table 4.2).

4.4 Number of Branches Per Plant

A comparison of the mean values of the branch number in the 4, 8, 12, 16 and 20 WAP is shown in Table 4.3. The data revealed that, there was no branch observed on the plants at 4 WAP.

At 8 WAP, the highest branch number observed due to R0A1 (0Gy + 0.01 % SA)(3.3 branches) was comparable with that due to other treatments that received R0A0 (3.0 branches) and that of R1A0, R1A1, R1A2 and R2A1 treatments (Table 4.3). The lowest branch number due to R4A3 and R4A4 (0.3 branch) was comparable with that due to all treatments that involved R3 and R4 (except R3A0 with 2.0 branches) combined with various SA concentrations. However, at 12 WAP, the lowest branch number due to R3A0 (3.0 branches) was significantly lower than that due to R3 and R4 combined with SA concentrations. At 16 WAP, the lowest branch number due to R1A4 (4.3 branches) was only significantly lower than that due to R2A2, R2A3, R2A4, R3A1, R3A2, R3A3 and R4A1 treatments. The highest leaf number was due to R3A2 treatment at 16 (15.0 branches) and 20 (27.0 branches) WAP and also in the combined ANOVA of the overall data. At 20 WAP, R0A4 treatment resulted in the lowest branch number which was comparable with that due to treatments that consisted of R0, R1 and R2A0 and R2A1

Table 4.3: Effect of Gamma Radiation and Sodium Azide on Number of Branches Per Plant of Pigeon Pea at Growth Stages.

Mutagen	Age of plant(WAP) Number of Branches per Plant					
	4	**8**	**12**	**16**	**20**	**Mean**
R0A0	0.0	3.0^{ab}	5.7^{c-g}	9.3^{a-g}	13.7^{ef}	6.33^{f-i}
R0A1	0.0	3.3^{a}	4.3^{e-h}	6.0^{d-g}	19.3^{cde}	6.60^{e-i}
R0A2	0.0	2.7^{abc}	3.3^{gh}	10.0^{a-g}	17.0^{c-f}	6.60^{e-i}
R0A3	0.0	2.3^{a-d}	4.7^{d-h}	5.3^{fg}	13.7^{ef}	5.20^{ij}
R0A4	0.0	2.7^{abc}	4.7^{d-h}	9.0^{a-g}	12.3^{f}	5.73^{hij}
R1A0	0.0	2.3^{a-d}	4.7^{d-h}	5.7^{efg}	17.3^{c-f}	6.00^{g-j}
R1A1	0.0	2.7^{abc}	9.0^{abc}	10.7^{a-f}	19.3^{cde}	8.33^{b-e}
R1A2	0.0	2.7^{abc}	7.3^{b-f}	9.7^{a-g}	17.7^{c-f}	7.47^{d-h}
R1A3	0.0	1.7^{cd}	6.0^{c-g}	7.7^{c-g}	15.0^{ef}	6.07^{g-j}
R1A4	0.0	1.7^{cd}	4.0^{fgh}	4.3^{g}	18.0^{c-f}	5.60^{hij}
R2A0	0.0	1.3^{cde}	5.7^{c-g}	8.3^{b-g}	16.7^{def}	6.40^{f-i}
R2A1	0.0	2.3^{a-d}	4.0^{fgh}	5.3^{fg}	15.0^{ef}	5.33^{ij}
R2A2	0.0	1.7^{cd}	8.3^{a-d}	14.0^{ab}	20.0^{b-e}	8.80^{a-d}
R2A3	0.0	1.7^{cd}	7.7^{b-f}	12.0^{a-d}	22.3^{a-d}	8.73^{a-d}
R2A4	0.0	1.7^{cd}	8.0^{b-e}	10.7^{a-f}	25.7^{ab}	9.20^{a-d}
R3A0	0.0	2.0^{bcd}	3.0^{h}	5.3^{fg}	18.7^{c-f}	5.80^{hij}
R3A1	0.0	1.3^{cde}	10.3^{ab}	13.0^{abc}	23.0^{a-d}	9.53^{abc}
R3A2	0.0	1.3^{cde}	9.0^{abc}	15.0^{a}	27.0^{a}	10.47^{a}
R3A3	0.0	1.3^{cde}	11.7^{a}	12.0^{a-d}	23.3^{abc}	9.67^{ab}
R3A4	0.0	1.7^{cd}	9.0^{abc}	$8.7b^{c-g}$	19.3^{cde}	7.73^{c-g}
R4A0	0.0	1.3^{cde}	7.0^{b-f}	10.0^{a-g}	22.3^{a-d}	8.13^{b-f}
R4A1	0.0	1.3^{cde}	8.7^{abc}	11.7^{a-e}	20.0^{b-e}	8.33^{b-e}
R4A2	0.0	1.0^{de}	10.3^{ab}	10.0^{a-g}	17.3^{c-f}	7.73^{c-g}
R4A3	0.0	0.3^{e}	6.3^{c-g}	6.0^{d-g}	15.7^{ef}	5.67^{hij}
R4A4	0.0	0.3^{e}	4.3^{e-h}	4.3^{g}	12.3^{f}	4.27^{j}
SE±	0.0	0.38	1.11	1.71	1.71	0.11

Means followed by the same letter(s) along column are not significantly different (P > 0.05). Note: R0 = 0 Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

treatments. However, other treatments of R2 and most of those consisting of R3 and R4 produced higher leaf numbers (Table 4.3).

The combined ANOVA of the data on branch number in pigeon pea showed that the highest number of branches due to R3A2 (10.47 branches) was only comparable with that due to R2A2, R2A3, R2A4, R3A1 and R3A3 treatments. The lowest branch number was due to R4A4 (4.27 branches) (Table 4.3).

4.5 Plant Height:

The mean comparison of plant height presented in Table 4.4 showed that plant heights in the various sampling dates differed significantly ($P \leq 0.05$). The plant heights were observed to fluctuate among treatments. Treatment R3A2 (150Gy+0.02%SA) produced the highest plant heights from 4 to 16 WAP and also when data was combined. Also, R4A2 had the lowest plant height from 4 to 16 WAP and in the combined ANOVA. At 4 WAP, the highest plant height observed due to R3A2 (150Gy + 0.02 % SA)(81.0 cm) was comparable with that due to other treatments that received R0A0 (0Gy + 0.00 % SA)(75.7 cm) and that of R0A2, R3A4 and R3A3 (Table 4.4). The lowest plant height due to R4A2 (200Gy + 0.02 % SA)(61.3 cm) was comparable with that due to all treatments except that of R0A0, R0A2, R2A1, R2A4, R3A2, R3A3, R3A4, R4A0 and R4A1 treatments.

Similarly at 8 WAP the lowest plant height due to R4A2 (200Gy + 0.02 % SA)(118.7 cm) was significantly lower than that due to most treatments involving R2 and R3 combined with SA concentration (Table 4.4).

Table 4.4: Effect of Gamma Radiation and Sodium Azide on Plant height (cm) of Pigeon Pea at Growth Stages.

Mutagen	Age of Plant (WAP) Plant Height per Plant					
	4	8	12	16	20	Mean
R0A0	75.7^{abc}	143.3^{a-e}	162.0^{b-e}	225.3^{f}	228.0^{f-j}	166.87^{e-i}
R0A1	64.0^{cd}	122.3^{def}	129.3^{f}	202.3^{h}	216.7^{hi}	146.93^{j}
R0A2	75.7^{abc}	140.3^{b-f}	160.7^{b-e}	239.0^{b-f}	257.7^{ab}	174.67^{c-f}
R0A3	69.7^{a-d}	131.0^{c-f}	148.0^{def}	191.3^{h}	253.3^{abc}	158.67^{i}
R0A4	69.0^{a-d}	132.3^{c-f}	164.7^{a-d}	193.3^{h}	245.3^{b-e}	160.93^{hi}
R1A0	64.7^{bcd}	136.0^{c-f}	163.3^{a-e}	225.0^{f}	245.7^{b-e}	166.93^{e-i}
R1A1	64.7^{bcd}	126.0^{def}	152.7^{c-f}	228.3^{ef}	248.3^{b-e}	164.00^{ghi}
R1A2	69.0^{a-d}	141.0^{b-f}	176.3^{abc}	204.0^{h}	238.7^{c-g}	165.80^{f-i}
R1A3	65.3^{bcd}	132.7^{c-f}	183.0^{ab}	226.7^{f}	232.7^{e-h}	168.07^{e-h}
R1A4	70.3^{a-d}	145.7^{a-e}	157.7^{b-e}	231.3^{def}	236.0^{c-g}	168.20^{e-h}
R2A0	72.0^{a-d}	151.3^{abc}	178.3^{abc}	248.7^{a-d}	237.0^{c-g}	177.47^{bcd}
R2A1	79.0^{a}	161.0^{ab}	190.3^{a}	245.7^{a-e}	246.7^{b-e}	184.53^{ab}
R2A2	73.0^{a-d}	143.0^{b-f}	167.7^{a-d}	247.7^{a-d}	246.7^{b-e}	175.60^{b-e}
R2A3	72.0^{a-d}	146.3^{a-d}	175.3^{abc}	226.7^{f}	253.3^{b-e}	174.73^{c-f}
R2A4	75.3^{abc}	151.7^{abc}	180.3^{ab}	256.7^{ab}	253.7^{abc}	183.53^{abc}
R3A0	72.0^{a-d}	144.3^{a-e}	168.3^{a-d}	250.0^{a-d}	251.3^{a-d}	177.20^{bcd}
R3A1	69.0^{a-d}	153.3^{abc}	181.0^{ab}	252.0^{abc}	244.7^{b-f}	180.00^{bcd}
R3A2	81.0^{a}	166.7^{a}	190.0^{a}	260.0^{a}	257.0^{ab}	190.93^{a}
R3A3	74.3^{abc}	145.7^{a-e}	171.3^{a-d}	240.0^{b-f}	265.7^{a}	179.40^{bcd}
R3A4	77.3^{ab}	152.3^{abc}	176.7^{abc}	247.3^{a-d}	247.3^{b-e}	180.20^{bcd}
R4A0	69.7^{a-d}	153.3^{abc}	181.7^{ab}	257.7^{ab}	256.7^{ab}	183.80^{ab}
R4A1	73.3^{a-d}	146.0^{a-e}	168.0^{a-d}	236.7^{c-f}	237.7^{c-g}	172.33^{d-g}
R4A2	61.3^{d}	118.7^{f}	129.0^{f}	196.3^{h}	226.7^{ghi}	146.40^{j}
R4A3	65.0^{bcd}	136.3^{b-f}	163.3^{a-e}	221.7^{ef}	234.3^{d-g}	164.13^{ghi}
R4A4	64.0^{cd}	121.3^{ef}	138.0^{ef}	206.7^{gh}	215.0^{i}	149.00^{j}
SE±	3.80	7.40	8.12	5.70	5.30	0.60

Means followed by the same letter(s) along column are not significantly different (P > 0.05). Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

At 12 WAP, the lowest plant height due to R4A2 (200Gy + 0.02 % SA)(129.0 cm) and R0A1 (0Gy + 0.01 % SA)(129.3 cm) was significantly lower than that due to R1, R2 and R3 combined with various concentrations of SA.

At 16 WAP, the lowest plant height due to R0A3 (0Gy + 0.03 % SA)(191.3 cm) was not significantly lower than those due to most R0 (0Gy) treatments and that due to R1A2 (50Gy + 0.02% SA)(204.0 cm). Most treatments that consisted of R1, R2 and R3 in combination with various concentrations of SA showed higher plant height than other treatments.

At 20 WAP, R4A4 treatment (200Gy + 0.04 % SA)(215.0 cm) resulted in the lowest plant height which was comparable with that due to treatments that consisted of R0A0, R0A1 and R4A2 treatments. The highest plant height due to R3A3 was comparable with that due to R0A2, R0A3, R2A4, R3A0, R3A2 and R4A0 treatments.

The combined ANOVA of the data on plant height showed that, the highest plant height due to R3A2 (150Gy + 0.02 % SA)(190.93 cm) was only comparable with that due to R2A1, R2A4 and R4A0 treatments. This was followed by that due to most treatments that consisted of R2 and R3 in combination with various concentrations of SA. The lowest plant height due to R4A2 was comparable with that due to R0A1 and R4A4 (Table 4.4).

4.6 Root Length:

Data recorded on root length is presented in Table 4.5. The root length differed significantly with treatments and particularly at 12 WAP showed that most treatments consisting of R2, R3 and R4 produced longer root length.

At 4 WAP and 12 WAP, the highest root length was that due to R2A1 (100Gy + 0.01% SA) with 16.3 cm and 40.0 cm respectively. While the least root length at 4 WAP was due to R4A3 (200Gy + 0.003% SA)(5.7 cm) compared to R0A0, R0A1, R0A2, R0A3, R0A4, R1A0, R1A1, R1A2, R1A4, R3A0, R3A1, R4A2 and R4A4.

At 8 WAP, the highest root length due to R2A0 (100Gy + 0.0% SA)(42.3 cm) was comparable to R1A2 and R1A4. The lowest root length recorded was due to R4A4 (200Gy + 0.004% SA)(22.3 cm) which was comparable with all the treatment that received R0 in combination with SA apart from other treatments.

In the 12 WAP, R0A0, R2A1, R2A4 and R4A4recorded the highest root length which was not significantly different from those due to R0A1, R1A4, R2A0, R2A2, R2A3, R3A0, R3A1 and R4A3. The least root length was that due to R1A0 which is comparable to R0A2, R0A3, R0A4, R1A1, R1A2, R1A3, R3A3 and R3A4.

The highest root length recorded at 16 WAP was that due to R4A4 (200 + 0.004% SA)(61.7 cm) and this was comparable to those of R2A1, R2A3 and R0A0 .While the least root length was that due to R0A2 (0Gy + 0.02% SA)(35.7 cm)

Table 4.5: Effect of Gamma Radiation and Sodium Azide on Root Length (cm) of Pigeon Pea at Growth Stages

	Age of Plant(WAP) Root Length per Plant					
Mutagen	**4**	**8**	**12**	**16**	**20**	**Mean**
R0A0	6.0^{f}	24.3^{ij}	40.0^{a}	56.7^{abc}	59.3^{a-d}	37.27^{b-f}
R0A1	9.7^{b-f}	25.0^{hij}	37.7^{ab}	48.0^{d-g}	54.0^{b-g}	34.87^{f-i}
R0A2	7.3^{def}	26.0^{g-j}	29.3^{d-h}	35.7^{k}	42.3^{h}	28.13^{n}
R0A3	7.3^{def}	28.7^{d-j}	25.3^{gh}	47.7^{d-g}	53.3^{b-g}	32.47^{h-l}
R0A4	8.3^{b-f}	26.0^{g-j}	28.3^{e-h}	39.7^{h-k}	45.0^{gh}	29.47^{mn}
R1A0	7.3^{def}	32.3^{b-g}	24.3^{h}	37.0^{ij}	46.7^{fgh}	29.53^{mn}
R1A1	8.0^{c-f}	34.0^{b-e}	27.7^{fgh}	44.7^{e-i}	56.0^{b-f}	34.07^{g-j}
R1A2	7.0^{ef}	37.7^{ab}	25.3^{gh}	37.7^{jk}	53.3^{b-g}	32.20^{i-m}
R1A3	10.7^{b-e}	35.0^{bcd}	27.3^{fgh}	50.0^{c-f}	61.0^{ab}	36.80^{c-g}
R1A4	8.0^{c-f}	37.7^{ab}	34.0^{a-e}	50.3^{c-f}	58.0^{a-e}	37.60^{b-f}
R2A0	10.0^{b-f}	42.3^{a}	34.7^{a-d}	47.3^{d-g}	55.7^{b-f}	38.00^{b-e}
R2A1	16.3^{a}	41.3^{a}	40.0^{a}	55.3^{abc}	62.7^{ab}	43.13^{a}
R2A2	11.7^{bcd}	27.0^{f-j}	37.3^{ab}	51.3^{b-e}	60.7^{abc}	37.60^{b-f}
R2A3	12.0^{bc}	25.0^{hij}	36.3^{abc}	57.3^{ab}	60.0^{a-d}	38.13^{bcd}
R2A4	12.7^{b}	29.7^{c-i}	40.0^{a}	51.0^{b-e}	62.0^{ab}	39.07^{bc}
R3A0	9.0^{b-f}	27.7^{e-j}	38.7^{ab}	53.0^{bcd}	58.7^{a-f}	37.40^{b-f}
R3A1	9.0^{b-f}	32.7^{b-f}	38.3^{ab}	46.7^{d-h}	50.7^{d-h}	35.47^{d-g}
R3A2	11.0^{b-e}	31.3^{b-h}	32.3^{b-f}	44.7^{e-i}	56.0^{b-f}	35.07^{e-i}
R3A3	12.0^{bc}	28.7^{d-j}	27.7^{fgh}	38.3^{ijk}	49.0^{e-h}	31.13^{klm}
R3A4	11.3^{b-e}	35.3^{bc}	33.3^{b-f}	41.0^{g-k}	53.3^{b-g}	34.87^{f-i}
R4A0	12.3^{bc}	33.3^{b-f}	31.0^{c-g}	42.0^{g-k}	51.0^{c-h}	33.93^{g-k}
R4A1	11.7^{bcd}	34.0^{b-e}	35.0^{a-d}	40.0^{h-k}	55.3^{b-f}	35.20^{d-h}
R4A2	9.0^{b-f}	27.3^{f-j}	32.3^{b-f}	41.7^{g-k}	46.3^{fgh}	31.33^{j-m}
R4A3	5.7^{f}	23.7^{ij}	34.3^{a-e}	43.3^{f-j}	47.7^{fgh}	30.93^{lm}
R4A4	8.3^{b-f}	22.3^{i}	40.0^{a}	61.7^{a}	67.0^{a}	39.87^{b}
SE±	1.30	1.89	1.90	1.88	2.83	0.18

Means followed by the same letter(s) along column are not significantly different (P > 0.05). Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

At 20 WAP, the highest root length due to R4A4 (200Gy + 0.04% SA)(67.0 cm) was not significantly different from those of R0A0, R1A3, R1A4, R2A1, R2A2, R2A3, R2A4 and R3A0.

The combined ANOVA of data on root length in Pigeon pea showed that the highest root length due to R2A1 (100Gy + 0.01% SA)(43.13 cm) was significantly higher than those of other treatments. The lowest mean value was that due to R0A2 (0Gy + 0.02% SA)(28.13 cm).

4.7 Yield Parameters

4.7.1 Days to 50% flowering

Data recorded for days to 50% flowering was observed to be significantly different among the treatments at $P \leq 0.05$ (Table 4.6). The highest days to 50% flowering was noted for Control treatment, R0A0 (0Gy + 0.0% SA)(108.7 days) which was similar to those due to R0A1 (0Gy + 0.01% SA)(93.7 days), R0A3 (0Gy + 0.03% SA)(101.3 days) and R4A4 (200Gy + 0.04% SA)(101.3 days) and which were significantly not different from R4A2, R4A3, R3A0 and R3A1 treatments. Meanwhile, the lowest days to 50% flowering observed with mutagenic treatment R1A0 (80.3 days) was only significantly lower than those due to R0A0, R0A3 and R4A4 (Table 4.6).

4.7.2 Number of seeds per pod

The mutagenic treatments R0A3 (0Gy + 0.03% SA)(9.0 seeds), R1A2 (50 Gy+0.02 % SA)(9.0 seeds) and R2A2 (100Gy + 0.02% SA)(8.3 seeds) resulted in the highest number of seeds per pod which was only significantly higher than those due to R3A2 (150Gy +

Table 4.6: Effect of Gamma Radiation and Sodium Azide on Some Yield Parameters of Pigeon Pea

Mutagen	DFF(Days)	NSP	NPP	PWT(g)	HSWT(g)	TGY kgha^{-1}	CP(%)
R0A0	108.7^{a}	6.7^{abc}	52.17^{bcd}	122.4^{h}	12.3^{ij}	636.00^{c-g}	18.9^{p}
R0A1	93.7^{abc}	8.0^{ab}	25.56^{kj}	186.1^{b-e}	13.7^{c-g}	418.00^{g-l}	18.6^{q}
R0A2	88.3^{bc}	8.0^{ab}	43.22^{ef}	188.1^{bcd}	12.4^{hij}	628.30^{c-h}	19.8^{o}
R0A3	101.3^{ab}	9.0^{a}	46.39^{de}	168.6^{b-g}	14.8^{a-d}	922.90^{b}	21.3^{g}
R0A4	82.0^{c}	6.7^{abc}	40.00^{efg}	169.9^{b-g}	13.8^{c-g}	540.50^{e-j}	20.0^{klm}
R1A0	80.3^{c}	8.0^{ab}	47.56^{dce}	170.2^{b-g}	14.5^{a-e}	801.40^{bcd}	19.4^{p}
R1A1	87.3^{bc}	8.0^{ab}	41.67^{efg}	139.8^{gh}	14.8^{a-d}	735.20^{b-e}	20.1^{klm}
R1A2	87.7^{bc}	9.0^{a}	27.89^{ikj}	181.3^{b-e}	12.8^{f-j}	482.60^{e-l}	24.0^{d}
R1A3	90.7^{bc}	7.7^{abc}	14.22^{l}	140.2^{gh}	13.9^{c-g}	234.90^{l}	21.8^{f}
R1A4	86.7^{bc}	8.7^{ab}	36.56^{fgh}	172.4^{b-f}	15.0^{abc}	699.90^{b-e}	21.3^{g}
R2A0	85.3^{bc}	8.0^{ab}	20.56^{kl}	155.8^{d-g}	15.4^{ab}	381.40^{h-l}	20.3^{jk}
R2A1	86.3^{bc}	9.0^{a}	72.72^{a}	286.8^{a}	13.2^{e-j}	1278.20^{a}	24.7^{b}
R2A2	89.7^{bc}	8.3^{ab}	40.17^{efg}	148.2^{fgh}	13.2^{e-j}	659.00^{c-g}	20.0^{lmo}
R2A3	89.3^{bc}	7.0^{abc}	31.11^{hij}	187.8^{bcd}	13.7^{c-h}	432.60^{f-l}	23.7^{de}
R2A4	90.0^{bc}	8.3^{ab}	37.61^{fgh}	164.6^{c-g}	14.8^{a-d}	684.20^{b-f}	24.4^{c}
R3A0	91.3^{abc}	5.0^{c}	31.11^{hij}	182.8^{b-e}	15.6^{a}	358.50^{i-l}	23.6^{e}
R3A1	93.0^{abc}	7.0^{abc}	51.89^{bcd}	196.0^{bc}	11.^{9j}	644.20^{c-g}	20.2^{jkl}
R3A2	87.3^{bc}	6.0^{bc}	51.67^{bcd}	184.4^{b-e}	13.9^{c-f}	639.70^{c-g}	20.6^{hi}
R3A3	81.7^{c}	8.0^{ab}	16.11^{l}	159.9^{d-g}	15.6^{a}	304.50^{jkl}	25.1^{a}
R3A4	81.7^{c}	7.7^{abc}	56.11^{b}	199.9^{b}	13.8^{c-g}	877.60^{bc}	18.7^{pq}
R4A0	83.3^{bc}	6.7^{ab}	34.17^{ghi}	186.6^{b-e}	12.6^{g-j}	442.00^{f-l}	20.4^{ij}
R4A1	85.0^{bc}	7.3^{abc}	54.44^{bc}	195.8^{bc}	14.6^{a-d}	866.40^{bc}	19.9^{lmo}
R4A2	93.7^{abc}	7.3^{abc}	31.83^{hij}	154.8^{efg}	14.1^{b-f}	499.20^{e-j}	20.4^{ij}
R4A3	94.0^{abc}	7.3^{abc}	35.556^{fgh}	179.0^{d-g}	15.6^{a}	595.40^{d-i}	20.0^{lmo}
R4A4	101.3^{ab}	8.0^{ab}	16.67^{l}	163.0^{b-f}	13.5^{d-i}	273.70^{kl}	20.7^{h}
SE±	4.21	0.80	2.47	9.30	0.25	9.37	0.09

Means followed by the same letter(s) along column are not significantly different (P >0.05)
Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.0% SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,
DFF = Days to 50% Flowering, NSP = Number of Seeds per Pod, NPP = Number of Pods per Plant, PWT = Pod Weight per Treatment, HSWT = Hundred Seed Weight Per Treatment, TGY =Total Grain Yield, CP = Crude Protein

0.02 % SA)(6.0 seeds) and R3A0 (150Gy + 0.00 % SA)(5.0 seeds) treatments. The lowest number of seeds per pod was observed with treatment R3A0 (150Gy + 0.00% SA)(5.0 seeds) (Table 4.6).

4.7.3 Number of pods per plant

The highest number of pods per plant observed with R2A1 (100Gy+ 0.01% SA)(72.72 pods) was significantly higher than that from the other treatments while the lowest number of pods per plant observed with R1A3(50Gy + 0.03% SA)(14.22 pods) was not significantly lower than that from R3A3 (150Gy + 0.03% SA)(16.11 pods), R4A4 (200Gy + 0.04% SA)(16.67 pods), and R2A0 (100Gy + 0.00% SA)(20.56 pods) (Table 4.6).

4.7.4 Pod weight

The observation on the Pod weight per treatment showed that there was significant difference between the mutagenic treatments means and the control at $P \leq 0.05$. The highest pod weight per treatment observed with R2A1 (100Gy + 0.01% SA)(286.8 g) was significantly higher than that from the other treatments. This was followed by that due to treatments that consisted of R3 in combination with SA and R4A0 and R4A1. The least pod weight per treatment observed with R0A0 (0Gy + 0.0% SA)(122.4 g) was only similar with that due to R1A3 and R2A2 treatments (Table 4.6).

4.7.5 Hundred seed weight

The difference in the hundred-seed weight per treatment was significant among the mutagenic treatments and the control ($P \leq 0.05$). The highest hundred-seed weight per treatment observed at R3A0 (150Gy + 0.00% SA)(15.6 g), R3A3 (150Gy+ 0.03%

SA)(15.6 g) and R4A3 (200Gy + 0.04% SA)(15.6 g) was similar to that due to R0A3, R1A0, R1A1, R1A4, R2A0, R2A4 and R4A1 treatments. The lowest observed with mutagenic treatment R3A1 (150Gy+ 0.00% SA)(11.9 g) was not significantly lower than that from R0A0 (0Gy + 0.00% SA) R0A2, R2A1, R2A2, and R4A0 treatments (Table 4.6).

4.7.6 Total grain yield (TGY)

The highest total grain yield observed in R2A1 (100Gy + 0.01% SA)(1278.20 $Kgha^{-1}$) was significantly higher than that due to all other treatments. This was followed by that due to R0A3 and most of the treatments consisting of R1, R2 and R3 in combination with the various concentrations of SA. The least grain yield observed with the treatment due R1A3 (50Gy + 0.03% SA)(234.90 $Kgha^{-1}$) was similar with that due to R0A1, R1A2, R2A0, R2A3, R3A0, R3A3, R4A0 and R4A4 (Table 4.6).

4.7.7 Determination of crude protein (CP)

The range of values obtained for crude protein is presented in Table 4.6below. The highest crude protein value was obtained from R3A3 (150Gy + 0.03% SA)(25.1 %) was significantly higher than that due to all the other treatments ($P \leq 0.05$). This was followed by that due to R2A1 (100Gy + 0.01 % SA)(24.7 %), R2A4 (100Gy + 0.04 % SA)(24.4 %), R2A3 (100Gy + 0.03 % SA)(23.7 %), R1A2 (50Gy + 0.02% SA)(24.0 %) and R3A0 (150Gy + 0.00 % SA)(23.6 %) treatments. The least crude protein obtained from treatment R0A1 (0Gy + 0.01% SA)(18.6%) was only similar to that due to R3A4 (150Gy + 0.04 % SA)(18.7 %) treatment (Table 4.6).

4.8 Correlation Analyses of Some Growth and Yield Components of Pigeon Pea

Correlation analyses carried out in respect of the traits studied in this study were between the growth and yield parameters are presented in table 4.7.

The germination percentage correlates positively with days to 50% flowering, number of seed per pod, number of pod per plants 100- seed weight and protein content. However, the correlations were not significant ($p > 0.05$).

The leaf number had significant correlations with number of branches ($p < 0.01$) and plant height ($p < 0.05$). The positive correlations were with number of branches, root length, plant height, number of seed per pod, pod weight, 100-seed and protein percentage, while the negative correlations were with germination percentage, day to 50% flowering, number of pod per plant total grain yield. Among the correlations, only the number of branches was strongly correlated.

The branch number had a weak, positive and significant ($p < 0.01$) relationships with leaf number and plant height. While, the correlations with other parameters were weak, the positive correlations were with root length, number of pods, pod weight, 100 - seed weight and protein percentage. The negative correlations were with germination percentage, days to 50% flowering, number of seed per pod and total grain yield. The correlation with germination was non-significant ($p > 0.05$).

The plant height correlated positively and significantly with leaf number at ($p < 0.05$), branch number and protein percentage ($p < 0.01$). It also correlates significantly but

Table 4.7: Correlation analyses of some growth and yield components of Pigeon pea

Variables	LN	BN	RLT	PLH	GP	DFF	NSP	NPP	PWT	HSWT	TGY
LN	1.000$^{n.s}$										
BN	0.304**	1.000$^{n.s}$									
RLT	0.089$^{n.s}$	0.096$^{n.s}$	1.000$^{n.s}$								
PLH	0.240*	0.320**	-0.165$^{n.s}$	1.000$^{n.s}$							
GP	-0.091$^{n.s}$	-0.227*	-0.060$^{n.s}$	-0.026$^{n.s}$	1.000$^{n.s}$						
DFF	-0.101$^{n.s}$	-0.118$^{n.s}$	0.227*	-0.287**	0.131$^{n.s}$	1.000$^{n.s}$					
NSP	0.085$^{n.s}$	-0.116$^{n.s}$	0.150$^{n.s}$	-0.005$^{n.s}$	0.205$^{n.s}$	0.016$^{n.s}$	1.000$^{n.s}$				
NPP	-0.077$^{n.s}$	0.060$^{n.s}$	-0.021$^{n.s}$	0.209$^{n.s}$	0.096$^{n.s}$	0.050$^{n.s}$	0.027$^{n.s}$	1.000$^{n.s}$			
PWT	0.184$^{n.s}$	0.093$^{n.s}$	-0.011$^{n.s}$	0.193$^{n.s}$	-0.135$^{n.s}$	-0.137$^{n.s}$	0.068$^{n.s}$	0.495**	1.000$^{n.s}$		
HSWT	0.048$^{n.s}$	0.115$^{n.s}$	0.024$^{n.s}$	0.069$^{n.s}$	-0.169$^{n.s}$	-0.005$^{n.s}$	-0.004$^{n.s}$	-0.226*	-0.098$^{n.s}$	1.000$^{n.s}$	
TGY	-0.026$^{n.s}$	-0.021$^{n.s}$	0.057$^{n.s}$	0.177$^{n.s}$	0.128$^{n.s}$	0.026$^{n.s}$	0.471**	0.858**	0.474**	-0.011$^{n.s}$	1.000$^{n.s}$
CP	0.132$^{n.s}$	0.196$^{n.s}$	0.272*	0.345**	-0.077$^{n.s}$	-0.092$^{n.s}$	0.115$^{n.s}$	-0.179$^{n.s}$	0.265*	0.193$^{n.s}$	-0.052$^{n.s}$

* - significant at 0.05

** - p<0.01

n.s - not significant

DFF = Days to 50% Flowering, NSP = Number of Seeds per Pod, NPP = Number of Pods per Plant, PWT = Pod Weight per Treatment, HSWT = Hundred Seed Weight per Treatment, TGY =Total Grain Yield, CP = Crude Protein, N = Number of Observations GP =Germination percentage, LN =Leaf Number, BN =Branch Number, RLT =Root Length, PLH =Plant Height

negatively with day to 50% flowering at ($p < 0.01$). The negative correlations were with germination percentage, root length, days to 50% flowering and number of seed per pod, while plant height correlates positively with leaf number, branch number, number of pod, pod weight, total grain yield and protein percentage. However, branch number and protein percentage were significantly correlated individually with plant height.

There was a positively significant relationship between root lengths and days to 50% flowering, and protein percentage, leaf number, number of seed per pod, 100- seed weight and total grain yield at $p < 0.05$ even though they were weak relationships. Meanwhile, correlations with other parameters were also weak. The negative correlations were with germination percentage, plant height, number of pods per plant and pod weight.

All the correlations with days to 50% flowering were weak. However, the relationships with root length were positive and significant ($p < 0.05$), while that with plant height was negative and significant ($p < 0.01$). Other positive correlations were with number of seeds per pod, number of pods per plant and total grain yield, while the negative correlations were with root length, number of seeds per pod, pod weight, 100- seed weight and protein percentage.

The plant height, root length, germination percentage, days to 50% flowering , number of pods per plant, pod weight, total grain yield and protein percentage correlates positively with number of seeds per pod, branch number, plant height and 100 – seed weight correlates negatively. The correlation of number of seeds per pod was strong, positive and significant at $p < 0.01$.

The number of pods correlates strongly, positively and significantly with pod weight and total grain yield ($p < 0.01$). Other positive correlations were with branch number, plant height, germination percentage, days to 50% flowering and number of seed per pod. Though, the correlations between number of pods per plant and 100- seed weight was negatively significant ($p < 0.05$), other negative correlations were with leaf number, root length and total protein percentage.

The pod weight correlates positively and significantly with number of pod per plant and total grain yield ($p < 0.01$). Other positive but weak correlations were with leaf number, branch number, plant height, number of seed per pod and protein percentage ($p < 0.01$). Though, correlation with protein percentage was at ($p < 0.05$). Pod weight correlates negatively with root length, germination percentage, days to 50% flowering and 100 - seed weight.

The 100 - seed weight correlates negatively and significantly with number of pod per plant ($p < 0.05$). All the correlations with 100 – seed weight were weak, however, the positive correlations with leaf number, branch number ,root length, plant height and protein percentage, while the germination percentage, days to 50% flowering, number of seed per pod, number of pod per plant, pod weight correlates negatively.

The total grain yield correlates strongly, positively and significantly with number of seed per pod, number of pod per plant and pod weight ($p < 0.01$). Other positive correlations were with root length, plant height, germination percentage and days to 50% flowering.

The leaf number, branch number 100 - seed weight and protein percentage correlates negatively.

Protein percentage had a strong, positive and significant correlations with plant height, at $p < 0.01$ and weak, positive significant correlations with root length and pod weight at $p < 0.05$. The leaf number, branch number, number of seed per pod and 100 – seed weight were also positively correlated, while germination percentage, days to 50% flowering, number of pod per plant and total grain yield correlates negatively.

4.9 Regression Coefficient Analysis

The Ordinary Least Square (OLS) model was applied to determine the contribution of the two mutagens; sodium azide and gamma radiation to the grain yield and protein content of the plant. The model was not found to be significant ($P > 0.05$). The coefficient of determination for the model was 0.017. For the protein content the coefficient of determination was 0.050 while the observed F-values for the models were 0.613 and 1.906 respectively. The obtained beta estimates for the yield is summarized in Table 4.8a while Table 4.8b presents the beta estimates for the protein mode.

From the tables the regression functional relationship between estimate factors and the yield of the plant could be estimated as Yield = 44.935 – 0.029 Sodium azide – 72.713 gamma radiation. The protein content could be express as Protein = 20.288 + 0.003 Sodium azide – 26.947 gamma radiation. From the tables, the contribution of the two factors was not significant to either the yield or protein content of the plant.

Table 4.8a: Summary of the regression estimates for the two factors contribution to the plant yield.

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	44.935	4.611		9.746	0.000
R	-0.029	0.029	-0.116	-0.989	0.326
A	-72.713	145.804	-0.058	-0.499	0.620

Dependent variable = Yield

R – Gamma Radiation

A – Sodium Azide

Table 4.8b: Summary of the regression estimates for the two factors contribution to the crude protein percentage.

Variables	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
(Constant)	20.288	0.493		41.120	0.000
R	0.003	0.003	0.105	0.911	0.365
A	26.947	15.602	0.198	1.727	0.088

Dependent variable = Protein

R – Gamma Radiation

A – Sodium Azide

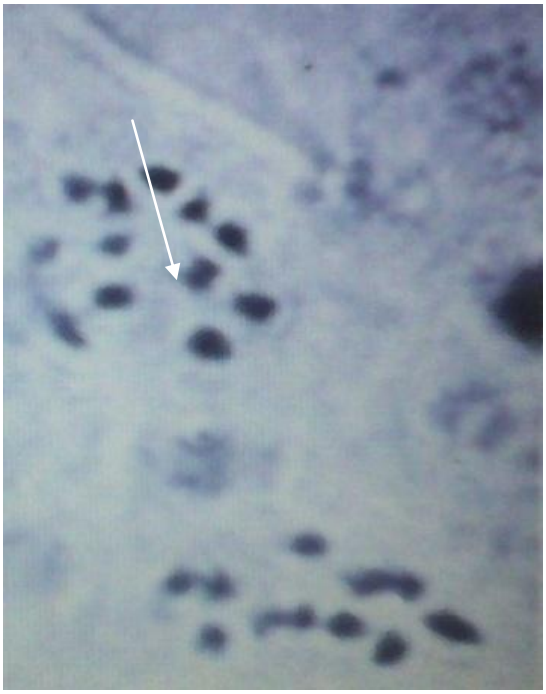
4.10 Observation of Meiotic Division

Analysis of the meiotic cell division in the pollen mother cells from pigeon pea plants grown from seeds treated with gamma rays and sodium azide visa-avis their combination offered a quick test for the effectiveness of the mutagens. Some chromosomal aberrations observed in this present study are shown below;

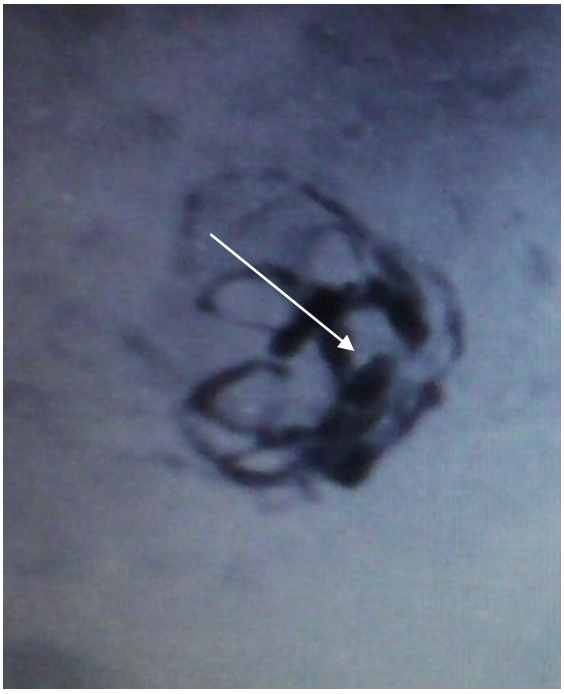
Plate I (a) reveals normal distribution of chromosomes at anaphase stage. Plate I (b) chromosomes are at resting or interphase stage in the control treatment. While in Plate I (c) the result also indicated normal separation of chromosomes at anaphase.

It must be stated here that the result obtained only revealed some chromosomal abnormalities in Plate II (a) Stickiness of chromosomes at telophase and Plate II (b and c) stickiness of chromosomes and disoriented or faulty polarization at anaphase.

Plate III (a) showing bridges at anaphase (b) single bridge and(c) showing stickiness of chromosomes at metaphase.



(a)



(b)

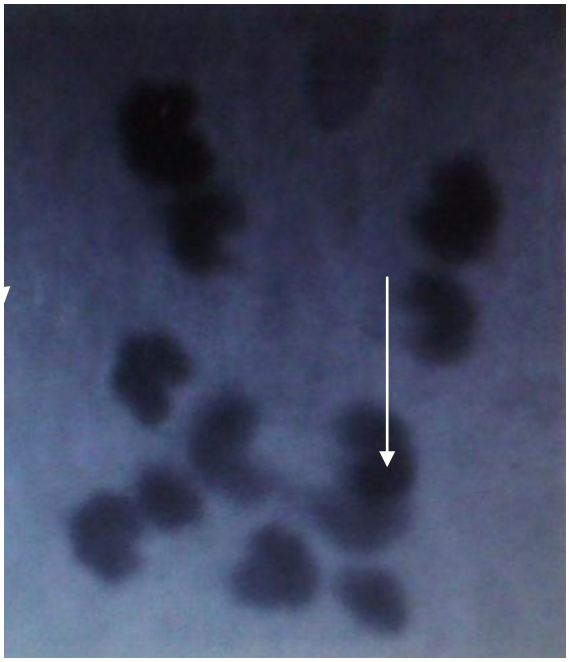


(c)

Plate I: Pollen mother Cells showing normal meiosis (control) (a) Normal distribution of chromosomes at anaphase (b) Diplotene bead – like chromosomes (c) Normal telophase II



(a)



(b)



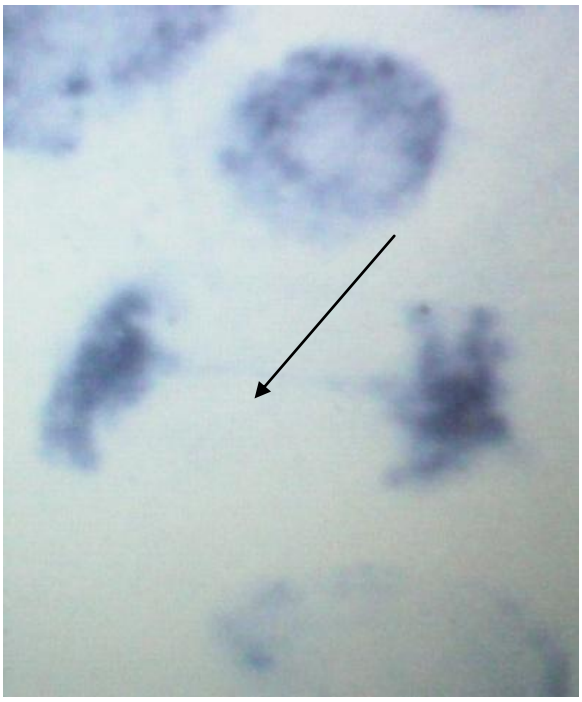
(c)

Plate II: Chromosomal abnormalities induced by gamma rays and sodium azide

(a) Chromosome in groups at metaphase (b) Pollen mother cell showing stickiness and precocious separation at metaphase (c) Sticky chromosomes at telophase I.



(a)



(b)



(c)

Plate III: Chromosomal aberration induced by Gamma rays and Sodium azide in (a) pollen mother cell showing bridges of chromosomes at anaphase (b) Pollen mother cell showing single bridge at anaphase I (c) Stickiness of chromosomes at metaphase.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

The seed germination percentage decreased with increasing doses of Gamma rays with the various concentrations of sodium azide in Pigeon pea. This clearly indicates that the mutagen had an inhibitory effect on seed germination. Similar inhibitory effects on seed germination by the mutagen have also been reported earlier by Apparao (2005) in cowpea, Khan and Wani (2006) in Mungbean.

The reduction in germination might be due to genetic and physiological processes inhibited by the mutagens. Decrease in percent seed germination in pigeon pea caused by Gamma Rays and Sodium azide could be attributed to physiological perturbations and partly to the chromosomal damages (Mensah *et al.*, 2007), Gaiward and Kothekar (2004) observed that with increase in concentration/doses, there was corresponding increase in damage to chromosomes in lentil after EI, NEU, EMS, SA and gamma rays treatments. According to Kumar and Yadav (2010), delayed and reduced seed germination could be due to delay or inhibition in physiological and biological process necessary for seed germination which includes enzyme activity. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009).

The increase in leaf number of plants under mutagens treatments with those receiving R2 and R3 with various concentrations of SA might be due to chromosomal aberrations that tend to produce an increase in certain morphological traits such as leaf number. Similar observation was reported by Khan *et al.* (2000) in Soya beans using ionizing radiation, with effect been dose-independent. The stimulatory effect might be due to an activation of growth hormones, auxin (Zaka *et al*., 2004).

There was increased branch number in most treatments (particularly, those consisting of R2 and R3) except in the mutagenic treatments with higher SA concentration R0A4 (0Gy+ 0.04% SA) and R4A4 (200Gy + 0.04% SA). This showed that the gamma ray at R4A4 (200Gy + 0.04%SA) exerted inhibitory effect on branches per plant, while lower treatment of SA showed profuse effect on branches per plant. Such mutations can be considered evolutionary conversion of the plant habit gene carrying substantial polygenic significance. Similar results were obtained by Chandirakala and Subbaraman (2010) who reported high magnitude of primary branches in pigeon pea.

There was increase in plant height with increasing gamma ray doses and sodium azide concentrations (especially, those consisting of R2 and R3 in combination with SA) compared to the Control except with mutagenic treatments R0A1 (0Gy + 0.01% SA) and R4A4 (200Gy + 0.04% SA), where there was reduction in plant height.

The increase in plant height by chemical and physical mutagens was ascribed to different factors resulting from the combined effect of the mutagens. Earlier studies by Markeen *et al.* (2007) reported reduction in plant height with higher doses of gamma rays, which was

attributed to gross injury caused at cellular level. The mean increase in plant height at maturity in the present study might be due to the alteration of their genome integrated by environmental signals as reported by Uno *et al.* (2001); probably by increasing the rate of cellular division and expansion at their meristematic regions. This is also in agreement with the findings of Hoballah (1999) who reported increase in plant height of Sesame due to radiation mutagenesis.

The reduction in root lengths in plants under R0, R1 and R4 in combination with SA concentrations was possibly due to the delay in root elongation. On the other hand, plants from seeds treated with 100Gy (R2) with its sodium azide concentrations showed increases in root length compared with other doses of radiation. This could be attributed to the reduced chromosomal disturbance during cell division and elongation.

The reduced number of days to 50% flowering in most of the treatments consistently shifted towards earliness. It is valuable in obtaining varieties associated with escape from pests, drought and other stress injuries that occur in late growing season. Maximum decreases in days to 50% flowering due to difference in the effects of mutagens which interfered with seed metabolism and onset of DNA synthesis as reported by Tambe (2009). However, the longer days to 50% flowering in the Control and treatments that consist of R0 (0Gy) was as a result of the absence of gamma irradiation/ SA treatment. Results obtained were in conformity with the work of Shinde (2007) in pigeon pea and Tambe (2009) in soya beans. There were positive relationships between days of 50% flowering

per plant, number of pods per plant, number of seeds per pod and seed yield. This position corroborates the earlier report of Udensi *et al*. (2012).

The number of seed per plant revealed that all the treatments, with the exception of R3A0 (150Gy + 0.0% SA), showed comparable increase thus indicating that SA has promoter effect on seed production. The enhancing effect may be due to sudden increase in the metabolic status of seedlings and increase in the activity of growth promoters' effects on seed production. Similar observation of increased number of seeds per pod was made by Biradar (2004), Shinde (2007) and Patil (2009).

The higher mean number of pods per plant observed at various concentration and the gradual decline at higher doses of gamma rays showed that higher concentrations of SA and dose of gamma radiation could result in the inhibition of physiological and biological processes necessary for pod formation which includes enzyme activity. Barshile *et al.* (2009) reported a similar observation in Soybeans.

Treatment with Sodium azide and gamma rays induced the production of higher pod weight per plant than the Control. The increase in pod weight due to all treatments of mutagens can be considered as evolutionary conversion of the plant habit genes carrying substantial polygenic significance stimulated by the mutagens (Barshille *et al.,* 2009). Number of pods per plant is one of the most important yield contributing traits which is closely and constantly correlated with the yield per plant. Similar increase in pod weight was observed by Khan *et al.* (2000).

The tendency of an increase in 100 - seed weight in pigeon pea by treatments that consisted of R1, R2, and R3 was probably due to the synergistic effects of the Sodium azide concentrations with the gamma doses which resulted in reduced injury at cellular level might have stimulated increase in 100 - seed weight. Such increase was reported by Biradar (2004), though contradicting Bale (1999) who observed a decrease in 100 seed weight.

Yield is a very important parameter in mutation breeding, because ultimately the plant breeder wants to improve the yield along with other beneficial traits, the observed increase in total grain yield per plant that received 0Gy + 0.03%SA, 50Gy +0.0%SA, 50Gy + 0.01%SA, 100Gy +0.01%SA, 100Gy + 0.03%SA,100Gy + 0.04%SA,150Gy + 0.04%SA, 150Gy + 0.01%SA, 150Gy + 0.02%SA, 150Gy +0.04%SA and 200Gy + 0.01%SA, could be attributed to the enhancing effect of the combinations of various doses of gamma rays with different concentrations of SA. However, the increase in mean values for quantitative traits could be due to the occurrence of polygenic mutations with cumulative effects according to Singh *et al*. (2000).

Correlation coefficients among most of the yield traits were statistically significant. Grain yield was positively correlated with number of pods per plant, number of seeds per plant, 100-grain weight. Pod weight was also positively correlated total grain yield and crude protein. Moreover root length also exhibited a significant positive association with grain yield which indicated efficient translocation of photosynthesis from source to sink. These results were in agreement with Kumar *et al.,* (2000); Singh *et al.,* (2006) and Bilgi (2006);

Singh and Chaudhary (1979) suggested that if the correlation coefficient between a causal factor and the effect (i.e. grain yield) is almost equal to its direct effect, then correlation explains the true relationship and direct selection through this trait will be effective.

In this present study, the mutagens had no direct positive effect on the yield traits, 0.326, 0.620, 0.365 and 0.088 respectively are statistically non-significant. On the other hand, negative coefficient -0.989, -0.499 for number of grain yield and crude protein percentage were in accordance with the findings of Abinasa *et al.* (2011).

Generally, there is a high performance in most of the mutagenic treatments over the Control treatment, with 18.9% crude protein. The higher seed protein content in plants with mutagenic treatments could be due to the genetic improvement brought about by the mutagenic treatments. Similar result was obtained by Shinde (2007) in Pigeon pea, Sagabe (2008) and Urdbean and Tambe (2009) in Soybean. It has been reported that protein production is directly linked with the quality of seeds; the better the quality of seed is, the more the production of protein. Therefore, in the case of increased protein content the seed quality also increased. It may also be due to interactions between genes and the environment (Singh *et al*., 1990). According to Khan *et al.* (2000), the improvement made in protein content through genetic manipulation could make it a good source of dietary protein to man. Protein is involved in chromosome organization needed for chromatic separation and segregation in plant.

Cytological analysis with respect to meiotic behaviour is considered one of the most dependable indices to estimate the potency of mutagens. It also provides a considerable

clue to assess sensitivity of plants for different mutagens. Physical and chemical mutagens are known to produce chromosomal aberrations leading to abnormal chromosome behaviour during meiosis.

In the present investigation, a vast array of meiotic aberrations was observed. All the mutagenic treatments induced different types of meiotic aberrations. The induction of cytological disturbances in the meiotic cell is of great value, as it results in genetic damage (possibly due to chromosomal aberrations) that gets transferred to the next generation (Kumar and Rai, 2007). Different types of chromosomal abnormalities observed during the present investigation have also been reported by different workers in various plant materials (Kumar and Verma, 2011). In the present study, the most frequent chromosomal aberrations in all the treatments were stickiness of chromosomes. Chromosomal stickiness is characterised by clustering of chromosomes during any phase of cell cycle and the number involved in such stickiness varied from two to whole chromosome complement and failed to disjunct individually. Stickiness could be due to the polymerization of nucleic acid occasioned by mutagenic treatments, leading to partial dissociation of nucleo-proteins and alterations in their pattern of organization. Stickiness has been common meiotic abnormality reported by various workers (Khan *et al.,* 2009); who suggested that stickiness might be due to disturbances in cytochemically otherwise balanced reactions. It could be also result from partial dissociation and altered pattern of organisation of nucleoproteins involved in chromosome organization leading to chromosome clumping.

Kumar and Rai (2007) suggested that stickiness occurred due to improper folding of chromosome fibres resulting in intermingling of fibres, with consequent chromosome attachment to each other by means of sub-chromatid bridges.

Precocious movement of chromosomes at metaphase stages observed in this present investigation might have resulted from disturbance to homology chromosome pairing or disturbed spindle mechanism as reported by Khan and Tyagi (2009).

Bridged chromosomes observed at anaphase during the present study might be due to sister chromatid exchange followed by delayed or failure of their separation at later stages. Bridge chromosomes might also occur as a result of delay in terminalisation, stickiness of chromosomal end or because of failure of chromosome movement (Bhat *et al.,* 2006). Kumar and Gupta (2009) reported that gene mutation or direct action of mutagen on the target protein, responsible for chiasmata terminalisation during diakinesis at meiosis -1, caused some structural defects in the protein which led to their improper functioning, thus resulting into bridges. Wani (2000) reported that gamma rays were more effective than chemical mutagens in causing chromosomal abnormalities.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The present investigation was conducted to elucidate the mutagenic effects of gamma rays and sodium azide in the local variety of pigeon pea, *Cajanus cajan* (L.) Millsp. Pigeon pea seeds were irradiated with five doses of gamma rays (0, 50, 100, 150 and 200Gy) and five concentrations of sodium azide (0.0%, 0.01%, 0.02%, 0.03% and 0.04%). The plants were raised during the rainy season of year 2011 at the Biological Sciences Departmental Garden, Ahmadu Bello University, Zaria.

The main objective of this study was to induce genetic variability in some quantitative traits and to isolate the promising mutants associated with increase in yield potentials of the crop. The significant findings are summarized as follows:

The mutagenic effect of the mutagens was evaluated on seed germination, number of leaves, branch number, plant height, root length and meiotic division in pigeon pea study of various quantitative traits

(a) Seed germination decreased with an increase in mutagenic treatments.

(b) Chromosomal aberrations or abnormalities increased with an increase in mutagenic treatments. Various meiotic aberrations induced by mutagens included stickiness and precocious separation of chromosomes leading to polyploidy plant with potential advantages (stronger progeny) over a diploid plant.

(c) The mean values for various quantitative traits showed stimulatory effects at 50Gy-150Gy with various concentrations of 0.01%-0.04% of SA showing earliness, profuse flowering and high yielding mutants.

(d) A positive correlation was observed between seed yield and many other quantitative traits like number of pods per plants, number of seeds per pod, and protein content.

6.2 Conclusions

The present findings lead to the following conclusions:

(a) Maximum frequency of mutations can be achieved in local variety of Pigeon pea by exposing it to lower doses and concentrations of gamma rays (below 200Gy) and sodium azide (below 0.04% S.A).

(b) The two mutagens affected the pigeon pea plant population morphologically as prominent Tall, High yielding, early flowering and profusely branching mutants were observed in the present experimental work.

(c) Beneficial genetic variability in yield parameters like increased number of seed/pods per plant, total and grain yield can be achieved by exposing pigeon pea to single or combined treatment of gamma ray (50Gy -150Gy) and SA (0.01%-0.04%).

(d) There are relatively High proteins content in the mutants by exposing pigeon pea to maximum doses and concentrations of sodium azide.

It is suggested that the mutants isolated in the present investigation would be of great utility in cultivation of Pigeon pea.

6.3 Recommendations

The overall results suggest that low doses/concentrations in both gamma rays and sodium azide gave superior performance in pigeon pea [*Cajanus cajan* (L) Millspaugh]. In view of this, it is recommended that lower doses/concentrations of two treatments (below 200Gy) and (0.04% SA) should be employed to enhance high grain yield/plant and high protein content.

Further work should be employed using lower doses/concentrations of gamma rays and sodium azide to determine the effects in inducing resistance against diseases and cooking time in pigeon pea.

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Appendix i



(a) (b)



(b) (d)

Plate iv: Pigeon pea plant on the field at 8 Weeks After Planting (WAP) (a) Control (b) Sodium azide treated plants (c) Gamma radiation treated plants and (d) Sodium azide and gamma radiation treated plants.