

**GENETIC VARIABILITY FOR YIELD AND OTHER AGRONOMIC TRAITS OF
GROUNDNUT(*ARACHIS HYPOGAEA* L.) AS INDUCED BY SODIUM AZIDE**

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**DEPARTMENT OF PLANT SCIENCE
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ZARIA, NIGERIA**

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**DEPARTMENT OF PLANT SCIENCE
FACULTY OF AGRICULTURE,
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ZARIA, NIGERIA**

FEBRUARY, 2020

DECLARATION

I declare that the work in this dissertation entitled ‘Genetic Variability for Yield and Other Agronomic Traits of Groundnut (*Arachis hypogaea* L.) as Induced by Sodium Azide has been carried out by me in the Department of Plant Science, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Ogban, Itunonun Ina

Name of Student

Signature

Date

CERTIFICATION

This dissertation entitled ‘Genetic Variability for Yield and Other Agronomic Traits of Groundnut (*Arachis hypogaea* L.) as Induced by Sodium azide written by me, meets the regulations governing the award of the degree of Masters of Science in Plant Breeding of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this research work to my Almighty and Everlasting God, the creator and owner of the entire universe.

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TABLE OF CONTENTS

TITLE PAGE	ii
DECLARATION	iii
CERTIFICATION	iv
DEDICATION	v
ACKNOWLEDGEMENT	vi
TABLE OF CONTENT	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xi
LIST OF PLATES.....	xii
LIST OF APPENDICES.....	xiii
ABSTRACT	xiv
CHAPTER ONE.....	1
1.0 INTRODUCTION.....	1
1.1 Research Problem.....	3
1.2 Justification.....	3
1.3 Objectives.....	4
CHAPTER TWO	5
2.0 LITERATURE REVIEW.....	5
2.1 Groundnut.....	5
2.2 Improvement of Groundnut Through Conventional Approaches.....	8
2.3 Mutation Breeding as Groundnut Improvement Tools.....	11
2.4 Mutagenic Agents for Creating Variation in Groundnut	14
2.4.1 Mutagenesis.....	Physical 14

2.4.2	Chemical Mutagens for Creating Variation in Groundnut.....	15
2.5	Suitability of Groundnut to Mutation Breeding	18
2.6	Frequency, Effectiveness and Efficiency of Mutagens.....	19
2.7	Heritability and Genetic Response Among Mutants.....	19
CHAPTER THREE		21
3.0 MATERIALS AND METHODS		21
3.1	Study Area.....	21
3.2	Experimental Materials and Description.....	21
3.3	Development of Experimental Population.....	22
3.4	Evaluation	22
3.4.1	Laboratory Evaluation.....	22
3.4.2	Field Evaluation of M_1	23
3.4.3	Field Evaluation of M_2	23
3.5	Data Collection	23
3.5.1	Field Data for M_1	23
3.5.2	Field Data for M_2	24
3.5.3	Statistical Analysis	25
3.5.4	Determination of effective doses.....	28
3.5.5	Estimation of Component of Variation.....	28
CHAPTER FOUR		30
4.0 RESULTS		30
4.1	Effect of Sodium azide on Germination of Two Groundnut Genotypes Conducted in Groundnut Improvement Laboratory Zaria, 2017.....	30
4.2	Analysis of Variance for Seedling Parameters of Two Groundnuts Genotypes Subjected to Sodium Azide Treatments in M_1	32
4.3.1	Mutation Frequency in M_1 generation	34
4.3.2	Mutagenic Effectiveness and Efficiency in M_1 Generation.....	34

4.4 Mean Performance of Seedling Triats in M ₁ Progenies of Mutants.....	37
4.5 Analysis of Variance for Agronomic Traits Measured at M ₂ Generation	37
4.6 Mean Performance of Agronomic Traits for the Top Ranking M ₂ Progeny of Mutants ...	41
4.7 Estimates of Component of Variation.....	42
4.8Superior Mutants Resulting From Treatment with Sodium Azide.....	45
CHAPTER FIVE	48
5.DISCUSSION	48
CHAPTER SIX.....	52
6.0SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	52
6.1Summary	52
6.2Conclusion	53
6.3Recommendations	53
References.....	54
List of Appendices	64

LIST OF TABLES

Table 3.1 Features of the selected groundnut genotypes used.....	21
Table 3.2. Form of analysis of variance (ANOVA) and expected mean squares for traits in M ₁	27
Table 3.3. Form of analysis of variance (ANOVA) and expected mean squares for the mutants in M ₂	27
Table 4.1 Mean squares for seedling parameters of two groundnuts genotypes subjected to Sodium azide treatments in M ₁	33
Table 4.2 Effect of different doses of Sodium azide treatment on the mutagenic frequency, effectiveness and mutagenic efficiency of M ₁ groundnut.....	36
Table 4.3 Mean performance of seedling traits in M ₁ progenies of mutants derived from SAMNUT 22 and SAMNUT 24 at different levels of treatment with Sodium azide treatment.....	38
Table 4.4 Mean squares for effects of Sodium azide on groundnut in M ₂ generation.....	40
Table 4.5 Effect of Sodium azide treatments on the mean performance of 15 top ranking M ₂ progeny of mutants.....	42
Table 4.6. Estimate of Variance Components for M ₂	44
Table 4.7 Superior mutants resulting from 6 hours of treatment with Sodium azide.....	46
Table 4.8 Superior mutants resulting from 12 hours of treatment with Sodium azide.....	47

LIST OF FIGURES

Figure1.1 Effect of Sodium azide treatment on the germination of two groundnut genotypes after 6hrs.....	31
Figure1.2 Effect of Sodium azide treatment on the germination of two groundnut genotypes after 12hrs.....	31

LIST OF PLATES

Plate 1. Reduced pod yield modified by mutagen.....	72
Plate 2. Increased pod yield modified by Mutagen.....	72
Plate 3. SAMNUT 22 Control.....	72
Plate 4. Increased number of branches and leaves modified by mutagen.....	72
Plate 5. Reduced number of branches and leaves modified by mutagen.....	72
Plate 6. SAMNUT 24 Control.....	72
Plate 7. SAMNUT 22 Control.....	73
Plate 8. Small size pods.....	73
Plate 9. Big size pods.....	73
Plate 10. Big seeds obtained as a result of mutation.....	73
Plate 11. Small seeds obtained as a result of mutation.....	73
Plate 12. SAMNUT 22 Control.....	73
Plate 13. Pictures of Laboratory evaluation.....	74
Plate 14. Cross section of the M ₂ groundnut population evaluated in Samaru 2018.....	74

LIST OF APPENDICES

Appendix A. Effect of Sodium Azide treatments on the mean performance of M ₂ progeny of mutants derived from SAMNUT 22 and SAMNUT 24 evaluated in IAR Samaru 2018.....	65
Appendix B. Summary Statistics for the Effect of Sodium Azide treatments on the mean performance of M ₂ progeny of mutants derived from SAMNUT 22 and SAMNUT 24 evaluated in IAR Samaru 2018.....	68
Appendix C. Names of mutant.....	69
Appendix D. List of plates.....	72

ABSTRACT

Groundnut improvement, through conventional method of breeding may not be able to create desired variability on which a robust breeding programme could be built. Also, groundnut as a self- pollinating crop naturally would have less variability in its gene pool. Thus, the present study was conducted (i) To determine the sensitivity of two groundnut genotypes to different treatment doses of Sodium azide (ii) To determine the effective treatment dose of Sodium azide for induction of variability among the genotypes, and, (iii) To evaluate variability for some agronomic traits modified by Sodium azide treatment. Two groundnut genotypes SAMNUT 22 and SAMNUT 24, obtained from IAR were treated with 10 mM, 20 mM, 30 mM, 40 mM, and 50 mM of Sodium azide concentration at the groundnut improvement lab for 6 and 12 hours. Each of the treated seeds and the controls were sown directly to well-prepared seed bed in the field to obtain the M_1 plants. During the off season, a set of 10 seed each were picked from each treatment for seed multiplication. A total of 24 M_1 families were evaluated in a Randomized Complete Block Design(RCBD) with two replications in 2018, while a total of 72 M_2 families were also evaluated in a 9 x 12 augmented design with 4 checks. Data were collected and significant differences ($P < 0.05$) were observed for the effect of Sodium azide treatment on emergence count, and number of pods per plot (NPP) for all genotypes. Highly significant differences ($P < 0.01$) were observed for number of days to 50% flowering, number of branches, plant height and 100 seed weight for all the genotypes. Highly significant ($P < 0.01$) difference for genotype by dose interaction was observed for number of pods per plots (NPP). SAMNUT 24($t = 1.2$) was more sensitive to Sodium azide treatment than SAMNUT 22($t=0.5$). The mutagenic frequency recorded in the present investigation ranged from 29 % to 5 %. The mutagenic frequency varied across the different concentration, also a decreasing trend with increase in concentration of the mutagen was observed. The mutation frequency was found to be low

possibly because of low physiological damage and the low response of groundnut at the maturity stage. The higher effectiveness and efficiency of Sodium azide treatment observed at 10 mM for 12 hours of SAMNUT 24 indicates that lower doses were more effective in inducing variability in agronomic traits of groundnut. A decrease in mutagenic effectiveness with increase in concentration dose of the mutagen was also observed across the genotypes this is as a result of the failure in proportional increase of mutation frequency with the increase in concentrations/doses of the mutagen. The differences in the mutagenic effectiveness of the genotypes studied indicate that the genotypes responded differently to Sodium azide treatment. The Mutagenic efficiency did not follow any particular trend (increase or decrease), it varied across the mutagenic treatments. A high significant difference was observed among treatments, controls and interaction of emergence count and number of seeds per pod (NSP) in the M_2 . Analysis of variance for the different traits revealed highly significant difference among the 2 genotypes for all the agronomic traits studied. The existence of these differences indicates the presence of sufficient genetic variability among the genotypes for these traits. The phenotypic component of variation estimates, were generally higher than the Genotypic component of variation estimates for all the studied traits except for plant height which had higher genotypic component of variation, thus indicating a level of environmental influence on the expression of the traits. The M_2 mutant, 10 mM6 SAMNUT 24 at 6 hours recorded the highest 100-seed weight (123.0 g) among the other mutants while 50 mM2 SAMNUT 22 at 6 hours recorded the highest shelling percentage (93.0). Mutant 50 mM1 SAMNUT 24 at 12 hours had the highest number of pods per plot (386.6), mutant 10 mM3 SAMNUT 24 at 6 hours had the highest plant height (33.6), mutant 40 mM2 SAMNUT 22 at 6 hours had the least days to 50% flowering (28), Mutant 20 mM4 SAMNUT 24 at 12 hours had the highest pod yield per plot. The mutants had higher and better value than their controls, thus the presence of variability induced by Sodium azide on the agronomic traits. For the improvement of cultivated groundnut, it is pertinent to

use diverse collections with more variability and also employ the use of varying concentration, for the purpose of variety development and for use in future breeding program.

CHAPTER ONE

1.0 INTRODUCTION

The groundnut (*Arachishypogaea*L.) belong to a specie in the legume family (Fabaceae). It is an important oilseed legume grown worldwide and is known by many other local names such as earth nuts, pea nuts, goober peas, monkey nuts, and pig nuts. It is grown both for domestic market and for export. The world groundnut production was estimated to be 47million metric tons in 2017, and the world groundnut exports totals approximately 1.9 million metric tons in 2017, FAOSTAT (2017). The world leading producers are China with 36 % of the global total, followed by India with 20%, other significant producers are USA followed by Nigeria in the fourth position and the largest producer in Africa(FAOSTAT,2018). Groundnut is the 5th most widely grown crop in sub-Saharan Africa behind maize, sorghum, millet and cassava, where it is grown exclusively for domestic use, either for consumption or as cash crop for smallholder farmersUSAD, (2013)

Groundnut is a nutritive crop with approximately 25% protein and 45 – 50% oil. The testa of groundnut seed is rich in vitamin B and it is used as a base ingredient for cosmetics. It also provides important ingredients in numerous industries for sweet, ice-cream, coating, peanut butter and bakery products(Skoric *etal*, 2008).Groundnut protein is of high biological value as it contains more protein than meat, about two and a half times more than eggs and far more than any other vegetable foods except soya bean and yeast(Skoric *etal*, 2008). The residue of the extraction process is used as commercial groundnut cake which is a concentrate feed for livestock and poultry. The nuts are eaten raw or after roasted as snacks. The green leaves or shoot makes excellent fodder and hay for animals Mensah *etal*,(2007)

Crop improvement by mutagenesis has been applied in a number of crops for yield improvement, creation of new cultivars, stress and drought tolerance, disease resistance and for horticultural or floriculture purposes(Rinse, 1985).

Chemical mutagens are one cause of mutations in living organisms. Many of such chemicals have clastogenic (chromosome damaging) effect on plants via reactive oxygen - derived radicals (Yuan and Zhang, 1993). These effects can occur both spontaneously and artificially following induction by mutagens. Chemical mutation generally produces induced mutation which leads to base pair substitution, especially GC-AT resulting in amino acid change, which changes the function of proteins but do not abolish their functions as deletions or frame shift mostly do (Van Harten, 1996). These chemo-mutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants.

Sodium azide (NaN_3) is a chemical mutagen and has been found to be one of the most powerful chemical mutagens that decrease cyanide resistance and respiration in tobacco callus (Wang *et al.*,2011). It is known to be highly mutagenic in several organisms, including plants and animals (Rinse,1985) and its mutagenic potential has been reported in many screening assays. Sodium azide is marginally mutagenic in different organisms (*let al.*, 1980), such as *Drosophila* and *Arabidopsis* (KamraandGallopudi, 1979; Gichner and Veleminsky, 1977). The mutagenicity is mediated through the production of an organic metabolite of azide compound (Owais and Kleinhofs, 1988) this metabolite enters into the nucleus, interacts with DNA and creates a point mutation in the genome. Being a strong mutagen in a plant, it affects the different partsof the plant and their growth development phenomena by disturbing the metabolic activity.

1.1 Research Problem

Though, groundnut is an economic crop with lots of industrial potentials and capacity to fit into the array of crops for food security and poverty eradication among the teeming population of the poor small holder farmers, the crop is faced with a number of challenges that has contributed to its decline and low production in sub-Saharan Africa (Misari *et al.*, 1980). Low yields, abiotic stress, pest and diseases are major problems facing the crop. Crop improvement, through conventional method of breeding may not be able to create desired variability on which a robust breeding programme could be built. Also, groundnut as a self-pollinating crop naturally, would have less variability in its gene pool and thus limiting the number of natural varieties for which breeders could screen and exploit for improvement purposes. Badigannava and Murty (2007)

1.2 Justification

Mutation breeding in crop plants is an effective approach in the improvement of crops having narrow genetic base such as groundnuts (*Arachis hypogaea* L.). The low genetic variability for the traits of importance in groundnut, and the polyploidy nature, are a bottleneck to the groundnut improvement (Nigam, 2000). The cultivated accessions of groundnut in the gene bank, and the advanced breeding lines in the breeding programs are the most frequently used sources of variability used as parents in hybridization. Often time's selection of an appropriate source to be used as parent in hybridization is challenging due to inadequate variability and amenability of the trait for improvement. Thus, much of the variability remains poorly used in improvement program Nigam (2000). Radio-mutagenesis is known to significantly improve the frequency of induced changes. It can thus be used to widen the genetic variability to be integrated in many varietal selections. Determination of effective concentration dose is prerequisite for mutation breeding and development of genetic variability, by induced mutation (Lukanda *et al.*,

2012). The quantum and quality of genetic variability is essential for continued crop improvement. Hence the present study which attempts to evaluate the genetic variability for yield and other agronomic traits of groundnuts as induced by Sodium azide.

1.3 Objectives

- I. To determine the sensitivity of two groundnut genotypes to different treatment concentrations of Sodium azide.
- II. To determine the effective treatment concentration of Sodium azide for induction of variability among the genotypes.
- III. To evaluate variability for some agronomic traits modified by Sodium azide treatment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Groundnut

Groundnut (*Arachis hypogea* L) is an important oilseed crop and grain legume grown worldwide. The groundnut seed has dual advantage of being important as a source of edible oil as well as protein. However, it is self-pollinating and possesses limited variability. Despite its long history of cultivation, broad sub-specific variability and wide geographic distribution of the cultivated groundnut, defects in its genetic composition with respect to requirement of man are wide spread and for many of these no remedial resources are known to exist among its varietal forms(Brock,1977).

The peanut, also known as groundnut or the goober and taxonomically classified as *Arachis hypogaea*L. is a legume crop grown mainly for its edible seeds. It is widely grown in the tropics and sub-tropics, being important for both small and large commercial producers. World annual production of shelled groundnut was 47 million metric tons in 2017 (FOASTAT,2017). The groundnut pods are formed underground rather than above ground, this characteristic made the botanist Linnaeus to assign the name hypogea, which means under the earth to it(FOASTAT,2017). Groundnut is the 5th most widely grown crop in the Sub-Saharan Africa behind maize, sorghum, millet and Cassava, where it is grown exclusively for domestic use, either for consumption or as cash crop for small holder farmers (USAD, 2013).

In Africa, groundnut seems to have been introduced by the Portuguese into Senegal and Gambia from where it spread to the different parts of Africa (FAOSTAT 2010). Ranking 4th among the world oilseed crops and 13th among food crops, is the cultivated groundnut (*Arachis hypogea* L.). It is an important cash crop currently grown in over eighty

countries/regions from 40°N to 40°S across tropical and warm temperate regions (Mcgill, 1983). In developing countries, a large portion of groundnuts are crushed for edible oil (Wang *et al.*, 2012). Foods uses of groundnut are predominant in developed nations where high oleate not only means better keeping quality but also brings about multiple health benefits, for example, reduced risk of cardiovascular disease, increased sensitivity to insulin, preventive effects on tumorigenesis, and amelioration of some inflammatory diseases (Fang *et al.*, 2012).

Nigeria has more area under groundnut cultivation in Africa followed by Sudan. Groundnut crop is widely grown in Nigeria covering over two million hectares of land with major concentration in the northern region of the country encompassing the Sahel (12°-14°N), Sudan (10°- 13°N) and Northern half of the Northern Guinea Savannah (8°- 11°N) where the rainy seasons last for a period of 75-150 days with annual precipitation ranging between 500mm-1400mm and pod yield of about 1000kg/ha (Olorunju *et al.*, 1999; Udom *et al.*, 2009).

Groundnut is and has always been an important crop in Nigeria where it is grown largely under smallholder system and rain-fed conditions (Echekwu, 2010). Until 1974, Nigeria was the leading exporter of groundnut and amounted for about 70% of total export earnings, making it then, the country's single export crop before the unprecedented epidemic of rosette in 1975 and the prevalence of rust and leaf spot diseases which resulted in the considerable decline of its production (Upadhyaya *et al.*, 2001).

Recently, the use of groundnut meal is becoming more recognized not only as dietary supplement for children on protein poor cereals-based diets but also as effective treatment for children with related malnutrition (Rao, 1988). Its protein content is of high biological

value as it contains more protein than meat, about two and half times more than eggs and far more than any other vegetable foods except soybeans and yeast (Rao, 1988).

In Nigeria, groundnut provides high quality cooking oil and is an important source of protein for both human and animal diet and also provides much needed foreign exchange by exporting kernels and cake (Nautiyal, 1999). As population continues to grow the demand for edible oil in many developing countries such as Nigeria will also continue to grow.

Groundnut will continue to be important in satisfying this growing demand because it is adaptable to a wide range of environments, from sandy soils of the sahel to a favorable irrigated area (FAO, 1994).

All parts of the groundnut plants are valuable (Abalu and Harkruss, 1978). The grains can be used to feed domestic birds. Before harvesting, the roots of groundnut fix atmospheric nitrogen by symbiosis association with the bacterium, (Rhizobium) thereby reducing the application of chemical fertilizer. Groundnut haulms also known as fodder, is regularly used to feed sheep, goats and cattle's, and also serve as fuel for heating (Brock, 1977). The most constituents of the groundnut are essential amino acids such as lysine and glutamine. Other components are crude fiber, crude protein and fat (FAO, 1994). In industries, groundnut has a variety of uses. Groundnut oil contributes to making paint, varnish, lubricating oil, leather dressings, polish, insecticides and nitroglycerin. Soap is also made from the sludge and or saponified oil, and many cosmetics contain groundnut oil and its derivatives. The protein portion is used in the manufacturing of plastics, wall boards, abrasives, cellulose (used in rayon and paper), and glue (FAO, 1994).

In most culinary uses, groundnut is classified as a nut because it behaves much more like nuts in the kitchen than other legumes, such as cowpea, soybeans common beans, etc.

Indeed, groundnut refers more to the underground location favored by the pods of this legume (Wang *et al.*,2012). This distinction is important, given that people with nut allergies can eat groundnut safely. Conversely, people who are allergic to the consumption of groundnut can eat other nuts with little or no health concern (Boshou *et al.*,2009) however, it should be noted that excessive consumption of groundnut, like virtually any other food, may result in unexpected metabolic challenges especially constipation. The value and utilization of groundnut as a crop for both nutritional purposes primarily depend upon the quality of its composition (Boshou *et al.*,2009).

2.2 Improvement of Groundnut Through Conventional Approaches.

Groundnut is an important oil seed crop that is known to have narrow genetic base (Knauff and Gorbet, 1989). The genetic diversity in groundnut is low due to origin of the crop through a single hybridization event between two diploid species followed by a chromosome doubling and crossing barriers with wild diploid species (due to ploidy differences)(Nigam,2000). The cultivated species is an allotetraploid while all wild *Arachis* species are diploid. The low genetic variability for the traits of importance and polyploidy nature are a bottleneck to the groundnut improvement (Nigam,2000).

The cultivated accessions of groundnut in the genebank, and the advanced breeding lines in the breeding programs are the most frequently used sources of variability used as parents in hybridization. Often time's selection of an appropriate source to be used as parent in hybridization is challenging due to inadequate variability and amenability of the trait for improvement. Thus, much of the variability remains poorly used in improvement program (Nigam, 2000).

Only three disease resistant parents were reported to exist in India, thus ensuring genetic diversity in farmers' fields is another important challenge to the breeder when limited

number of sources is known. Understanding the nature of variability of a trait is also important to select the breeding scheme to be employed for the improvement of the target trait (Reddy, 1988).

The genotype by environment has considerable influence on the progress of crop improvement and hence an important aspect for consideration. In groundnut, a majority of target traits of economic importance are polygenic and are highly influenced by environment that hinders the achievable genetics gains in breeding programs. Genetic analysis of yield revealed high influence of environment on pods yield (Zang *et al.*, 2011). High yielding cultivars with the least genotype by environment interactions are normally desirable. However, when a cultivar is to be selected for a specific environment, the genotype by environment interaction is desirable for maximizing production.

The breeding methods used for self – pollinated crops are applied in groundnut breeding. They include, mass selection, pedigree, bulk, single seed descent, and back cross method. Introduction and mass selections played an important role in the beginning, but later hybridization followed by selection in segregating generations following different methods were predominantly practiced in breeding improved groundnut varieties. Emasculation and pollination procedures of hybridization are cumbersome and the success rate of making crosses is generally low, particularly when carried out by inexperienced hands. Another major challenge in groundnut breeding like in many other crops is the time (8 years or more) lag between hybridizing two parents and identification of an improved breeding line for release as variety. In countries like India, Vietnam, Indonesia and African countries, lack of adoption of new varieties has been a major bottleneck to improved groundnut yields (Knauff and Gorbet, 1989).

Reliable and repeated phenotyping remains the key to the success of any crop improvement program, whether following conventional or molecular breeding approach (Halward *et al*, 1991) Several critical decisions in the process of breeding rely on results obtained from phenotyping. Phenotyping is important, first to identify a suitable source / donor for the trait and second for selection and advancing the plants/progenies through the generations in the breeding cycles. Selecting a plant/ progeny with desired combination of traits and rejecting the undesirable remains a challenging task in breeding programs as selections have to be done on a large number of plants/ progenies with due consideration to a large number of traits simultaneously.

Following the breeding procedures and using various techniques of phenotyping, several improved varieties were developed and released for cultivation worldwide. Since 1986, national partners in 36 countries in collaboration with International Crop Research Institute for the Semi – Arid tropics (ICRISAT) have developed and released 136 groundnut varieties in their respective countries (IPGRI, 1992). The genetic gain for yield in India came from improvement in seed size, seed weight and number of pods per plant (Ratnakumar *et al*,2012). Reddy, (1989) reported that improved varieties alone contributed to 30 % yield increase in India since 1967.

Following extensive screening of 5000 breeding lines and germplasm in wilt sick plots in china and Indonesia, many genotypes with varying levels of resistance have been identified and using them, bacterial wilt resistance cultivars were released in China, Indonesia, and other South East Asian countries that have offered protection against loses caused by the disease (Mehen *et al* 1993: Wang *et al.*, 2012).International Crop Research Institute for the Semi – Arid tropics(ICRISAT) made significant efforts in developing advance breeding lines that are resistance to rust and late leaf spot (LLS) and drought resistance linesresistance(Branch and Kvien,1992). The improved lines were released for cultivation

in several countries of Asian and Africa or have been used as parents in national breeding programs(Singh *et al*,2003).In China, for example several new groundnut cultivars with improved productivity and some level of tolerance to aflatoxin contamination are extensively used in production in combination with integrated management approaches for aflatoxin management(Boshou *et al*, 2009).

2.3 Mutation Breeding as Groundnut Improvement Tools.

Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents(Roychowdhury *et al.*, 2012).

Mutation breeding offers the possibility of inducing desired attributes that cannot be found either in nature or have been lost during evolution (Roychowdhury and Tah, 2013).

Mutation breeding is an alternative technique that was used in many areas of groundnut improvement program since conventional breeding program through crossing and selection is difficult in groundnut (Sorouet *et al.*, 1999).

Mutation breeding employs three types of mutagenesis. These are (i) induced mutagenesis, in which mutation occur as a result of irradiation (gamma rays, X-rays, ion beam, etc) or treatment with chemical mutagens; (ii) site directed mutagenesis, which is the process of creating a mutation at a defined site in a DNA molecule;and (iii) insertion mutagenesis, which is due to DNA insertions, either through genetic transformation and insertion of T-DNA or activation of transposable elements (Kharkwal, 2012)

Plant breeding requires genetic variation of useful traits for crop improvements. However, multiple mutants alleles are the source of genetic diversity for crop breeding as well as functional analysis of the targeted gene in many cases. The key point in mutation breeding

is the process of identifying individuals with a target mutation, which involves two major steps; mutant screening and mutant confirmation(Forster and Shu, 2012). Mutant screening is a process involving selection of individuals from a large mutated population that meet specific selection criteria, e.g. early flowering, disease resistance as compared to the parents. However, these selections are often regarded as putative mutants or false mutants. Mutant's confirmation on the other hand is the process of re-evaluating the putative mutants under a controlled and replicated environment using large samples. Through this process, many putative mutants are revealed to be false mutants. In general, the mutations that are important in crop improvement usually involve single bases and may or may not affect protein synthesis (Mba , 2013)

The history of plant mutation can be traced back to 300 BC with reports of mutant crop in China(Kharkwal, 2012, Van Harten, 1996). Mutation as a mechanism of creating variability were first identified by Hugo de Vries in the late nineteenth century, while experimenting on the rediscovery of Mendel's laws of inheritance(Kharkwal, 2012). He considered this variability as heritable changes by mechanism, very distinct from segregation and recombination. He described this occurrence as swift changes in organisms, which were hereditary and thus produced relatively large effects on the phenotypic appearance of organism. He then coined the term ' mutation' and presented an integrated concept concerning the occurrence of sudden, shock-like changes (leaps) of existing traits which leads to development of a new species and variation. Radiation – induced mutation as a novel genetic variability in plants advanced as a field after the discovery of the mutagenic action of X-rays demonstrated in maize, barley and wheat by Stadler(Stadler, 1990).

The first commercial mutant variety was produced in tobacco in 1934. Prior to 1995, Acquah, (2006), reported 77 cultivars that were developed via mutagenesis. In 1995,

the number of commercially released varieties increased to 484. This number has sharply increased since with new mutants varieties being continuously reported in different continents. Some of the plants include: fruit trees(e.g apple, citrus, peach), ornamentals (e.g chrysanthemum, dahlia,poinsettia), food crops(e.g rice, barley, wheat, corn, pea) etc. Agronomic traits modified due to mutation breeding include lodging resistance, early maturity, winter hardiness, product quality (e.g, protein and lysine content) and numerous ornamental mutants.Bhagwat *et al.*, (1997) reported radiation induced 12 mutants of groundnut varieties were improved with distinct morphological differences. Theses mutants and the parents showed characteristics bond differences Mensah and Obadoni (2007) reported mutagenic effects of different concentration of sodium azide on groundnut genotype, increased the possibility of evolving higheryield variants through increased heritability and genetic gain(Ahmed and Mohammed 2009), and Badigannavar and Murty (2007) reported a high heritability associated with a high genetic advance for plant height, pod yield and seed yield in gamma rays induced mutants of groundnut at M8 generation. Kaveraand Nadaf (2008) observed highly significant variation for palmitic, steric, oleic, linoleic, and arachidic oil on ethyl methane treated M4 mutants of groundnut with increased oil content. The oil content was reported to be of high quality than the parents.

Mondal *et al*, (2006) isolated 47 breeding mutants with various morphological traits at M3 through gamma rays and sodium azide mutagenesis of them. TGM 192M had high oil content with increased oleic/linoleic acid (O/L) ratio than parents.

As a breeding tool, mutagenesis became very popular from the 1950s onward when a large range of crop and ornament plant species were predominantly treated by irradiation to increase trait variation (Oldach,2011)

2.4 Mutagenic Agents for Creating Variation in Groundnut

Agents of artificial mutations are called mutagens. They are generally grouped into two broad categories, namely: chemical mutagens and physical mutagens. Traditionally, to induce mutations in crops, planting materials are exposed to physical and chemical mutagenic agents. Mutagenesis can be performed with all types of planting materials, e.g. whole plants usually seedlings and *in vitro* cultured cells. Nevertheless, the most commonly used plant material is seed. Multiple forms of plant propagules, such as bulbs, tubers, corms and rhizomes and more recently, the induction of mutations in vegetatively propagated plants is becoming more efficient as scientists take advantage of totipotency (ability of single cell to divide and produce all of the differentiated cells in organism to regenerate into whole plants) using single cells and other forms of *in vitro* cultured plant tissues (Mba,2013).

2.4.1 Physical Mutagenesis

In the past 80 years, physical mutagens, mostly ionizing radiations have been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis (Mba *et al*,2012). X-rays were the first physical mutagen to be used to induce mutations. Since then, various subatomic particles (neutrons, protons beta particles and alpha particles) have been generated using nuclear reactors (Acquaah,2006). It has high penetrating potentials and is hazardous. However, it can be used for irradiating whole plants and delicate materials such as pollen grains. Various mutants have been developed through gamma radiation (Mahadevappa *et al*, 1983). The mutagenic effect results mostly from DNA double strand breaks. The mutants show higher potential for improving plant architecture leading to better crop improvement and are used as a complementary tool in plant breeding (Khin,2006). Gamma rays have a shorter wave length and therefore, possess more energy than protons and x-rays, which give them ability to

penetrate deeper into tissue (Amano,2006). Ionizing radiations cause mutations by breaking chemical bonds in the DNA molecule, deleting a nucleotide, or substituting it with a new one (Acquaah,2006). He also pointed out the importance of radiation being applied at the proper dose, a factor that depends on radiation intensity and duration of exposure. The exposure may be chronic (continuous low dose administered for a long period) or acute (high dose over a short period). The quality of mutation (proportion of useful mutations) is not necessarily positively correlated with dose rate. It is common knowledge that high dose may not necessarily yield the best results (Acquaah,2006). The mutagen dose used should be a compromise between mutation load and the chance to find desirable mutations, and this greatly depends on the cost effectiveness of selection. Screening of larger mutant populations that originate from a lower mutagen dose may be feasible for traits with simple phenotypic selection criteria, such as early maturity(Patilet al, 2009). On the other hand, screening the same population for complex phenotypic traits such as seed protein quality would not be feasible(Patil et al., 2009). The advantage of using physical mutagenesis compared to chemical mutagenesis is the degree of accuracy and sufficient reproducibility, particularly for gamma rays which have a uniform penetrating power in tissues(Yamaguchi et al,2003).

2.4.2 Chemical Mutagens for Creating Variation in Groundnut

Chemical mutagens like ethyl methane sulphate, (EMS), Ethidium bromide, acryflavin (Levy, 1976), diethyl methane sulphate (DES), N-nitroso-N-methyl-Urea and sodium axide, have been used to create genetic variability in groundnut (Mensah and Obadoni., 2007).

The effect of chemical mutagens on plant materials is generally considered milder(Acquaah,2006). An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. The ratio of mutational to undesirable

modifications is generally higher for chemical mutagens than for physical mutagens (Acquaah, 2006). Usually, the material is soaked in a solution of the mutagen to induce mutations. However, chemical mutagens are generally carcinogenic and therefore, extra care must be taken for health protection during the procedure. Material and safety data sheets for the specific chemical mutagen chosen should be carefully read and the agent should be appropriately inactivated before disposal. Despite the large number of mutagenic compounds, only a small number has been tested in plants (Wani *et al.*, 2014). Among them, only a very restricted group of alkylating agents has found large application in plant experimental mutagenesis and plant mutation breeding. Over 80% of the registered new mutant plant varieties reported in the International Atomic Energy Association (IAEA) database (IAEA, 2015) obtained via chemical mutagenesis were induced by alkylating agents. Of these, three compounds are significant: ethyl methane sulphonate (EMS), 1-methyl-1-nitrosourea and 1-ethyl-1-nitrosourea, which account for 64% of these varieties. One of the most effective chemical mutagenic groups is the group of alkylating agents which reacts with the DNA by alkylating the phosphate groups as well as the purines and pyrimidines (Acquaah, 2006).

Another group is that of the base analogues (they are closely related to the DNA bases and can be wrongly incorporated during replication). A clear advantage of the point mutations created by chemical mutagens is their potential to generate not only loss-of-function but also gain-of-function phenotypes, if the mutation leads to a modified protein activity or affinity, like tolerance to the

herbicide glyphosate or sulphonylurea shown in the legume *Medicago truncatula* (Oldach, *et al.*, 2008). The concentration of the mutagen, the length of treatment and the temperature at which the experiment is carried out affect the efficiency of mutagenesis. As chemical

mutagens are very reactive, it is important to use fresh batches of the chemical(s) that have been appropriately stored.

Another chemical commonly used in creating variation in plants is Sodium azide. It is a very potent mutagen in microorganism and a very efficient mutagen in barley as well as in some other crop species; however, it is marginally mutagenic in mammalian systems and not at all mutagenic in *Neurospora* species. The mutagenicity of Sodium azide is mediated through the production of an organic metabolite of azide (Gaikwad and Kothekar, 2004). The high frequency of mutations that were induced by this chemical, the low frequency of chromosome aberrations, and its low toxicity for human health, makes sodium azide a particularly efficient mutagen that is very useful for practical (Kleinhofs *et al.*, 1978).

Azeez *et al.*, 2014, conducted a study on “The alkylating effect of Sodium azide on two varieties of groundnut (SAMNUT10 and SAMNUT 20) on the nut and nutrient content”. The result of the quantitative / vegetative and seed related character evaluated at maturity shows that there was significant difference in the response of two groundnut varieties to Sodium azide concentration. Generally, early maturing plants were obtained with all treatments concentrations in relation to control, nonetheless, higher doses of 40-50mM plants were significantly faster in maturity for both varieties and treatment, concentrations.

The primary yield evaluation test was conducted in 2011 in Laixi, Shandong, with three mutant lines derived from Huayu 22 (a Virginia type peanut cultivar) seeds performed well. 11-L36, a line developed through treatment of Huayu 22 peanut seeds with 0.39% Sodium azide, out yielded the local control Fenghua 1 by 27.04% (kernel yield). 11-L39 and 11-L40, both bred through treatment of Huayu 22 peanut seeds with 0.39% diethyl sulphate (DES), had 37.60% and 22.60% more kernel yield than Fenghua 1. Groundnut variety “Golden” has been developed through induced mutation following pedigree method from M₁ to M₇ generations. Groundnut mutants with different traits have been developed

through induced mutation and their direct and indirect utilization has resulted in the release of commercial varieties (Murty *et al*, 2004). Improvement in seed yield and its components through induced mutation have also been reported in chickpea and mung bean (Khattak *et al* 2007) ;Khattak *et al.*, 2008). “Golden” manifested improvement in the form of increase in seed size and pods per plant. Large– seeded mutant breeding lines was also detected in the “Georgia Brown” cultivar of peanut (Branch,2002). The distinct character of “Golden” is its reddish seed coat color which distinguishes it from the rest of the groundnut varieties cultivated in the area. Earlier many seed coat colors have been identified through induced mutations in groundnut (Suvendu *et al.*, 2007). Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and varieties. While many researchers found chemical mutagens to be more effective than physical ones (gojones and Reddy 2000; Bhat *et al*,2005), and many other researchers found the reverse case (Zeerak,1991). A number of workers have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetative and sexually propagated plants (Kleinhofs *et al.*,1978). Hence this study will be employing the use of sodium azide, to create mutation in groundnut.

2.5 Suitability of Groundnut to Mutation Breeding

Groundnut has been found suitable for mutagenesis as a result of its self-pollinating specie and the easily exposed embryo to mutagens (Nigam,2000). The low genetic variability present in groundnut due to its origin through a single hybridization event, between two diploid species followed by a chromosome doubling and crossing barriers with wild diploids species (due to ploidy differences)has been a major problem for groundnut improvement Janila *et al.*, (2013). Consequently, introgression of some of the economic important traits through natural and /or interspecific hybridization is limited (Busisiwe *et*

al., 2015) it is therefore important to introduce mutagenesis in groundnut in order to create genetic variability.

2.6 Frequency, Effectiveness and Efficiency of Mutagens

Mutation frequency provide vital information about how often a mutation may be expressed in a particular genetic group(Yoon *et al.*,2009). Mutation frequency is defined as the proportion of mutant cells in a population. It is distinguished from mutation rate which relates to the rate at which mutation events arise, and is generally expressed as event per cell division. Mutation frequency is simply the ratio of mutants / total cells in the population.Mutagenic effectiveness refers to the rate of point mutations relative to dose, whereas mutation efficiency refers to the rate of point mutations relative to other biological effects induced by the mutagen. The biological damage was measured in terms of injury (reducing height), lethality (% survival) at 30 days after sowing in the field and emergence count. The term effectiveness refers to the frequency of mutations induced by unit dose of mutagens, whereas efficiency refers to the proportion of mutations in relation to other associated undesirable biological effects like lethality induced by the mutagens. Determination of the effective concentration dose is prerequisite for mutation breeding and development of genetic variability by induced mutation (Mondal *et al.*, 2006).

2.7 Heritability and Genetic Response Among Mutants

Heritability expresses the reliability of the phenotypic value, as a guide to breeding.Characters with high heritability can therefore be improved rapidly through selection than those with low heritability, since the later are influenced by environmental factors. Prakash *et al.*,(2000), evaluated 91 spreading groundnut cultivars and observed heritability in broad sense was high for pod yield, oil percentage and 100-kernel weight. The association of high heritability and high genetic advance was observed in pod yield, pods and 100-kernel weight. (Hamid *et al.*, 2006) also obtained higher heritability and

genetic advance in percentage of mean for plant height, pod number, kernel and pod yield. Mensah and Obadoni (2007) reported increase in genetic parameters such as heritability and genetic gain for pods per plant and seed per plant of groundnut under chemical treatment (Sodium azide) at M₂ generation and indicated the possibility of evolving higher yield variants through proper crop selection. Badigannavar and Murty (2007) reported a high heritability associated with a high genetic advance for plant height, pod yield and seed yield in gamma rays induced mutants of groundnut at M₈ generation. Singh(2005)studied 163 genotypes of groundnut for different characters. High heritability and high percentage of genetic advance were recorded for number of pods and pod yield per plant.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Area

The research was conducted at the Institute for Agricultural Research Farm Samaru, Zaria (11°11'N 7°38'E, 686m above sea level, 1200 mm annual rainfall) situated in the northern Guinea savannah of Nigeria. The average annual rainfall is about 105kmm which is distributed within 160 days (May – September) and the dry season period starts at about middle of October to the end of April. The relative humidity varies greatly, falling during the dry season and rising during the rainy season with an annual relative humidity of 54%.

3.2 Experimental Materials and Description

The experimental materials comprised of two genotypes of groundnuts, SAMNUT 22 and SAMNUT 24. The features of the materials are as described in Table 3.1.

Table 3.1 Features of the selected groundnut genotypes used

S/N	Parameters	SAMNUT 22	SAMNUT 24
1.	Source	IAR. Zaria	IAR. Zaria
2.	Maturity	Medium maturing (115-120 days)	Extra-early maturing (80-90 days)
3.	Pod yield (t/ha)	2.5t/ha	2-2.5t/ha
4.	Haulm yield (t/ha)	4-5t/ha	2.5-3t/ha
5.	Oil content (%)	51%	53%

*IAR- Institute for Agricultural Research. Annual report 2011

3.3 Development of Experimental Population

Two hundred (200) seeds each of SAMNUT 22 and SAMNUT 24 were soaked in freshly prepared Sodium azide solution at 10mM, 20mM, 30mM, 40mM and 50mM concentration levels for 6hours and 12hours, According to Azeez, *et al*(2014). Another set of 200 seeds were soaked in water for the same hours; this will serve as the control. After the desired soaking time elapsed, the seeds were brought out and rinsed thoroughly in running tap water. A set of 30 seeds each were picked from the treated seeds and used for germination / sensitivity test, while the remaining seeds were sown directly to well-prepared seed beds of 15m long using a spacing of 25cm inter row. Each plot was labeled appropriately. The chemical preparation was done at the Tissue culture laboratory using Beakers, measuring cylinder, distilled water, weighing balance, magnetic stirrer and bottles. The soaking and germination test was done at the groundnut improvement laboratory using petri dishes and filter papers. One hundred (100) treated seeds, each of the different treatments were sown in the field. Each row of 15m represented a treatment. This was done to raise the M_1 generation, the M_1 seeds were planted to raise the M_2 plants.

3.4 Evaluation

3.4.1 Laboratory Evaluation

Thirty (30) seeds were picked from each of the treatments that is; 10 mM, 20mM, 30mM, 40mM, and 50mM to conduct the sensitivity and germination test in the groundnut improvement laboratory. A set of 30 untreated seeds were also picked to serve as control. Fifteen(15) seeds were placed in a petri dish which has slightly watered filter paper. Germination count was done on daily bases and the final count was done at the end of 10 days.

3.4.2 Field Evaluation of M₁

The M₂ generation was raised by growing M₁ seeds to progeny rows. The field was sprayed with glyphosate to suppress the growth of weeds before planting. The M₁ seeds were sown in the field at a spacing of 30cm within rows and 75cm between rows. The plant progenies were carefully evaluated and screened from the day of emergence for agromorphological and seedling traits. Plots were laid out using Randomized Complete Block Design (RCBD) with two replications. Each plot (one row, 5m long) corresponds to a treatment per genotype. Manual hoe weeding was carried out at three and six weeks after sowing. Ridge moulding was done at 8 weeks after sowing to allow for the penetration of pods. This was followed by hand-pulling of weeds at 10 weeks after sowing. Fertilizer application was done immediately after the first hoe weeding. NPK 15-15-15 and single super phosphate at the rates of 30 kg/ ha-1 and 60 kg/ ha-1 respectively were applied. Harvesting was carried out at maturity by carefully digging out the pods. This was followed by manual picking of the pods.

3.4.3 Field Evaluation of M₂

Seventy-two (72) M₂ Plant progenies were evaluated in the field using a 9 x 12 augmented design. The seeds were sown at a spacing of 0.75m between rows and 0.30m within rows. The plot size was a single 5m row seed bed. The recommended cultural practice as done in M₁ was followed in the M₂ evaluation.

3.5 Data Collection

3.5.1 Field Data for M₁

The following seedlings data were collected:

- **Laboratory germination percentage:** This was done by counting the number of germinated seeds on daily basis for 10 days in the laboratory. A percentage was then gotten at the end of 10 days.
- **Emergence rate:** Emerged plants were counted at 15th day after sowing and the percentage calculated using the following formula:

$$\text{Emergence rate} = \frac{\text{Number of plants emerged}}{\text{Total number of seeds sown}} \times 100$$

- **Survival percentage:** Observation on the survival of seedlings was recorded 30 days after sowing (DAS) and survival percentage calculated by using the following formula:

$$\text{Survival Percent} = \frac{\text{Number of seedlings at 30 DAS}}{\text{Total number of germinated seeds}} \times 100$$

- **Seedling height (cm):** Height of the main axis from ground level to the apical leaflet was measured 30 days after sowing.

3.5.2 Field Data for M₂

The following yield and morphological data were collected

- **Emergence rate:** Emerged plants were counted on the 15th day after sowing and the percentage calculated using the following formula:

$$\text{Emergence rate} = \frac{\text{Number of plants emerged}}{\text{Total number of seeds sown}} \times 100$$

- **Plant height (cm):** Height of the main axis from ground level to the apical leaflet for each plant was measured at maturity.
- **Number of branches per plant:** Number of branches borne on the main axis for each plant was recorded.

- **Days to 50% flowering:** The time taken in days from sowing for 50% of the population to produce flowers.
- **Number of pods per plot:** The total number of matured pods per plot was recorded at the time of harvest.
- **Number of seeds per pod:** Dried pods were shelled and the number of seeds in a pod were counted.
- **100 seeds weight (g):** The weight of 100 seeds collected.
- **Shelling percentage (%):** This is the proportion of the seeds to the shell of groundnut. This was calculated as follows:

$$\text{Shelling percentage} = \frac{\text{Seed weight}}{\text{Pod weight}} \times 100$$

- **Pod yield per plot (g) :** Total yield of pods per plot was measured.

3.5.3 Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) to test the significance of difference among the genotypes using the general Statistical Analysis System (SAS) software package. Multiple comparisons of the various means were conducted based on the procedure described by Dunnett, (1985). The mean value, Coefficient of Variation and LSD at 5 % level of probability were estimated for each trait.

The statistical model used for the analysis of variance is the Generalized Linear Model (GLM) for randomized complete block design described by Kaps and Lamberson,

(2004).**Note:** This model was used separately to analyze for 6 hours and 12 hours' treatment respectively.

$$Y_{ijk} = \mu + G_i + D_j + R_k + (GD)_{ij} + E_{ijk}$$

Where,

Y_{ijk} = an observation in treatment i and block j

μ = the overall mean

G_i = Effect of treatment i th variety, $i = 1, 2$

D_j = Effect of j th Conc of Soduim azide $j = 1, 2, 3, 4, 5$

R_k = Effect of k th replication, $k = 1, 2$

$(GD)_{ij}$ = interaction effect of the i th variety with j th dose.

ε_{ijk} = random error with mean 0 and variance σ^2

Both varieties and doses was treated as fixed effects, since the varieties and doses were selected for the purpose of the study (Obi, 1986).

Table 3.2. Form of analysis of variance (ANOVA) and expected mean squares for traits in M₁

Source of variation	Degree of Freedom	Mean squares	EMS
Replications	(<i>r</i> - 1)		
Genotypes	(<i>g</i> - 1)	<i>m_g</i>	$\sigma_e^2 + rd\sigma_g^2 + \sigma_e^2 + rd\sigma_{dg}^2 + \sigma_e^2$
Doses	(<i>d</i> - 1)	<i>m_d</i>	$\sigma_e^2 + rg\sigma_d^2 + \sigma_e^2 + rd\sigma_{dg}^2$
Genotypes × doses	(<i>g</i> - 1)(<i>d</i> - 1)	<i>m_{dg}</i>	$\sigma_e^2 + rd\sigma_{dg}^2$
Error	(<i>dgr</i> - 1)	<i>m_e</i>	σ_e^2

This ANOVA table was used separately to analyze for 6 hours and 12 hours' treatments.

Where;

r = number of replications

g = number of genotypes

σ_e^2 = error variance σ_g^2 = Genetic variance among genotypes

σ_d^2 = Dosage variance among genotypes σ_{dg}^2 = genotype x doses variance among varieties

Subscript = observed mean squares of the subscripted effect

Table3.3. Form of analysis of variance (ANOVA) and expected mean squares for the mutants in M₂.

Source of Variation	Degree of Freedom	Mean Squares	EMS
Block	(<i>b</i> - 1)		$\sigma_e^2 + 4\sigma_b^2$
Entries	(<i>e</i> - 1)	<i>m₅</i>	
Check	(<i>c</i> - 1)	<i>m₄</i>	$\sigma_e^2 + \sum_{i=1}^4 U_{i^2..} - \frac{1}{4} \left(\sum_{i=1}^4 U \right)^2$
Mutants	(<i>m</i> - 1)	<i>m₃</i>	$\sigma_e^2 + \sigma_g^2$

Check x Mutants	1	m_2	
Error	$(b-1)(c-1)$	m_1	σ_e^2

Where;

b = number of blocks; e = number of entries

c = number of checks; m = number of mutants

σ_e^2 = error variance;

σ_g^2 = Genetic variance among mutants

σ_b^2 = variance among blocks

m Subscript = observed mean squares of the subscripted effect.

3.5.4 Determination of Effective Doses

1. Estimation of Mutation Frequency

Mutation frequency was estimated using the formula described by Sasikala and Kamala (1988).

Mutation frequency (%) = number of mutants / Total number of plant scored X 100

2. Estimation of Mutagenic Effectiveness and Efficiency

The mutagenic effectiveness and efficiency were estimated using the formula suggested by Sasikala and Kamala (1988).

Mutagenic effectiveness = Mutagenic efficiency / Dose X 100

Mutagenic efficiency = Mutation frequency/ percentage lethality X 100

3.5.5 Estimation of Component of Variation

Variance components were estimated from the expected mean squares using observed mean squares. Thus:

$$\sigma_g = m_3 - m_1 \sigma_{ph}^2 = \sigma_g^2 + \sigma_e$$

Phenotypic and genotypic component of variation were computed using the formula given by Singh and Chaudhary (1985).

$$\text{Genotypic component of variation (G.C.V)} = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

$$\text{Phenotypic component of variance (P.C.V)} = \frac{\sqrt{\sigma_{ph}^2}}{\bar{X}} \times 100$$

Where;

$$\sigma_g^2 = \text{genotypic variance}$$

$$\sigma_{ph}^2 = \text{phenotypic variance}$$

$$\bar{x} = \text{mean of character}$$

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Sodium Azide on Germination of Two Groundnut Genotypes Conducted in Groundnut Improvement Laboratory Zaria,2017.

The effect of Sodium azide on germination of two groundnut genotypes are as shown in Figure 1:1. and Figure 1.2. The groundnut SAMNUT 22 treated with 10mM of Sodium azide at 6 hours had the highest germination percentage (80%) while treatments at 20 mM, 30 mM 40mM and 50mM had lower germination percentage. At 12 hours, the same genotype SAMNUT 22 had the highest germination of 85% at 10mM while the lowest germination percentage was recorded at a concentration of 50mM. On the other hand, SAMNUT 24 treated with 10mM at 6 hours had 50% as the highest germination percentage and 20 % at 50mM as the lowest. At 12 hours, the highest percentage was recorded at 20mM (80%) and the lowest at 30mM. From the figures below, the error bars tend to be overlapping thus indicating a non-significant difference among the genotypes. The result of the germination test and T- test carried out shows that SAMNUT 24 is more sensitive to Sodium Azide treatment than SAMNUT 22. T test SAMNUT 22 = 0.5, T test SAMNUT 24 = 1.2

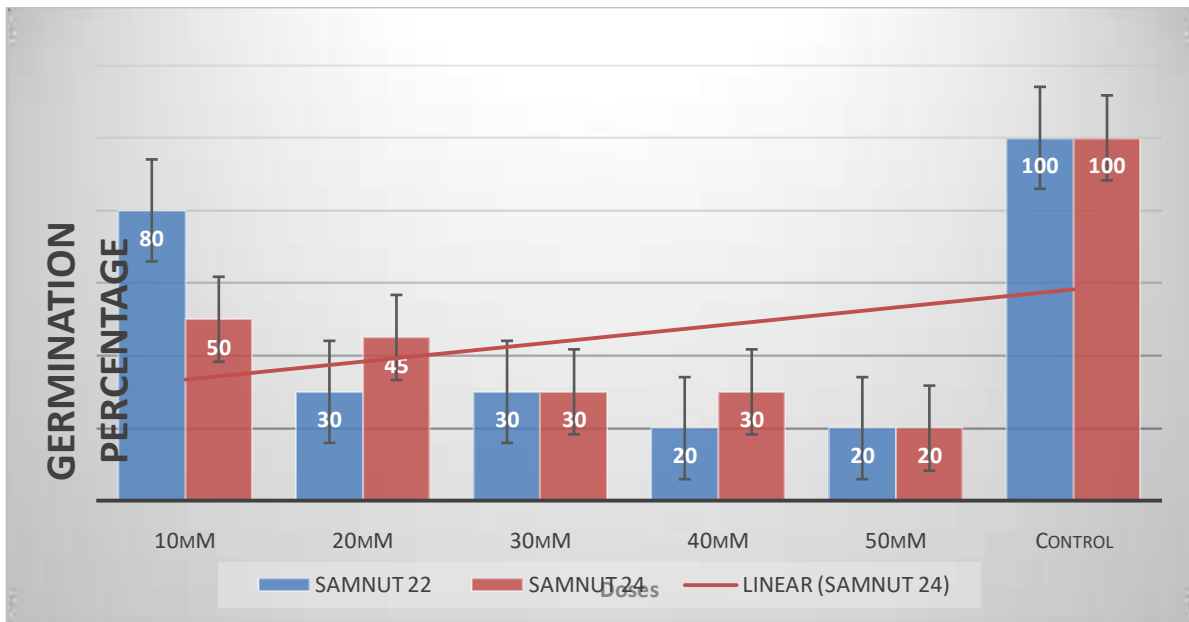


Figure1.1 Effect of Sodium Azide treatment on the germination of two groundnut genotypes after 6hrs.

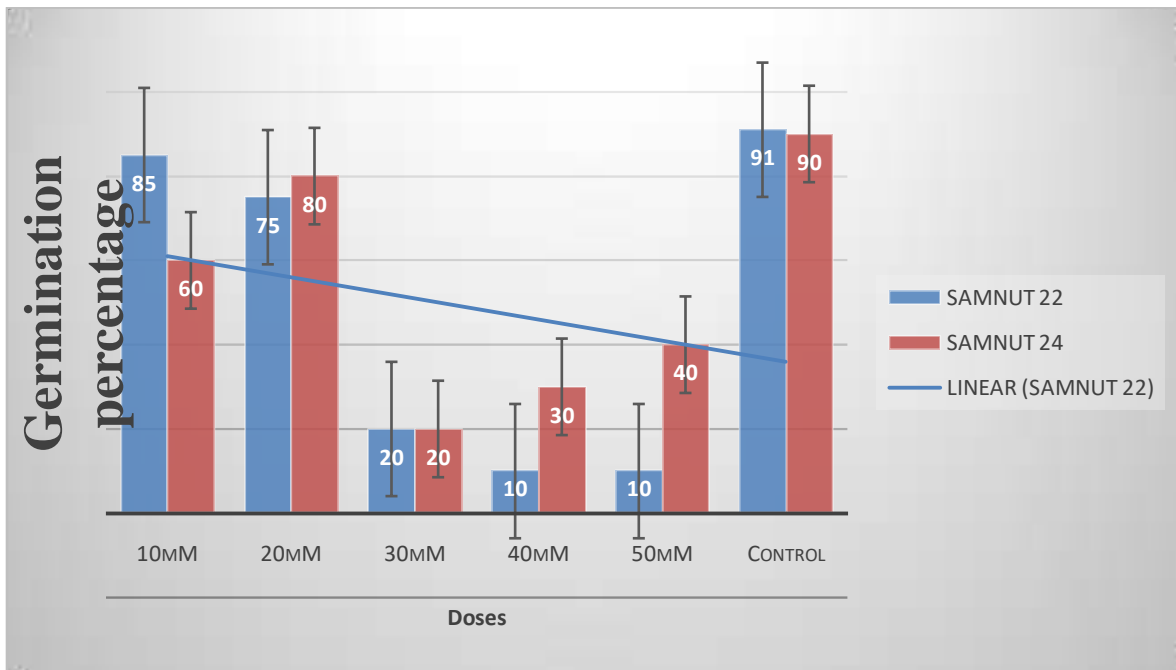


Figure 1.2 Effect of Sodium azide treatments on the germination of two groundnut genotypes after 12 hours.

4.2 Analysis of variance for seedling parameters of two groundnuts genotypes subjected to Sodium azidetreatments in M₁

The analysis of variance resultis presented in Table 4.1. The result shows that there were significant differences ($P < 0.05$) for the effect of Sodium azidetreatment on emergence count, for the two genotypes and for doses. Significant difference was also observed for number of leaves for the two genotypes, seedling height among replication and the interaction between genotype and doses, as well as leaf length among doses.

Table 4.1 Mean squares for seedling parameters of two groundnut genotypes subjected to Sodium azide treatments in M₁

Source of Variation	Df	Emergence count	Survival rate %	Seedling height (cm)	Number of Leaves	Leaf Lnt	Leaf width
Replication	1	2.52	40.35	3.22*	176.66	2.10	0.68
Genotypes	1	11.82*	61.18	1.73	601.00*	0.77	0.54
Doses	5	54.18*	18.44	1.32	216.67	3.69*	0.81
Genotype X Doses	5	4.79	199.63	2.25*	275.36	2.39	0.77
Error	23	9.34	2314.17	1.85	348.63	3.16	0.96

*= Significant (P < 0.05) **= Significant (P < 0.01)

4.3.1 Mutation frequency in M₁ generation

The frequency of mutations in M₁ progenies are presented in Table 4.2. The effect of Sodium azide treatment on the two groundnut genotypes studied can be observed on plant type (dwarf/tall), leaf size (small/ large) and seed size. The mutagenic frequency recorded in the present investigation ranged from 5% to 29 %. The mutation frequency varied across the different concentration. For SAMNUT 22 at 6hrs, the highest mutation frequency was recorded at 10mM (14), while the lowest frequency was recorded at 50mM. For SAMNUT 24 at 6 hrs, the highest mutation frequency was recorded at 10 mM and 20 mM with values of 15 and 17 respectively, while the lowest frequency was recorded at 40 and 50mM (7). At 12hrs, SAMNUT 22 recorded the highest mutation frequency at 20mM (29) and the lowest frequency at 50mM (5). SAMNUT 24 at 12 hrs recorded the highest frequency at 50mM (14.) while 30mM had the lowest frequency (6).

4.3.2 Mutagenic effectiveness and efficiency in M₁ generation

The results obtained from the present study are given in table 4.2. The mutagenic effectiveness ranged from 1 to 6, while mutagenic efficiency ranged from 2 to 45. A decrease in mutagenic effectiveness with increase in concentration dose of the mutagen was observed across the genotypes. The Mutagenic efficiency did not follow any particular trend, it varied across the mutagenic treatments. SAMNUT 22 treated with 10mM of Sodium azide at 12hrs had the highest effectiveness (4) and highest efficiency (33.33), while 40mM and 50mM had the lowest effectiveness (2) and efficiency (11). For SAMNUT 24 at 6 hrs, the highest effectiveness and efficiency were recorded at 10mM (3) and 20mM (31) respectively, while the lowest values were observed at 30mM (1) effectiveness and 17efficiency respectively). At 12hrs SAMNUT 22 had the highest effectiveness and efficiency at 20mM (6) and 50mM (45), while the lowest values were recorded at 50mM (1) and 40mM (9). SAMNUT 24 at 12 hrs had it highest effectiveness

at 10mM (3) and efficiency at 30mM (33) while the lowest values were recorded at 30mM (1) and 20mM (10). The result indicates that 10mM was the most effective and efficient Sodium azidedose for creating variability.

Table 4.2 Effect of different doses of Sodium azide treatment on the mutagenic frequency, effectiveness and mutagenic efficiency of M₁ groundnut

Treatment	Number of Mutants	Mutation Frequency(%)	Mutation Effectiveness	Mutagenic Efficiency
6 Hours (SAMNUT 22)				
10 mM	2	14	4	33
20 mM	2	14	3	33
30 mM	2	13	3	29
40 mM	1	9	2	11
50 mM	1	9	2	11
Mean	1.6	12	3	24
SD	0.5	3	1	12
CV	0.3	0.2	0.3	1
6 Hours (SAMNUT 24)				
10 mM	2	15	3	29
20 mM	2	17	3	31
30 mM	2	7	1	17
40 mM	1	7	1	2
50 mM	1	7	1	20
Mean	1.6	11	2	23
SD	0.5	5	1	6
CV	0.3	1	1	0.3
12 Hours (SAMNUT 22)				
10 mM	1	14	3	8
20 mM	2	29	6	15
30 mM	2	18	4	22
40 mM	1	11	2	9
50 mM	1	5	1	45
Mean	2	15	3	20
SD	0.9	9	2	15
CV	0.5	0.6	0.7	0.8
12 Hours (SAMNUT 24)				
10 mM	2	13	3	6
20 mM	1	10	2	10
30 mM	1	6	1	33
40 mM	1	8	2	13
50 mM	2	14	3	33
Mean	2	10	2	19
SD	0.9	3	2	13
CV	0.5	0.3	1	0.68

4.4 Mean Performance of Seedling Traits in M₁ Progenies of Mutants

The effect of Sodium azide treatment on seedling traits of the two groundnut genotypes are presented in Table 4.3. Generally, the performance of the seedling traits decreased with the increase in the dose of the mutagenic agent in both genotypes. Example, the emergence count went down from 16.0 to 10.5 at 6hrs in SAMNUT 22. This is also the same trend with survival rate, seedling height, number of leaves, leaf length and leaf width.

Survival rate at 6hrs for SAMNUT 22 varied from 82.12 (40mM) to 95.00(30mM) while at 12 hrs, it varied from 72.23 (40mM) to 91.67(10mM). Survival rate for SAMNUT 24 at 6hrs, varied from 72.82(50mM) to 93.22(10mM) while at 12 hrs, it varied from 81.17(50mM) to 89.73(10mM). Emergence count at 6hrs for SAMNUT 22 varied from 10.50(40mM) to 16.00(10mM). At 12hrs for SAMNUT 22, emergence count varied from 8.00 (10mM) to 14.50 (50mM). For SAMNUT 24 at 6hrs, emergence count varied from 13.5 (10mM) to 16.00 (40mM). At 12hrs, emergence count varied from 8.50 (20mM) to 17.00 (10mM). Seedling height for SAMNUT 22 at 6hrs varied from 6.10(20mM) to 7.10 (30 mM). At 12hrs the seedling height varied from 5.75(10 mM) to 7.50(30mM). Seedling height for SAMNUT 24 at 6hrs varied from 6.10(20 mM) to 10.60 (10 mM). SAMNUT 22 at 6hrs had the highest number of leaves ie 53.11(10 mM), 7.40 as leaf length and 3.30 leaf width, while 50mM recorded the lowest number of leaves ie 38.50, 4.40 leaf length and 3.00 leaf width. SAMNUT 24 treated with 10 mM at 6hrs had 46 leaves, 6.6 as leaf length and 3.5 leaf width. While 30mM had the lowest number of leaves ie 25, 4.7 leaf length and 3.1 leaf width. SAMNUT 22 treated with 30 mM at 12 hrs had 46 leaves, 6.2 leaf length and 3.1 leaf width, while 20mM had the lowest number of leaves ie 27 leaves, 4.7 leaf length and 2.9 leaf width. SAMNUT 24 treated with 40 mM at 12 hrs had 45 leaves, 6.5 leaf length and 4.1 leaf width, while 10mM and 20mM had the lowest number of leaves ie 28, 50mM had 4.1 leaf length and 2.5 leaf width.

Table 4.3 Mean performance of seedling traits in M₁ progenies of mutants derived from SAMNUT 22 and SAMNUT 24 at different levels of treatment with Sodium azide

TREATMENT	EMERGENCE COUNT	SURVIVAL RATE	SEEDLING HEIGHT (CM)	NUMBER OF LEAVES	LEAF LENGTHS	LEAF WIDTH
SAMNUT 22						
at 6hrs						
Control	11.50	91.89	7.65	39.00	5.40	2.50
10 mM	16.00	95.89	8.60	53.11	7.40	3.30
20mM	12.50	89.52	7.97	44.11	6.41	3.11
30mM	14.00	85.00	7.40	44.22	6.41	3.12
40mM	10.50	82.12	7.15	40.00	5.40	3.11
50mM	13.50	83.34	7.10	38.50	4.40	3.20
SAMNUT 24						
at 6hrs						
Control	13.50	90.88	6.45	29.00	6.10	3.10
10mM	16.50	93.22	10.60	46.00	6.60	3.50
20mM	15.00	89.37	6.10	29.00	5.10	3.00
30mM	15.50	78.84	7.35	25.00	6.50	3.10
40mM	13.50	73.03	6.50	35.00	4.70	3.10
50mM	14.00	72.82	7.20	36.00	6.40	3.40
SAMNUT 22						
at 12hrs						
Control	12.00	88.89	6.95	35.40	5.80	2.81
10mM	14.50	91.67	5.75	37.11	5.40	3.21
20mM	8.50	90.91	6.20	27.00	6.22	3.11
30mM	11.50	89.93	7.50	46.00	5.50	3.21
40mM	9.00	72.23	6.60	34.50	5.44	3.11
50mM	8.00	72.50	7.35	43.30	4.70	2.90
SAMNUT 24						
at 12hrs						
Control	13.00	86.61	9.00	28.00	7.30	3.20
10mM	17.00	89.73	6.75	28.00	5.30	4.10
20mM	8.50	86.67	6.10	28.00	6.50	3.10
30mM	16.00	85.24	7.10	31.00	5.50	2.50
40mM	11.00	89.52	6.80	45.00	4.70	3.80
50mM	13.00	81.17	7.45	35.00	4.10	3.60
MEAN	12.77	87.55	7.12	35.59	5.80	3.15
CV	23.92	10.64	19.16	15.15	8.77	8.98
LSD	5.32	4.77	2.76	4.79	0.46	0.25

4.5 Analysis of Variance for Agronomic Traits Measured at M₂Generation

The result from the analysis of variance for the M₂ generation are presented in Table 4.4. Significant differences were observed in number of branches, seedling emergence count, number of pods per plot, 100 seed weight and pod yield among entries and checks, emergence count, number of pods per plot and pod yield per plot had high significant difference among treatments.

Table 4.4 Mean squares for effects of Sodium azide on groundnut in M₂ generation

Source	DF	Days to 50% flowering	Emergence count	Seedling height (cm)	Plant height (cm)	Number of branches	NPP	NSP	Shellin (%)	100 seed weight (g)	Pod yield per plot(g)
Block	8	46.94	11.11	2.49	118.82	10.67	3667	4.42	90.64	172.6	238.5
Entries	75	20.28	14.65*	0.97	18.48	10.93	6682*	1.13	23.79	214.5*	297.4*
Check	3	12.52	28.74**	0.74	47.17	34.92*	3482	7.89*	25.34	281.0*	345.3*
Mutants	71	20.50	11.89*	0.99	17.09	10.03	6568*	1.16	23.75	201.7	269.5
Check vs Mutant	1	28.17	168.89**	0.08	31.13	3.18	24406**	0.00	21.34	924.2*	1448.3**
Error	24	26.60	4.57	1.39	86.41	10.65	3556	4.42	49.96	204.1	285.6

** = 0.01, *0.05,

4.6 Mean Performance of Agronomic Traits for the Top Ranking M₂ Progeny of Mutants

The mean performance of agronomic traits in the M₂ progenies of mutants is presented in Table 4.5. Emergence count ranged from 1.1 for mutant 59 to 14.4 for mutant 11 with a pooled mean of 7.66. Seedling height ranged from 3.5 for mutant 20 and 55 to 6.1 for mutant 64 with a pooled mean of 5.61. Number of branches ranged from 1.9 for mutant 67 and 62 to 12.9 for mutant 64 with a pooled mean of 7.9. Days to 50 % flowering ranged from 36 days to 39 days with a pooled mean of 38.17. Plant height ranged from 6.1 for treatment 53 to 26.6 for mutant 55 with a pooled mean of 23.48. Number of pods per plot ranged from 23.6 for mutant 53 to 306.6 for mutant 68 with a pooled mean of 146.12. Number of seeds per pod was the same for all treatments (2). Shelling percentage ranged from 58 for mutant 64 to 80 for mutant 10 with a pooled mean of 69.41.100 Seed weight ranged from 56 for mutant 62 to 114 for mutant 53 with a pooled mean of 88.92. While Pod yield per plot ranged from 75.64 for mutant 62 to 164.53 for mutant 59 with a pooled mean of 134.59.

Table 4.5 Effect of Sodium azide treatments on the mean performance of 15 top ranking M₂ progeny of mutants

Mutants	RSI	Emergence count	Seedling height (cm)	Days to 50% flowering	Number of branches	Plant height (cm)	NPP	NSP	Shelling (%)	100 seed weight (g)	Pod Yield Per Plot(g)
9	14	5.9	5.8	36	11.9	21.4	157.1	2	71	63	99.11
11	5	14.4	4.4	36	2.9	13.8	208.6	2	69	79	126.98
16	15	10.6	4.0	36	4.9	21.2	182.1	2	75	86	127.88
20	9	12.6	3.5	36	6.9	21.6	266.1	2	69	70	131.20
26	6	8.6	4.3	36	4.9	23.5	192.1	2	62	65	109.18
28	4	3.6	5.7	39	3.4	18.5	102.9	2	59	77	100.67
40	11	6.1	3.7	39	18.9	10.0	80.6	2	63	97	115.54
42	3	5.4	5.6	36	4.4	21.2	116.6	2	62	70	96.82
53	12	6.1	5.4	39	7.9	6.1	23.6	2	73	114	87.43
55	10	9.4	3.5	39	5.4	26.6	295.9	2	65	63	131.06
59	13	1.1	5.8	39	3.9	7.1	184.9	2	80	92	164.53
62	2	8.6	4.4	36	1.9	13.2	85.4	2	73	56	75.64
64	8	6.4	6.1	36	12.9	20.6	190.1	2	58	65	105.72
67	1	5.6	4.9	36	1.9	14.8	153.1	2	70	68	103
68	7	4.9	3.9	36	3.9	20.4	306.6	2	76	89	159.52
Mean	8	7.66	5.61	38.17	7.59	23.48	146.1	0	69.41	88.92	134.59
LSD	2.00	2.78	0.87	3.88	2.68	4.97	62.19	0	4.81	11.99	99.42
CV	0.53	28.97	22.9	14.7	41.04	41.41	40.97	0	11.17	15.75	88.22

NNP = number of nuts per pod, NPP = number of pods per plot, RSI = Rank summation index

Note For Mutants name refer to appendix

4.7 Estimates of Components of Variation for M₂

The result of the genotypic and phenotypic component of variation is presented in table 4.6. The Phenotypic component of variation (PCV) was generally higher than the Genotypic component of variation (GCV) for all traits measured except for plant height. The GCV varied from 0 for number of seed per pod to 37.60 for number of pods per plot, while PCV varied from 0 for number of seeds per pod to 55.5 for number of pods per plot. The phenotypic variance component contributed more to total environmental variance in days to 50 % flowering, emergence count, seedling height, number of branches per plant, number of pods per plant and 100 seed weight, While genotypic variance component contributed more to the total environmental variance in plant height, number of seeds per pods and shelling percentage.

Table 4.6. Estimate of Variance Component for M₂

Traits	σ_g^2	σ_{ph}^2	σ_e^2	Mean	GCV(%)	PCV(%)
Days to 50% flowering	6.10	20.50	26.60	38.17	6.47	11.86
Emergence count	7.27	11.84	4.57	7.66	35.20	44.92
Seedling Height(cm)	0.40	0.99	1.37	5.61	11.27	17.73
Plant height(cm)	69.32	17.09	86.41	23.48	35.46	17.60
Number of branches	0.62	10.03	10.65	7.59	10.37	41.73
Number of pods per plot	3012	65.68	3556	146.12	37.60	55.50
Number of seeds per pod	3.26	1.16	4.42	0.00	0.00	0.00
Shelling %	26.21	23.75	49.96	69.41	7.40	7.70
100 seed weight(g)	2.40	201.7	204.10	88.92	1.70	15.90
Pod yield per plot (g)	1178	1463.2	285.6	134.59	25.50	28.42

σ_g^2 = genetic variance, σ_{ph}^2 = phenotypic variance, σ_e^2 = environmental variance, GCV = genotypic component of variation, PCV = phenotypic component of variation.

4.8 Superior Mutants Resulting from Treatment with Sodium azide

The mean values of top performing mutants for the agronomic traits in M₂ generation are presented in Table 4.7 and 4.8. Mutants with improved agronomic traits than the parents were identified from this study. The mutant, 10mM6 SAMNUT 24 at 6hours recorded the highest 100-seed weight (123.0 g) among the mutants while 50mM2 SAMNUT 22 at 6hours recorded the highest shelling percentage (93.0). The mutant 50mM1 of SAMNUT 24 at 12 hours had the highest number of pods per plot (386.6), while 10mM3 SAMNUT 24 at 6hours had the highest plant height (33.6), the mutant,10mM4 of SAMNUT 24 at 6hours had the highest number of branches (18.9), while 40mM2 SAMNUT 22 at 6hours had the least days to 50% flowering (28), mutant 50mM1 of SAMNUT 22 at 12hours had the highest seedling height (9.7) while 40mM2 SAMNUT 24 at 6hours had the highest emergence rate (15.6). Mutant 20 mM4 of SAMNUT 24 at 12 hours had the highest pod yield per plot (173.02).

Table 4.7 Superior mutants resulting from 6 hours of treatment with Sodium azide

MUTANTS	Emergen ce count	Seedling height	Days to 50% flowering	Number of Branches	Plant height	NPP	Shelling %	100 seed weight (g)	Pod Yield Per plot(g)
SAMNUT 22 at 6Hours									
10 mM1	11.1	5.9	39	7.9	26.0	110.0	71	71	96.44
20 mM1	14.4	3.8	49	6.9	32.7	306.6	72	76	146.52
20 mM2	7.6	3.8	39	6.9	24.9	243.4	79	109	164.98
30 mM1	8.6	6.2	39	10.9	24.2	44.6	58	122	132.56
40 mM1	10.9	7.6	36	8.7	30.7	286.4	76	72	137.87
40 mM2	8.4	6.8	28	9.0	27.9	157.1	80	92	128.13
50 mM2	2.4	7.7	29	6.2	25.6	186.4	80	93	99.11
Control	6.6	5.9	37	9.8	27.2	146.7	68	97	122.30
SAMNUT 24 at 6 hours									
10 mM2	10.9	4.0	36	3.9	21.8	232.1	75	84	137.38
10 mM3	11.4	8.3	29	8.2	33.6	87.4	76	114	134.10
10 mM4	6.9	6.9	52	18.7	24.4	71.1	65	75	91.35
10 mM5	7.6	3.6	36	5.9	26.0	217.1	74	72	121.93
10 mM6	10.6	4.0	36	4.9	21.2	182.1	73	86	127.88
20 mM1	12.4	5.6	32	7.9	24.9	260.9	75	76	136.01
20 mM3	15.4	5.6	29	8.2	28.2	211.4	64	86	134.62
30 mM1	12.6	3.5	36	6.9	21.6	266.1	69	70	131.20
30 mM3	6.9	4.5	36	4.9	26.4	194.1	70	82	126.64
40 mM1	10.6	4.4	39	17.9	20.1	105.4	68	67	91.24
40 mM2	15.6	5.6	39	11.9	19.9	83.4	74	71	90.18
40 mM4	8.6	6.5	36	4.9	23.5	192.1	62	65	109.18
40 mM5	5.4	6.5	32	6.9	29.7	165.9	76	85	123.16
50 mM2	6.9	4.7	56	4.9	26.7	140.1	65	92	124.22
50 mM3	6.4	5.9	32	7.9	33.4	80.9	69	82	100.61
Control	4.6	5.8	39	5.9	24.6	110.0	70	56	127.33
Mean	7.66	5.61	38.17	7.59	23.48	146.1	69.41	88.92	134.59
LSD	2.78	0.87	3.88	2.68	4.97	62.19	4.81	11.99	99.42
CV(%)	28.92	22.9	14.7	41.04	41.41	40.97	11.17	15.75	88.22

NPP= number of pods per plot

Table4.8 Superior mutants resulting from 12 hours of treatment with Sodium azide

MUTANTS	Emergence count	Seedling height (cm)	Days to 50% flowering	Number of Branches	Plant height (cm)	NPP	Shelling %	100 seed weight (g)	Pod Yield per Plot(g)
SAMNUT									
22 at 12 hours									
10mM1	7.6	6.8	39	16.9	29.0	291.0	70	63	129.93
10mM2	8.4	8.8	29	8.2	15.8	96.4	74	120	142.17
10mM3	10.4	7.3	29	8.2	18.3	70.4	80	81	97.19
20mM1	6.6	3.0	49	11.9	29.4	122.1	72	100	128.08
20mM3	10.9	8.1	52	6.7	6.7	45.1	78	90	100.37
30mM1	5.6	6.2	29	12.9	18.5	233.4	73	79	132.68
30mM2	9.4	6.1	36	6.9	10.2	174.6	67	103	143.16
40mM2	6.1	6.7	39	18.8	10.0	80.6	63	97	115.54
50mM1	5.4	9.7	36	4.4	21.2	116.6	62	70	96.82
Control	4.3	5.9	40	9.8	28.0	106.2	67	95	119.52
SAMNUT									
24 at 12 hours									
10mM1	12.6	5.0	39	7.7	26.9	157.9	59	113	149.32
10mM2	6.9	6.5	39	7.4	30.8	164.1	71	76	113.74
10mM3	10.6	6.4	39	7.2	22.1	287.9	58	98	164.22
10mM5	12.9	4.7	36	9.9	29.6	254.1	76	82	140.44
10mM6	13.4	6.9	32	7.9	15.3	44.1	73	123	133.14
10mM7	12.4	6.1	32	9.9	19.1	244.9	65	93	165.33
20mM1	5.9	6.6	39	7.2	27.3	22.1	64	88	93.08
20mM2	8.4	7.4	29	7.9	22.7	36.4	77	99	107.37
20mM3	6.1	5.4	39	7.9	6.1	23.6	71	82	87.43
20mM4	2.1	5.7	39	2.9	30.7	256.6	73	114	173.02
20mM5	9.4	3.5	36	5.4	26.6	295.9	65	63	131.06
20mM6	10.6	5.9	39	7.4	21.1	272.9	46	72	134.77
30mM2	15.4	5.5	36	5.9	18.3	125.9	74	80	108.96
30mM3	8.4	6.5	32	7.9	31.1	128.1	68	78	107.46
30mM5	16.6	6.3	39	13.7	28.7	186.6	72	72	114.92
40mM2	11.6	4.9	39	2.9	23.1	94.1	79	102	116.70
50mM1	14.4	6.8	36	7.9	28.3	386.1	76	72	161.80
50mM2	11.9	6.3	36	6.7	24.7	77.1	71	82	99.73
Control	8.1	5.3	39	5.9	23.3	246.4	70	95	151.67
Mean	7.66	5.61	38.17	7.59	23.48	146.1	69.41	88.92	134.59
LSD	2.78	0.87	3.88	2.68	4.97	62.19	4.81	11.99	99.42
CV(%)	28.92	22.9	14.7	41.04	41.41	40.97	11.17	15.75	88.22

NPP= number of pods per plant.

CHAPTER FIVE

5.0 DISCUSSION

The effect of chemical mutagen (Sodium azide) treatment on the agro-morphological traits measured indicates Sodium azide doses induced variations in the groundnut genotypes. The reduction in survival of the two genotypes observed as Sodium azide doses increased indicates that the genotypes were sensitive to treatments. The result of the laboratory germination test, indicates that SAMNUT 24 was more sensitive to Sodium azide treatment than SAMNUT 22. Mutagenic sensitivity is known to be influenced by a variety of factors, which include moisture content, temperature, pH, concentration of catalytic ions, various pre and post-treatment conditions, genetic constitution of the material, strength of the mutagen used and duration of the treatment (Konzak *et al.*, 1965; Kavera 2008). It can also be attributed to the level of differentiation of rudimentary plant parts at the time of treatment on one hand and the extent of damage to the growth components like rate of cell division, cell elongation, various hormones and biosynthetic pathways related to growth and development on the other hand (Burghate *et al.*, 2013). In addition, the sensitivity of peanut seedlings and growing plants may have been brought about by its well differentiated seed embryo (Joventino, 1984).

The mutagenic frequency recorded in the present investigation varied across the different concentration. A decreasing trend with increase in concentration of the mutagen was also observed. This is in agreement with the findings of Badere and Chaudhary (2007) in linseed; and Dixit and Dubey (1986) in lentil, who reported a dose dependent decrease in mutagenic frequency and effectiveness. The mutation frequency was found to be low possibly because of low physiological damage and the low response of groundnut at the mature stage which agrees with the findings of Joventino (1984) which stated that the low response of groundnut

to fast neutron could most probably be due to the tetraploid genome and the somatic competition that invariably occurs between normal and mutated cells during the growth stages of the M₁ plants. The morphological aberration used in the determination of the mutation frequency includes; seedling traits such as emergence count, survival rate and seedling heights. The effectiveness and efficiency of mutagens are pre-requisite for induction and utilization of mutations. Efficient mutagenesis is the production of desirable changes with minimum undesirable changes. In the present study, the higher effectiveness and efficiency of Sodium azide treatment at 10 mM 12 hours of SAMNUT 24 indicates that lower doses were more effective in inducing variability in agronomic traits of groundnut. Begum and Dasgupta (2010) also reported lower doses of mutagens that were effective in causing polygenic variability in various quantitative characters of sesame treated with gamma rays and EMS. A decrease in mutagenic effectiveness with increase in concentration dose of the mutagen was also observed across the genotypes. This is as a result of the failure in proportional increase of mutation frequency with the increase in concentrations/doses of the mutagen. The differences in the mutagenic effectiveness of the genotypes studied indicate that the genotypes respond differently to Sodium azide treatment. The mutagenic efficiency did not follow any particular trend, it varied across the mutagenic treatments. A similar result was reported by Bhosle and Kothekar (2010) in cluster bean and Gaikward and Kothekar (2004) in Lentil. The results are in agreement with the findings of Roychowdhury *et al*, (2004) in mung bean and Dhanavel *et al*. (2008) in cowpea.

The lowest germination percentage and survival rate was observed at 12 hours of SAMNUT 24, which indicates that it was more sensitive to Sodium azide treatment than SAMNUT 22. This reduction in survival rate could be as a result of the physiological disturbance or

chromosomal damage to the cells of the plant by the mutagen as reported by (Mensah and Obadoni, 2007), and Kavera (2008).

Analysis of variance for the different traits revealed that generally, there was highly significant difference among the mutants for all the agronomic traits studied. The existence of these differences indicates the presence of sufficient genetic variability among the genotypes for these traits. This is most likely because these genotypes might have been affected by natural selection. The present findings are in agreement with the findings of Kadam *et al.* (2007), Khote *et al.* (2009) and Ladole *et al.* (2009).

The mean performance for number of pods per plot was higher than the rest of the traits measured. Environmental factors were most likely to be responsible for these variations. Unfortunately, these are variables which the breeder has very little control about. The choice of genotypes and breeding procedure to be adopted in an improvement programme will depend upon the mean performance and on the magnitude and quality of genetic variability present (Eckebili *et al* 1977). Most of the traits evaluated had values with wide range to the mean differences observed. This agrees with the findings of (Knauft and Wynne, 1995) which reported that variation exists among peanut genotypes for different attributes. This highly significant variation in plant attribute within a population suggests that selection is possible for each character. The overall picture suggests that high genetic variability or diversity exists among the genotypes studied. In other words, they are diverse in their genetic base.

In this study, the phenotypic component of variation (PCV) estimates were higher than the genotypic component of variation(GCV) estimate for all the traits measured except for plant

height which had higher genotypic component of variation. The high PCV estimates observed for the measured traits indicate a level of environmental influence on the expression of the traits. However, the significant GCV estimates obtained indicates the presence of high level of genetic variability among the mutants. The values of GCV and PCV were relatively high for the number of pods per plot thus, suggesting wide spectrum of genotypic variation for this trait. The result also agrees with the findings of Meta (2007) who also reported high magnitude of both GCV and PCV for number of pods per plot in groundnut. Emergence count, Plant height, number of branches and days to fifty percent flowering recorded moderate value of GCV due to high genetic variability among the genotypes thus implying these materials can be selected for further breeding. This result is in agreement with result obtained by Meta (2007) and John *et al.*, (2008), who also reported moderate magnitude of GCV for plant height. The higher estimates of GCV and PCV have been earlier reported by John *et al.*, (2008), Meta (2007) and Ladole *et al.* (2009). Moderate magnitudes of GCV and PCV were observed for plant height, number of branches, emergence count and days to fifty percent flowering. Abimiku and Bello (2010), revealed that genotypic coefficient of variation helps to measure the range of variability in a character and provide a measure to compare variability present in a population.

The mutants identified from this study performed better than the controls in most of the traits considered. Mutant 40 mM2 of SAMNUT 22 at 6 hours had 28 days to flowering compared to the control which had 37 days to flowering, while the mutant 10 mM of SAMNUT 24 at 12 hours had 123 for 100 seed weight compared to the control which had 95. Mutant 20 mM4 of SAMNUT 24 at 12 hours recorded the highest pod yield per plot. The presence of this superior mutants indicates that sodium azide was able to create genetic variability in the groundnut genotypes, thus providing possibility for future selection and improvement of

these traits. Mensah and Obadoni (2007) reported such increase in variability for pod yield per plots and seed per plant at M₂ generation in groundnut.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Crop improvement by mutagenesis has been applied in a number of crops for yield improvement, creation of new cultivars, stress and drought tolerance, disease resistance and for horticultural or floricultural purposes. Induced mutations have been used to improve major crops that are mainly propagated by seeds and to introduce novel genetic variability in ornamental crops. Chemical mutagens are one cause of mutations in living organisms. Many of such chemicals have clastogenic (chromosome damaging) effect on plants via reactive oxygen -derived radicals. These effects can occur both spontaneously and artificially following induction by mutagens.

Two genotypes of groundnut were treated with five doses of Sodium azide. The treated seeds were planted in well prepared seed beds on the field to produce M₁ plants. Seedling traits were measured for the assessment of sensitivity of the genotypes to Sodium azide treatments. The M₁ families generated as well as the parental genotypes were evaluated on the field, and the following results were obtained:

1. The seedling growth parameters such as seedling height, survival rate, and emergence count, decreased gradually with increasing concentration of Sodium azide treatment indicating sensitivity of the genotypes to Sodium azide treatment.
2. SAMNUT 24 was more sensitive to Sodium azide treatment than SAMNUT 22.

3. Sodium azide concentration (10 mM) was found to be the most effective and efficient dose for creating variability in this study.

4. The mutants recorded higher variability than the controls, indicating that Sodium azide treatment was able to create additional variability in the groundnut genotypes. The mutant, SAMNUT 24 at 12 hours 10mM recorded the highest 100-seed weight (123.0 g) among the mutants while at 50mM, the highest number of pod per plant was gotten.

6.2 Conclusion

The result of the germination/ sensitivity test carried out in the laboratory shows that SAMNUT 24 is more sensitive to Sodium azide treatment than SAMNUT 22.

It was also observed that 10mM even though the lowest level of concentration, was the most effective and efficient Sodium azide dose for creating variability in this study.

Mutants with improved performance in the agronomic traits, example mutant 45 had 287 number of pods per plot and large seed size, Example mutant 48 had 123 as 100 seed weight, mutant 54 had 173 pod yield per plot and early maturity etc were identified.

Sodium azide therefore could be utilized to increase variability in groundnut, which ultimately increased the possibility of isolating beneficial mutants for groundnut improvement.

6.3 Recommendations

For the improvement of cultivated groundnut, it is pertinent to use diverse collections with more variability and also employ the use of varying concentration, for the purpose of variety development and for use in future breeding programs.

Mutants with improved agro-morphological traits have been isolated in this research work, it therefore becomes imperative that the isolated mutants be incorporated into the current national groundnut improvement program.

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Appendix A

Effect of Sodium Azide treatments on the mean performance of M₂ progeny of mutants derived from SAMNUT 22 and SAMNUT 24 evaluated in IAR Samaru 2018

Mutants	RSI	Emergence count	Seedling height (cm)	Days to 50% flowering	Number of branches	Plant height (cm)	NPP	NNP	Shelling (%)	100 seed weight (g)	Pod yield per plot
1	48	11.1	5.9	39	7.9	26.0	110.6	2	71	71	96.44
10	36	2.4	7.7	29	6.2	25.6	186.4	2	80	93	135.87
11	5	14.4	4.4	36	2.9	13.8	208.6	2	69	79	126.98
12	16	10.9	4.0	36	3.9	21.8	232.1	2	75	84	137.38
13	76	11.4	8.3	29	8.2	33.6	87.4	2	76	114	134.10
14	57	6.9	6.9	52	18.7	24.4	71.1	2	65	75	91.35
15	20	7.6	3.6	36	5.9	26.0	217.1	2	74	72	121.93
16	15	10.6	4.0	36	4.9	21.2	182.1	2	75	86	127.88
17	37	12.4	5.6	32	7.9	24.9	260.9	2	75	76	136.01
18	26	4.9	4.7	36	4.9	25.6	188.1	2	73	112	155.26
19	44	15.4	5.6	29	8.2	28.2	211.4	2	64	86	134.62
2	39	5.6	6.2	39	3.4	28.7	127.9	2	59	98	127.42
20	9	12.6	3.5	36	6.9	21.6	266.1	2	69	70	131.20
21	63	5.9	4.5	39	15.7	22.7	186.1	2	75	115	157.80
22	22	6.9	4.5	36	4.9	26.4	194.1	2	70	82	126.64
23	23	10.6	4.4	39	17.9	20.1	105.4	2	68	67	91.24
24	42	15.6	5.6	39	11.9	19.9	83.4	2	74	71	90.18
25	50	7.6	5.8	39	4.4	29.5	150.9	2	54	101	135.71
26	6	8.6	4.3	36	4.9	23.5	192.1	2	62	65	109.18
27	52	5.4	6.5	32	6.9	29.7	165.9	2	76	85	123.16
28	4	3.6	5.7	39	3.4	18.5	102.9	2	59	77	100.67
29	33	6.9	4.7	56	4.9	26.7	140.1	2	65	92	124.22

3	41	2.1	5.3	52	12.9	24.5	108.6	2	71	87	11.98
30	46	6.4	5.9	32	7.9	33.4	80.9	2	69	82	100.61
31	72	7.6	6.8	39	16.9	29.0	291.4	2	70	63	129.93
32	35	8.4	8.8	29	8.2	15.8	96.4	2	74	120	142.17
33	27	10.4	7.3	29	8.2	18.3	70.4	2	80	81	97.19
34	70	6.6	3.0	49	11.9	29.4	122.1	2	72	100	128.08
35	21	2.6	4.0	49	9.9	21.1	35.1	2	64	102	110.07
36	47	10.9	8.1	52	6.7	6.7	45.1	2	78	90	100.37
37	28	5.6	6.2	39	12.9	18.5	233.4	2	73	79	132.68
38	18	9.4	6.1	36	6.9	10.2	174.6	2	67	103	143.16
39	34	6.4	6.7	45	8.9	22.0	40.9	2	68	82	91.41
4	68	14.4	3.8	49	6.9	32.7	306.6	2	72	76	146.52
40	11	6.1	3.7	39	18.9	10.0	80.6	2	63	97	115.54
41	66	2.4	9.7	45	2.9	27.8	45.9	2	72	107	117.56
42	3	5.4	5.6	36	4.4	21.2	116.6	2	62	70	96.82
43	66	12.6	5.0	39	7.7	26.9	157.9	2	59	113	149.32
44	69	6.9	6.5	39	7.4	30.8	164.1	2	71	76	113.74
45	39	10.6	6.4	39	7.2	22.1	287.9	2	58	98	164.22
46	54	9.4	7.0	42	3.9	25.3	199.4	2	65	95	140.86
47	73	12.9	4.7	36	9.9	29.6	254.1	2	76	82	140.44
48	43	13.4	6.9	32	7.9	15.3	44.1	2	73	123	133.14
49	25	12.4	6.1	32	9.9	19.1	244.9	2	65	93	165.33
5	61	7.6	3.8	39	6.9	24.9	243.4	2	79	109	164.98
50	51	6.1	4.5	52	6.7	28.0	49.6	2	71	94	105.41
51	48	5.9	6.6	39	7.2	27.3	22.1	2	64	88	93.08
52	58	8.4	7.4	29	7.9	22.7	36.4	2	77	99	107.37
53	12	6.1	5.4	39	7.9	6.1	23.6	2	71	82	87.43
54	64	2.1	5.7	39	2.9	30.7	256.6	2	73	114	173.02
55	10	9.4	3.5	36	5.4	26.6	295.9	2	65	63	131.06
56	16	10.6	5.9	39	7.4	21.1	272.9	2	46	72	134.77
57	24	7.6	4.7	39	6.9	22.2	270.6	2	64	86	148.24
58	37	8.1	4.9	39	7.4	22.5	100.6	2	75	86	109.14

59	13	1.1	5.8	39	3.9	7.1	184.9	2	80	92	164.53
6	65	8.6	6.2	39	10.9	24.2	44.6	2	58	122	132.56
60	19	15.4	5.5	36	5.9	18.3	125.9	2	74	80	108.96
61	52	8.4	6.5	32	7.9	31.1	128.1	2	68	78	107.46
62	2	8.6	4.4	36	1.9	13.2	85.4	2	73	56	75.64
63	75	16.6	6.3	39	13.7	28.7	186.6	2	72	72	114.92
64	8	6.4	6.1	36	12.9	20.6	190.1	2	58	65	105.72
65	31	4.9	4.7	39	5.9	27.3	107.4	2	69	92	116.70
66	54	11.6	4.9	39	2.9	23.1	94.1	2	79	102	123.64
67	1	5.6	4.9	36	1.9	14.8	153.1	2	70	68	103.21
68	7	4.9	3.9	36	3.9	20.4	306.6	2	76	89	159.52
69	60	14.4	6.8	36	7.9	28.3	386.1	2	62	73	161.80
7	74	10.9	7.6	36	8.7	30.7	286.4	2	76	72	137.87
70	62	11.9	6.3	39	6.7	24.7	77.1	2	71	82	99.73
71	59	11.9	5.4	39	9.9	24.6	106.1	2	69	83	107.40
72	44	6.9	5.8	39	7.2	21.8	146.7	2	79	88	121.74
73	54	6.6	5.9	37	9.8	27.2	110.0	2	68	97	122.30
74	30	4.6	5.8	39	5.9	24.6	136.2	2	70	96	127.33
75	29	4.3	5.9	40	9.8	21.8	106.6	2	67	95	119.52
76	32	8.1	5.3	39	5.9	23.3	246.4	2	70	95	151.67
8	71	8.4	6.8	28	9.0	27.9	157.1	2	80	92	128.13
9	14	5.9	5.8	36	11.9	21.4	157.1	2	71	63	99.11
Mean	Mean	7.66	5.61	38.17	7.59	23.48	146.12	0	69.41	88.92	134.59
LSD	LSD	2.78	0.87	3.88	2.68	4.97	62.19	0	4.81	11.99	99.42
CV	CV	28.97	22.9	14.7	41.04	41.41	40.97	0	11.17	15.75	88.22

NNP = number of seeds per pod, NPP = number of pods per plant, RSI = Rank Summation Index.

Appendix B

Summary Statistics for the Effect of Sodium Azide treatments on the mean performance of M₂ progeny of mutants derived from SAMNUT 22 and SAMNUT 24 evaluated in IAR Samaru 2018

Traits	Range	Mean	LSD	CV(%)
E. Count	1.10-16.60	7.66	2.78	28.97
S. Ht	3.50-9.70	5.61	0.87	22.90
DFF	28.0-52.0	38.17	3.88	14.70
NB	1.9-18.90	7.59	2.68	41.04
PH	6.1-33.60	23.48	4.97	41.41
NPP	22.1-386.1	146.12	62.19	40.92
NNP	2.00	0.00	0.00	0.00
SH %	54.00-80.0	69.41	4.81	11.17
100 SWT	63.0-123.0	88.92	11.99	75.75

E.count = emergence count, S.Ht = Seedling height, DFF = days to fifty percent flowering, NB = number of Branches, NNP = number of nuts per pod, SH% =shelling percentage, 100 SWT = 100 seed weight

Appendix C

Names of Mutants.

Mutant	Mutant Name
1	6hrs SAMNUT 22 10mM1
2	6hrs SAMNUT 22 10mM2
3	6 hrs SAMNUT 22 10mM3
4	6hrs SAMNUT 22 20mM1
5	6hrs SAMNUT 22 20mM2
6	6hrs SAMNUT 22 30mM1
7	6hrs SAMNUT 22 40mM1
8	6hrs SAMNUT 22 40mM2
9	6hrs SAMNUT 22 50mM1
10	6 hrs SAMNUT 22 50mM2
11	6hrs SAMNUT 24 10mM1
12	6hrs SAMNUT 24 10mM2
13	6hrs SAMNUT 24 10mM3
14	6hrs SAMNUT 24 10mM4
15	6hrs SAMNUT 24 10mM5
16	6hrs SAMNUT 24 10mM6
17	6hrs SAMNUT 24 20mM 1
18	6hrs SAMNUT 24 20mM 2
19	6 hrs SAMNUT 24 20 mM3
20	6 hrs SAMNUT 24 30mM1
21	6 hrs SAMNUT 24 30 mM2
22	6 hrs SAMNUT 24 30 mM3
23	6 hrs SAMNUT 24 40 mM1
24	6 hrs SAMNUT 24 40 mM2

25	6 hrs SAMNUT 24 40 mM3
26	6 hrs SAMNUT 24 40 mM4
27	6 hrs SAMNUT 24 40 mM5
28	6 hrs SAMNUT 24 50mM1
29	6 hrs SAMNUT 24 50mM2
30	6 hrs SAMNUT 24 50mM3
31	12 hours SAMNUT 22 10 mM1
32	12 hours SAMNUT 2210mM2
33	12 hours SAMNUT 2210mM3
34	12 hours SAMNUT 2220mM1
35	12 hours SAMNUT 22 20 mM2
36	12 hours SAMNUT 2220mM3
37	12 hours SAMNUT 2230mM1
38	12 hours SAMNUT 2230mM2
39	12 hours SAMNUT 22 40 mM1
40	12 hours SAMNUT 2240mM2
41	12 hours SAMNUT 22 40 mM3
42	12 hours SAMNUT 2250mM1
43	12 hours SAMNUT 24 10 mM1
44	12 hours SAMNUT 24 10mM2
45	12 hours SAMNUT 24 10mM3
46	12 hours SAMNUT 24 10 mM4
47	12 hours SAMNUT 24 10mM5
48	12 hours SAMNUT 24 10mM6
49	12 hours SAMNUT 24 10mM7
50	12 hours SAMNUT 24 10 mM8
51	12 hours SAMNUT 24 20mM1
52	12 hours SAMNUT 24 20mM2
53	12 hours SAMNUT 24 20mM3
54	12 hours SAMNUT 24 20mM4
55	12 hours SAMNUT 24 20mM5
56	12 hours SAMNUT 24 20mM6
57	12 hours SAMNUT 24 20 mM7

58	12 hours SAMNUT 24 20 mM8
59	12 hours SAMNUT 24 30 mM1
60	12 hours SAMNUT 24 30mM2
61	12 hours SAMNUT 24 30mM3
62	12 hours SAMNUT 24 30mM4
63	12 hours SAMNUT 24 30mM5
64	12 hours SAMNUT 24 40 mM1
65	12 hours SAMNUT 24 40mM2
66	12 hours SAMNUT 24 40 mM3
67	12 hours SAMNUT 24 40 mM4
68	12 hours SAMNUT 24 40 mM5
69	12 hours SAMNUT 24 50mM1
70	12 hours SAMNUT 24 50mM2
71	12 hours SAMNUT 24 50 mM3
72	12 hours SAMNUT 24 50 mM4
73	12 hours SAMNUT 24 50 mM5
74	6 hours SAMNUT 22 Control
75	6 hours SAMNUT 24 Control
76	12 hours SAMNUT 22 Control
77	12 hours SAMNUT 24 Control

Appendix D

List of plates



Plate 1. Reduced pod yield modified by Mutagen



Plate 2. Increased pod yield modified by mutagen



Plate 3. SAMNUT 22 Control



Plate 4. Increased number of Control branches and leaves modified by mutagen



Plate 5. Reduced number of branches and leaves modified by mutagen



Plate 6. SAMNUT 24



Plate 7. SAMNUT 22 Control



Plate 8. Small size pods

Plate 9. Big size pods



Plate 10. Big seeds obtained as a result of mutation. **Plate 11. Small seeds obtained as a result of mutation.** **Plate 12. SAMNUT 22 Control**



Plate 13. Pictures of laboratory germination test



Plate 14. Cross section of the M₂ groundnut population evaluated in Samaru 2018

