

**EFFECTS OF FAST NEUTRON, ETHYL METHANE SULPHONATE,
NITROUS ACID AND SODIUM AZIDE ON
THE AGROMORPHOLOGICAL TRAITS OF FOXTAIL MILLET
(*SETARIA ITALICA* [L.] P. BEAUV.)**

BY

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**DEPARTMENT OF BOTANY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA**

DECEMBER, 2016

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By

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BOTANY**

**DEPARTMENT OF BOTANY
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NIGERIA**

DECEMBER, 2016

DECLARATION

I declare that the work in this dissertation entitled “Effects of Fast Neutron, Ethyl Methane Sulphonate, Nitrous Acid and Sodium Azide on the Agromorphological Traits of Foxtail Millet (*Setaria italica* [L.] P. Beauv.)” has been carried out by me in the Department of Botany. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Esson Akolo Elijah

Signature

Date

CERTIFICATION

This dissertation entitled “EFFECTS OF FAST NEUTRON, ETHYL METHANE SULPHONATE, NITROUS ACID AND SODIUM AZIDE ON THE AGROMORPHOLOGICAL TRAITS OF FOXTAIL MILLET (*SETARIA ITALICA* [L.] P. BEAUV.)” by Akolo Elijah ESSON meets the regulations governing the award of the degree of MASTER OF SCIENCE IN BOTANY of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my mother Mrs. Alice Sunday Esson whom God has used to be a pillar in my educational pursuit and my elder brother; Esson Albert who sacrificially supported me during the course of this programme.

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To God Almighty, Father of our Lord Jesus Christ, the fountain of all being and bliss, who fills everything in every way.

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ABSTRACT

This study was carried out to determine the effects of fast neutron, ethyl methane sulphonate, and nitrous acid and sodium azide foxtail millet on the agronomic traits at different concentrations. Seeds of foxtail millet were obtained from Institute for Agricultural Research, Ahmadu Bello University, Zaria and treated with different doses/concentrations of Fast Neutron, EMS, Nitrous Acid and Sodium Azide and untreated seeds (control). The treated seeds were sown in a completely randomized block design (CRBD) to raise the M_1 generation which were advanced to M_2 generation and the following data were collected; germination percentage, plant height, number of leaves, leaf length, leaf width, fresh weight, dry weight, days to 50% flowering, panicle length, panicle weight and 1000 seeds weight. Data obtained were analyzed using SAS version 9.1. Variations were observed in the M_1 that showed significant difference ($P \leq 0.05$) in plant height, leaf length, leaf weight, and panicle length where control performed better in most characters for fast neutrons. The results of M_2 generation of fast neutrons treatment showed increase in plant height (33.08cm to 46.65cm), leaf length (16.66cm to 18.47cm), leaf width (1.38cm to 1.68cm), panicle length (5.42cm to 6.70cm), panicle weight (1.61g to 2.51g) and 1000 seed weight (2.86g to 3.55g). EMS treatment at M_1 generation recorded significant ($P \leq 0.05$) variation for percentage germination (95%), leaf length (34.87cm), leaf width (1.50cm), fresh weight (2.60g), dry weight (1.73g), days to fifty percent flowering (34 days), panicle length (5.67cm) and panicle width (1.35g) with 0.4% EMS showing highest mean values for most traits. EMS treatment of M_2 generation had high values at 0.1% for plant height, number of leaves, leaf length, leaf width, panicle length, panicle weight and 1000 seed weight. Nitrous acid showed variation for plant height, number leaves, fresh weight, dry weight, days to 50% flowering, panicle length and panicle weight with 0.1% and 0.4% best for yield parameters. Sodium azide showed significant difference decrease ($P \leq 0.05$) in germination percentage (47.50%), plant

height (26.40cm), leaf length (10.04cm) compared to the control at M₁ generation. Results from M₂ generation showed significant variation ($P \leq 0.05$) for germination percentage, plant height, leaf length, leaf width, dry weight, panicle length, panicle weight and 1000 seed weight with highest mostly at par with the control except panicle weight (1.5g to 22.24g) and 1000 seed weight (2.81g to 3.59g) showing increase. Heritability values were high (greater than 60%) for most traits studied for all mutagens. Mutagenic frequency, effectiveness and efficiency were highest for EMS at 0.1% concentration (Mutagenic frequency 7.50% effectiveness 18.75% efficiency 7.50%) and sodium azide at 0.2% concentration (Mutagenic frequency 7.00%, effectiveness 1.84% and efficiency 1%). EMS was found to be more effective and efficient in inducing mutation in foxtail millet than sodium azide. Increased genetic variability and high heritability for agronomic traits of foxtail millet treatment with fast neutron, EMS, and nitrous acid and sodium azide provides great scope for further selection in breeding programme for foxtail millet, while mutants with low biological damages and high mutation frequency can be developed at low concentration of EMS and sodium azide.

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LIST OF ABBREVIATIONS

%= percentages

cm=centimetre,

g=gram,

\geq =greater than,

\leq =less than,

min= minutes,

FN=Fast neutrons,

EMS= Ethyl methane sulphonate,

NA=Nitrous Acid,

SA=Sodium azide,

M₁ = First mutant generation,

M₂= Second mutant generation.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background of the Study

Foxtail millet (*Setaria italica*) is one of the oldest cultivated grain crops and is thought to have been domesticated from the wild species, green foxtail (*Setaria viridis*) more than 8,000 years ago in northern China (Barton, 2009). Foxtail millet is of Poaceae family, Panicoideae tribe, distributed widely around warm and temperate regions in Asia, Europe, North America, Australia, and North Africa and used as grain or forage (Austin, 2006). Foxtail millet is a self-pollinating crop with chromosome number of $(2n=18)$ (Sivaraman and Ranjekar, 1984). It is one of the most important food crops of the semi-arid tropics, primarily because of its favourable growth characteristics under high temperature and limited rainfall conditions (Brink and Belay, 2006). In the tropics, foxtail millet is grown at an altitude of 2,000m to 3,300m altitude and it does not tolerate frost. In China and India, it is mainly grown in areas with an annual rainfall of 400 to 800 mm. Foxtail millet is an underutilized, drought-tolerant crop that stands to become much more important in a potentially much warmer and dryer future environment (Diao, 2011). Foxtail millet has rapid growth rate in tropical climates and is among the plant with high water efficiency. This crop is produced in poor and low efficiency soils of Europe and tropical and sub-tropical regions of Asia. (Majid *et al.*, 2012).

Foxtail millet (*Setaria italica*) is rich in minerals and phytochemicals (FAO, 2008), it is a nutritious food ingredient in traditional diet, especially for people in Europe, Asia and Africa (Xuewei *et al.*, 2015). Drought and heat hardiness support suitability of foxtail millet for growth in the semi-arid tropics, and the nutritional profile suggests this grain also has potential as a poultry feed ingredient. The nutritional characteristics of foxtail millet are interesting for poultry

feed because of its relatively high protein content (10.4–15.6%), favorable amino acid profile, considerable energy density (ME of approximately 3,303 kcal/kg), and apparent absence of major ant nutritional factors like amylase inhibitors, glucosinolates, polyphenols, and tannins. reported that foxtail millet could replace 100% of corn in the diet of broiler birds without a negative effect on performance(Rama Rao *et al.*, 2004).

Induced mutation has great relevance in raising superior plant types in different crop plants. Most of the mutations are lethal or semi-lethal and do not have any practical value possibly due to doses monitored or mutagens employed. Selection of efficient mutagens and their concentration/dosage is prerequisite for successful improvement of certain qualitative and quantitative characters. Mutagenesis is usually done to create genetic variability not available in the gene pool or to repair specific deficiency of an otherwise outstanding genotype (Ambli and Mullainathan, 2014). Genetic variation can be artificially induced by the use of physical and chemical mutagens as they affect a wide range of chromosomal alterations resulting into abnormal behaviour during mitosis and meiosis leading to various degrees of sterility and inducing various structural changes in the chromosomes. The most common form of sterility is the occurrence of non-functional gametes.

Mutation induced changes in chromosomes are the primary basis of genetic variation. Thus, cytogenetical investigation of mitosis and meiosis is an important source of information regarding distortion due to mutagens as they deal with the genetic material, the chromosomes, and more appropriately the DNA which controls the phenotype. It also provides a considerable clue to assess the sensitivity of plants for different mutagens and to ascertain the most effective mutagens and their treatment doses for a given crop to realize maximum results (Bharathi *et al.*, 2014).

Mutations are tools being used to study the nature and basis of plant growth and development, thereby producing raw materials for genetic improvement of crops (Adamu and Aliyu, 2007). Induced mutations can rapidly cause quantitative and qualitative variability in inherited traits in crops (Maduli and Mishra, 2007). Mutagenesis is one of the most critical steps for genetic studies as well as selective breeding. Various mutagenic agents are used to induce favourable mutations at high frequency which include ionizing radiation and chemical mutagens (Ahloowalia and Maluszynski, 2001). Successful mutant isolation largely relies on the use of efficient mutagens. In plant research, a chemical mutagen, ethyl Methanesulfonate (EMS) produces single base substitutions with different mutation spectra (Sri Devi and Mullainathan, 2011). These chemical mutagens induce a broad variation of morphological and yield structure traits in comparison to normal plants (Salim *et al.*, 2009).

1.2 Statement of Research Problem

Exotic plant species are usually associated with narrow genetic base which makes breeding and selection of this crops for desirable traits in a breeding programme almost impossible. Great variety of work has been done on foxtail millet, Forage foxtail millet was developed Australia (Fletcher *et al.*, 1996) and a pure lines have also been developed in India (Maciel *et al.*, 1995). Genetic characterization (Feng *et al.*, 2012) and modification (Travis and Manish, 2015). However successful development of stable and high-yield varieties is rarely reported and the value of induced mutation effects on the agronomic trait this plant has not been fully exploited.

1.3 Justification

Feeding the ever increasing human population is a major challenge facing mankind. This is particularly true in the semi-arid tropics which include the African savanna and Sahel, and significant parts of Asia, Australia and the Americas, To achieve self-sufficiency of cereal

production within the semi-arid tropics, genotypes with tolerance, particularly to extreme conditions of drought and heat, need to be developed one way is the genotypes of fully domesticated cereals native to the semi-arid regions must be improved to provide high and stable yields under the fluctuating environmental conditions of the region.

Foxtail millet is largely a self-pollinating species. Out crossing rates have been estimated from 0.0 to only 1.4% for plants separated by 0.30 m (Till-Bottraud *et al.*, 1992), the traditional hybridization for new cultivar appears very difficult due to the miniature size of the floral organs.

Crop improvement depends on the genetic variability; In the process of breeding crop plants, the progress obtainable tends to be limited by the variability present in nature, so that further progress in the breeding becomes more difficult. This study will help to create variability which in turn introduces novel genetic variability in foxtail millet which will be useful in further breeding of better varieties of the crop in order to address current challenges faced by farmers.

Mutagenic effectiveness and efficiency are two different properties, which are important in mutation breeding programmes. Knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations (Kumar and Mani, 1997). Mutagenic effectiveness is a measure of the mutations induced per unit dose of a mutagen (time \times concentration/dose), while mutagenic efficiency gives an idea of genetic damage (mutation) in relation to the total biological damage caused in M_1 generation (Singh, 2011). Although, both are two different properties but the usefulness of any mutagen in plant breeding programme depends on both of them. It is not necessary that an effective mutagen shall be an efficient one also (Khan *et al.*, 2005).

Heritability (H) is an approximate measure of the expression of a character (Kumar and Dubey, 2001). Falconer and Mackay (1996), defined heritability as the measure of the correspondence between breeding values and phenotypic values. Thus, heritability plays a predictive role in breeding, expressing the reliability of phenotype as a guide to its breeding value. It is the breeding value which determines how much of the phenotype would be passed onto the next generation (Tazeen *et al.*, 2009)

1.4 Aim

To evaluate the mutagenic effects of Fast Neutrons, Ethyl Methane Sulphonate, Sodium Azide and Nitrous Acid on the agronomy and morphology of Foxtail millet

1.5 Objectives

- i. To determine the effect of fast neutrons, ethyl methane sulphonate, sodium azide and nitrous acid on the agronomic traits of Foxtail millet
- ii. To determine the optimum concentration that will create useful variability in the agronomic traits for improved the yield of foxtail millet with minimum lethality.
- iii. To determine heritability in agronomic traits in (M_2) generation
- iv. To determine the mutagenic efficiency and effectiveness of ethyl methane sulphonate and sodium azide

1.6 Hypothesis

- i. Fast neutrons, ethyl methane sulphonate, sodium azide and nitrous acid do not have any significant effects in the agronomic traits of Foxtail millet.
- ii. Fast neutrons, ethyl methane sulphonate, sodium azide and nitrous acid have no beneficial effects on agronomic traits of foxtail millet.

- iii. Agronomic traits of foxtail millet treated with fast neutrons, ethyl methane sulphonate, nitrous acid and sodium azide are not heritable
- iv. Ethyl methane sulphonate and sodium azide are not efficient and effective in inducing mutation in foxtail millet.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Foxtail Millet

Millets represent the small-seeded group of the Poaceae family. Their similarities are that they are grown under extreme environmental conditions and therefore, especially suited to areas with inadequate moisture or short-growing cycle and poor soil fertility (Baker, 2003). The most widely- cultivated ones are pearl millet [*Pennisetum glaucum* (L.) R. Br.], finger millet [*Eleusine coracana* (L.) Gaertn], tef [*Eragrostis tef* (Zucc.) Trotter], fonio or acha [*Digitaria exilis* (Kippist) Stapf and *D. iburua* Stapf], foxtail millet [*Setaria italica* (L.) P. Beauvois], proso millet [*Panicum miliaceum* (L.)], barnyard millet [*Echinochloa crusgalli* (L.)P. Beauvois] and kodo millet [*Paspalum scrobiculatum* (L.)]. Foxtail millet [*Setaria italica* (L.) P. Beauv.] is a cereals of the Panicoideae tribe, grown widely around warm and temperate regions in Asia, Europe, North America, Australia, and North Africa, used for grain or forage (Austin 2006).

2.1.1 Description of plants

Foxtail millet (*Setaria italica*) is believed to be native to southern Asia and usually considered as one of the oldest cultivated millets (Oelke, 1990). It is an annual, warm-season crop that grows 10-20cm tall which produce one or more tillering shoots (Dekker, 2009). The stems are leafy, and more slender than those of pearl millet (Lee and Henning, 2014). The inflorescence yellowish or

purplish is composed of a main stalk with many side branches. The seedheads are dense and bristly and the oval, convex seed grain can be of variety of colors (Cash *et al.*, 2002).

2.1.2 Adaptation

Foxtail millet is grown in cooler, dryer regions than other millets (Koch, 2002). It can grow in sandy to loamy soils with pH from 5.5–7. It will grow rapidly in warm weather and can grow in semi-arid conditions; however, it has a shallow root system that does not easily recover from drought (Hancock Seed, 2014). The crop requires approximately 1/3 less water than corn (Koch, 2002) and has a high level of tolerance to salinity (Krishnamurthy *et al.*, 2014). It can grow at higher elevations (1500 m) as well as in plains (Baltensperger, 1996).

2.1.3 Nutritional Value and Other Uses

Foxtail millet like other millets is a rich source of human and livestock nutrition in developing countries (NAS, 1996). Millet generally has high amount of vitamin, calcium, iron, potassium, magnesium, and zinc (Leder, 2004). In addition to being nutritious, millets are also considered as healthy food. The grains of most millet do not contain gluten; a substance that causes celiac disease or other forms of allergies (Leder, 2004). Foxtail millet and other millet species (namely, kodo-, finger-, proso-, little- and pearl- millet) were shown to have an anti-proliferative property and might have a potential in the prevention of cancer initiation (Chandrasekara and Shahidi, 2011), due to the presence and quantity of phenolic extracts (Rao *et al.*, 2011). Foxtail millet protein improves insulin sensitivity and cholesterol metabolism through an increase in

adiponectin concentration thereby making it beneficial food component in obesity-related disease such as type 2 diabetes and cardiovascular diseases (Choi *et al.*, 2005).

Foxtail millet is grown for hay. Like all millets, this species is fast growing and produces relatively high yields with no danger of producing toxic levels of prussic acid (Lee and Henning, 2014). Foxtail millet can be harvested 75–90 days after planting (DAP) (Cash *et al.*, 2002). It can produce good quality (Koch, 2002). It is similar to other warm-season grasses in terms of forage quality (Baltensperger, 1996) and performed as well as corn in broiler chicken diets and could replace 100% of corn in broiler feed without negatively affecting protein digestibility or bird health (Boroojeni *et al.*, 2011).

Its water-use efficiency is one of the highest of any crop, thus making it a good choice for use in semi-arid environments (Koch, 2002). Mixing foxtail millet with cowpea (*Vigna unguiculata*) is a good cover crop option (Creamer and Baldwin, 1999). Because foxtail millet is fast growing and produces more biomass than annual rye, it is sometimes the preferred choice for restoration of mine lands or steep slopes (Burger *et al.*, 2009). Many granivorous birds are attracted to millet and it is often included in finch and exotic bird seed mixes. It is also planted in food plots for deer, turkey, quail, and dove (Hancock Seed Co., 2014).

2.1.4 Ethnobotany

Foxtail millet has a long history of cultivation for human consumption in China dating back to the Neolithic Era over 4,000 years ago (Baltensperger, 1996). It is the most important millet species currently grown in China (Baltensperger, 1996). It was once a staple crop in the northern Philippines and eventually lost its dominance to pond-field cultivation of rice and increased

production of swidden plots of sweet potato (Bodner and Gereau, 1988). Along with pearl millet and finger millet, foxtail millet is one of the most important millets in the semi-arid tropics of Asia and Africa (CGIAR, 2014). In the United States, foxtail millet was not often or widely grown until after colonial times (Oelke *et al.*, 1990).

2.1.5 Seeds and plant production

Foxtail millet is self-pollinated that produce seed in 75–90 days (Hancock Seed, 2014). There is a very quick transition period from vegetative growth to flower development (Baltensperger, 1996). Flowering occurs top-down on each of the stemmed branches.

2.2 Mutation in Crop Plant Breeding

Mutation can be defined as a sudden heritable alteration in the structure or sequence of DNA of an organism which is not caused as a result of segregation and recombination of genes. Mutation in plants is any heritable change in the idiotypic constitution of sporophytic or gametophytic plant tissue, not caused by normal genetic recombination or segregation (Harten, 1998). These changes in our target plant can be passed on to progeny and used for human benefit through breeding. While recombination of alleles gives rise to a certain degree of variation which already present in the genome, it is not capable of creating novel trait. Mutations therefore create genetic variability in a closed population.

2.2.1 Importance of mutation in creating variation

Variation is the source from which plant breeders are able to produce new and important cultivars. Alleles of varying forms at given loci in a population can be selected and fixed within a new individual or line. We depend on recombination and independent assortment of favorable alleles to produce new and unique individuals from which to select and produce the lines that will serve as our cultivars. While recombination of alleles provides offspring with presumably selectable variation for the spectrum of traits exhibited, it is only capable of creating new combinations of traits already existing. Recombination does not of itself produce novel traits. Exploiting natural or induced genetic diversity is a proven strategy in the improvement of all major food crops, and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability (Martin *et al.*, 2009). Through breeding and selection, species can have improved yield, quality, taste, size and resistance to disease and plants adapt to diverse climates and conditions (William, 2007). Mutation can occur spontaneously in nature or are induced by man.

2.3 Types of Mutation

2.3.1 Naturally occurring mutations

Mutations occur spontaneously in natural settings quite frequently. They can happen due to mistakes made during cell replication or exposure to mutagens such as radiation. It is estimated that a mutation occurs every 10^{-8} base pair per generation in eukaryotic genomes (Drake *et al.*, 1998). In corn (*Zea mays*), mutations occur from 10^{-6} to 5×10^{-4} per base pair per generation (Stadler, 1930). Those that can be track easily in the offspring are mutations occurring in either the gametes or cells that give rise to the gametes. Mutations in somatic cells cannot be passed on to future generations, and so are only important in vegetative propagated species.

2.3.1.1 *Transposable Elements*

Transposable elements are a special class of mutagen. They are self-replicating segments of DNA that excise and/or insert themselves within the genome. Also known as transposons, these strange sequences were first proposed by the pioneering Barbara McClintock working on maize (McClintock, 1948). Transposable elements, unlike other forms of mutagenesis, do not act upon the genome in a completely random fashion. Rather, they have certain “hot-spots” where they are more likely to insert or replicate themselves. By their insertion or deletion, they act upon the genes in which they are located or those adjacent to them. Transposable elements can cause gene disruptions, protein product alterations, or large-scale genome rearrangements. If inserted into the intron of a gene, they can cause transcriptional inefficiency (Hartwell *et al.*, 2008).

2.3.2 Induced mutation

An induced mutation is any change within the genome of an organism caused by physical or chemical agents called mutagens. The use of ionizing radiation and chemical mutagens has been used extensively in the study of genetics and contributed a great deal to our understanding of biology as a whole. Since the discovery of this, mutagens have been widely used in fields such as; biology, medicine, agriculture etc. (Ranch, 2001). From 1930 to 2014, more than 3200 mutagenic plant cultivated varieties have been released with highest number in cereals (FAO, 2014; Schouten and Jacobsen 2007).

2.4 Types of Induced Mutation

2.4.1 Ionizing radiation

Ionizing radiation includes ultra-violet (UV) light, X-ray, Gamma rays, and neutrons. These high-energy forms of radiation cause double-strand breaks of the DNA double helix. Once pieces of the DNA are broken, cellular repair mechanisms stitch the pieces back together. These DNA

repair systems can only handle low rates of radiation, however, and increases in the rate of exposure to ionizing radiation causes permanent mutations to occur and accumulate in an organism's genome. Radiation causes deletions of nucleotides from the DNA sequence. These deletions can cause reading-frame shifts, inactive protein products, or faulty transcripts. This typically results in null mutations, which are those in which a particular gene is inactivated. In the mutagenic treatment of plant material, doses can range from as low as 2 Gy for cell cultures or leaf tissues, to as high as 700 Gy for seed material (Ahloowalia and Maluszynski, 2001).

2.4.1.1 Fast neutrons

Mutation breeding employing fast neutron irradiation (FNI) has been used to develop new varieties (Sodkiewicz and Sodkiewicz, 1999) and is widely used for the induction of mutations (Zhang *et al.*, 2002) resulting in a significant increase in the yield of major crops, including chilli (Swaminathan, 1998). Research in *Capsicum annum* and *Capsicum frutescens* showed that fast neutrons caused significant variation in characters such as plant height, number of leaves/plant different yield traits in *C. annum* in which the number of fruits/plant and number of seeds/fruit the width and length of fruit the weight of fruit different irradiation exposure period (Falusi *et al.*, 2012)

Wang *et al.* (2015) who worked on peanut observed that as the irradiation dosage increased, the frequency of both somatic embryo formation and plantlet regeneration decreased. The regenerated plantlets were grafted onto rootstocks and were transplanted into the field. Later, the mature seeds of the regenerated plants were harvested. The M₂ generation plants from most of the regenerated cultivars exhibited variations and segregation in vigor, plant height, branch and pod number, pod size, and pod shape. Fast neutrons has also been reported to increase percentage

germination in sesame (Falusi *et al.*, 2013) and stimulate ambrosin, damsine, chlorophyll content and amino acid in *Ambrosia maritima* shoot grown under water stress (Hanan and Eman, 2014)

2.4.2 Chemical mutagens

Chemical mutagens affect the DNA molecule through chemical reactions within the genome. Base analogs are chemicals with similar properties to the DNA bases. They can be incorporated by the cell into the genome, replacing the proper base. Alkylating agents such as Ethyl Methane Sulfonate (EMS), react with guanine or thymine by adding an ethyl group which causes the DNA replication machinery to recognize the modified base as an adenine or cytosine, respectively. This base substitution typically does not result in reading frame shifts, but instead causes altered forms of a triplet sequence. Changing a single base within a coding region causes either a nonsense codon which stops transcription or an altered codon which changes the amino acid transcribed, which can de-activate, reduce efficiency of, or produce a new protein. Nitrous acid, a deaminating agent, exerts its effect by the removing amino groups of the adenine, cytosine and guanine residues of the nucleic acid (causing chemical alterations) as well as cross-links of undefined structures, deletions (Burnotte and Verly, 1971; Miller, 1972). When the cell replicates this altered area, it matches adenine to the deaminated cytosine, and cytosine to the deaminated adenine, resulting in similar effects to that of alkylating agents.

The last type of chemical mutagen, intercalating agents, causes deletions, reading frame shifts, or random base insertions. These compounds insert themselves into the DNA between adjacent base pairs, thus disrupting replication and transcription machinery.

2.4.2.1 Ethyl Methane Sulphonate

EMS is one of the potent alkylating chemical mutagen for chemical mutagenesis. EMS is more effective than physical mutagens (Bhat *et al.*, 2006). Different biological traits such as seed germination, pollen fertility, and survival e.t.c. have been studied earlier by different authors after the chemical mutagen treatment on different plants reported a radio protective effect of Decrease in seed germination, growth and survival with increasing concentrations of EMS in *Phaseolus lanatus* (Kumar *et al.*, 2003). Linearly decrease in seed germination has also been reported by in mungbean (Khan and Wani, 2004). A similar result was observed by Watto *et al.* (2012), after studying the mutagenic variability by EMS in Basmati rice. Selvaraj and Jaykumar (2004) observed decrease in seed germination, pollen fertility and general variation in quantitative traits with increasing concentrations of EMS on sunflower. Basu *et al.* (2008) observed improvement of different quantitative traits like pod length and number of pods in M₃ plants of Fenugreek (*Trigonella foenum-graecum* L.) after treating with different concentrations of EMS. Selvaraj *et al.* (2012) studied different quantitative characters like plant height, number of days for first flowering etc after treatment of EMS in *Jatropha curcas* L. They observed that height decreased with increasing concentrations of EMS while the number of days for first flowering considerably reduced at lower concentrations while as the same increased at higher concentrations. Decrease in quantitative traits at higher concentrations of EMS has been reported by Kozgar *et al.* (2010) in *Vigna radiata* and *Vigna mungo*. Wani *et al.* (2012) reported the increase of various quantitative characters like number of pods per plant and 100 seeds weight at lower concentrations of EMS in M₃ generation in chickpea (*Cicer arietinum* L.).

2.4.2.2 Sodium Azide

Sodium azide, a well-known respiratory, catalase and peroxidase inhibitor has been shown to be a potent chemical mutagen in both higher and lower organisms (Nilan *et al.*, 1973). It is a

common bactericide, pesticide and industrial nitrogen gas generator which is known to be highly mutagenic in several organisms (Grant *et al.*, 1994; Rines, 1985). The mutagenicity created by sodium azide is mediated through the production of an organic metabolite of azide compound, presumably azidoalanine. The production of this metabolite was found to be dependent on the enzyme O-acetylserine sulfhydrylase (Khan *et al.*, 2005). Sodium azide is mutagenic mechanism used for the improvement of economic characters in rice, wheat, barley and sorghum (Maluszynski *et al.*, 2009).

Studies in *Capsicum annum* showed that sodium azide was useful in creating variability at low concentration. Decrease in germination percentage, chlorophyll content, plant height, root length shoot length at high concentration. However, increase sugar content and total protein content increase at higher concentration (Umar *et al.*, 2012). Secondary metabolites synthesis was increased compared to the untreated seeds of *P. odontadenius* with a more important synthesis in phenolic compounds (Nakweti *et al.*, 2015). The high inhibitory effects secondary metabolite against growth of medically important parasites suggest that sodium azide in plant breeding for increasing production of secondary metabolite for medical and pharmaceutically importance. In another work, sodium azide also has been reported reduces the germination percentage, root length and shoot length; however, at low concentration it was not different with control (Srivastava and Singh, 2011). The magnitude of genotypic and phenotypic variability, heritability and genetic gain for various polygenic traits were also decreases with the increases in concentration of sodium azide. However, yield attributing characters showed both positive and negative shift in mean than those of control.

Similarly, increase in the seedling height, number of leaves, high frequency of paracytic stomata, higher stomatal index and density on the abaxial leaf surface and large stomata in seedlings induced with sodium azide in *Jatropha* (AbdulRahaman *et al.*, 2013).

2.4.2.3 Nitrous acid

Nitrous acid has been shown to deaminate nucleobases in vivo in DNA, (Zimmermann *et al.*, 1966). Atmospheric nitrous acid was observed to affect the physiological of pine trees (Sakugawa and Cape, 2007). They found that exposure to nitrous acid over two months affects photosynthesis and nutrient status of pine trees, by increase in the carbon to nitrogen ratio. Previous studies have described the growth-modulating properties of NO and its interaction with auxin in modulating root growth and developmental processes (Shapiro, 2001). Nitrous acid has been reported to promote seed germination, photomorphogenesis, mitochondrial activity, leaf expansion, root growth, stomatal closure, fruit maturation, senescence, and iron metabolism, defense response, playing key roles in the activation of defense genes e.g., pathogenesis-related protein 1 (Wendehenne *et al.*, 2001). In another work, nitrous acid was reported to decrease germination, seedling plant height, number of leaves, tiller/plant, and leaf length were significantly increase with time of exposure. All treatments induced early maturity but with low seed set and yield among 6 and 8 hour treatment time of 0.1% plants in fonio millet. Generally, the performance of the treated plants was better with short exposure time and four hour of treatment. (Animasaun *et al.*, 2014). Similarly, significant increase was observed when *Vicia faba* was treated for 24 hours with nitrous acid in number of branches/plant, pods/plant, seed weight rate, seed yield, and protein yield (AL-Shamma and Sahib, 2014).

2.5 Use of Mutation in Plant Breeding

Mutagenesis, the act of inducing mutations within an organism's genome, has been used in plant breeding since Muller's discovery of the mutagenic effects of X-rays on *Drosophila* flies

(Muller, 1927). The first crop species to be mutagenized was barley by Stadler, who began using X-rays to induce mutations independently of Muller at around the same time (Stadler, 1928). More than 3218 varieties have been released worldwide that have been derived either as direct mutants or from their progenies in 214 plant species. Around 492 and 110 varieties have been released in legumes and oil seed crops, respectively. Induction of mutations with radiation has been the most frequently used method for development of mutant varieties (FAO., 2014).

2.5.1 Disease resistance

A mutant displaying resistance against race 40 of stem rust was isolated following X-irradiation in the popular wheat variety Pb C 591 (Bhatia *et al.*, 1961). Multiple resistance to rusts was induced in *Triticum aestivum* varieties (Lalbahadur and Kharchia Local) with nitrosomethyl urea treatment. These mutants displayed resistance to several races of leaf rust at seedling stage. Besides, adult plant resistance was also found in these mutants for yellow rust, black rust and brown rust (Sawhney *et al.*, 1977). Some of these mutants displayed yield advantage, which was proved to be due to their ability to ward off rust infection. In cotton, a jassid-resistant line was isolated through mutation breeding of the highly susceptible variety 'Mescilla Acala' (Jagathesan, 1963). In groundnut, treatment with gamma ray combined with EMS and NaN₃ resulted in induction of resistance against two fungal, the early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Phaeoisariopsis personata*) in which individually or combined can cause more than 50% yield loss (Venkatachalam *et al.*, 1991). Cotton germplasm has been enriched with a number of useful mutants carrying resistance for bacterial blight and fusarium wilt disease, in addition to mutants for weak fiber attachments, high ginning out turn, and lint percentage (Gustafsson, 1940). Multi- location on-farm trials of the mutant line indicate significant increases in yield for farmers, without symptoms of the disease (Gustafsson, 1960).

2.5.2 Physiological trait

Mutation breeding was applied to alter grain colour of wheat. A light coloured grain mutant 'Sharbati Sonora' was obtained from 'Sonora 64' while a similar light grain colour mutant 'Pusa Lerma' was derived from another popular line 'Lerma Rojo 64A'. 'Pusa Lerma' with its high resistance to stem rust and semi-hard white grains was released for cultivation in peninsular India (Swaminathan, 1964). 'Sharbati Sonora' was early besides having amber grains and hence was found to be suitable for late planting. Amber-grained mutants were also induced by gamma rays in a high yielding dwarf variety Tonari 71 of *Triticum aestivum* (Sawhney *et al.*, 1977). These mutants were late by about six days, and also displayed colourless auricles and waxy ears and leaves. In rice several dwarf mutants were induced following chemical and physical mutagenesis (Singhet *et al.*, 1979). In tobacco Patel and Swaminathan⁴⁰ recorded significant improvement in nicotine content in mutants induced through X-rays and ³²P. (Patel *et al.*, 1986).

2.6 Cytological Aberrations

Auerbach and Robson (1942) were the first to report on induction of cytological aberrations. They reported the induction of mutations and cytological aberrations in *Drosophila* by mustard gas. The occurrence of rod bivalents, ring bivalents and univalents by mutagens was earlier reported by Mansour (1994); Bione *et al.* (2002), and Vinita *et al.* (2004). Bhat *et al.* (2007) observed different cytological abnormalities after EMS treatment of seeds of two varieties of *Vicia faba* L. The different meiotic abnormalities were stickiness, univalents, multivalent, laggards, unorientation, precocious separation of chromosomes at metaphase and bridges. Khan and Tyagi (2009) reported bridges and laggards in soybean after treatment with gamma rays and EMS. Ashutosh *et al.* (2012) reported different cytological abnormalities in *Catharanthus roseus* L. root meristem cells after treatment with EMS. The chromosomal anomalies observed include

condensation, persistent nucleolous, fragmentation, C- metaphase, bridge, laggard, cleft and binucleolated cells(Wani *et al.*,2013) observed various meiotic abnormalities like stickiness, univalents, precocious segregation, laggards, bridges, cytomixis and so on in *Trigonella foenum-graecum* L. after treating it with different doses/concentrations of gamma rays, EMS and SA. They found that gamma rays induced more meiotic abnormalities followed by EMS and SA.

2.7 Mutagenic Efficiency and Effectiveness

Mutagenic effectiveness and efficiency are two different properties, which are important in mutation breeding programmes. Knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations (Kumar and Mani, 1997). Mutagenic effectiveness is a measure of the mutations induced per unit dose of a mutagen (time \times concentration/dose), while mutagenic efficiency gives an idea of genetic damage (mutation) in relation to the total biological damage caused in M₁ generation (Konzak *et al.*, 1965; Khan and Wani, 2006 and Singh, 2011). Although, both are two different properties but the usefulness of any mutagen in plant breeding programme depends on both of them. It is not necessary that an effective mutagen shall be an efficient one also (Khan *et al.*, 2005). Various factors like biological, environmental and chemical ones modify the effectiveness and efficiency of different mutagens and the mutation rate (Blixt, 1970). Mutagenic effectiveness and efficiency were also found to depend upon mutagen type and the genotype. There have been a number of reports revealing that the effectiveness and efficiency of mutagens vary to a greater extent in various crop plants as in clusterbean (Velu *et al.*, 2008), cowpea (Dhanavel *et al.*, 2008; Girija *et al.*, 2013), garden pea (Sharma *et al.*, 2010; Solanki, 2005), limabean (Kumar *et al.*, 2003), mungbean (Singh, 2007; Goyal *et al.*, 2009), It has been noticed that among the monofunctional mutagens, while methylating agents are more toxic and thus have to be used

only at lower concentrations ethylating agents, being less toxic, can be applied at relatively higher concentrations to yield more mutations. Khan and Wani (2005) found that the order of mutagens based on effectiveness was $MMS > SA > EMS$ whereas on the basis of their efficiency, it was $EMS > MMS > SA$. SA most effective mutagen and EMS in mugbean (Wani *et al.*, 2011).

2.8 Heritability Estimate

The estimate of heritability acts as a predictive instrument in expressing the reliability of phenotypic values (Usharani and Kumar, 2016). Therefore, it helps the plant breeders to make selection for a particular character when heritability is high in magnitude (Unche *et al.*, 2008). High phenotypic and genotypic variances in the quantitative traits indicate better chances for selection to be successful. The genotypic coefficient of variation measures the range of genetic variability shown by the plant trait and helps to compare the genetic variability present in various traits. However, with the genotypic coefficient of variation alone it is not possible to determine the amount of variation that is heritable. The heritable portion of the variation is usually determined with the aid of heritability estimates. The values of heritability increased and differed from trait to trait. Heritability is a property not only of a character but also of the population and the environment to which the genotypes are subjected to. Therefore, its ultimate value depends on the magnitude of all the components of variance (Bhareti *et al.*, 2011). The high estimates of heritability in quantitative traits has been found to be useful from the point of view of plant breeding, as this enables selection to be based on phenotypic performance. Selection will be rewarding for improvement of such traits, where high heritability is associated with the trait under consideration.

The fertile branches per plant, pods per plant, 100-seed weight and seed yield per plant exhibits high heritability in *Vigna radiate* treated with EMS (Wani and Khan, 2006). Kaul and Kumar (1983) obtained low heritability values for grain yield in rice. Kumar (2008) recorded high heritability for number of seeds per pod, days to flowering, yield per plant, 100 grain weight, number of pods per plant and harvest index in pea. Heritability was high almost all the doses of gamma ray and EMS treatments (Usharani and Kumar, 2016). High heritability indicates that these traits are under the control of additive gene action and directional selection for these traits could be effective for desired genetic improvement (Malik *et al.*, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was conducted in the botanical garden of the department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria, (Lat 11° 11' N; Long 7°N 38' Altitude 660m above sea level). Samaru lies in the northern guinea savanna agroecological zone of Nigeria with a mean annual rainfall of about 1100mm. Rainfall is essentially between April and September and a mean

daily temperature of 27°C. The coldest months were November-January. The experiments were conducted from July, 2015 to February, 2016.

3.2 Source of Materials

Healthy, pure and dry seeds (moisture content 10-12%) of foxtail millet were obtained from Institute for Agricultural Research/Faculty of Agriculture, Ahmadu Bello University, Zaria, Kaduna State

3.3 Mutagenic Treatment

3.3.1 Physical mutagens

Foxtail millet seeds were exposed to fast neutrons AmBe source with a flux of $1.5 \times 10^4 \text{cm}^2 \text{s}^{-1}$ at Center for Energy Research and training, Ahmadu Bello University, Zaria for 0min., 90min., 180min., 270min. and 360min.

3.3.2 Chemical mutagens

The treatment with chemical mutagens was conducted in the postgraduate laboratory of the Department of Biological Science, Ahmadu Bello University, Zaria. Seeds of foxtail millet were presoaked in distilled water for 4hours. Presoak seeds in water some hours prior to mutagenic treatment allows the mutagen to diffuse more rapidly to the tissues of interest (meristems) (Foster and Shu 2011). The seeds were soaked for 4hours in different concentrations of the chemical mutagens; Ethyl Methane Sulphonate (0.00%, 0.1%, 0.2%, 0.3% and 0.4%), Sodium Azide (0.00%, 0.1%, 0.2%, 0.3% and 0.4%), and Nitrous Acid (0.00%, 0.1%, 0.2%, 0.3% and 0.4%). After the treatment period, the treated seeds were thoroughly washed in running tap water to remove the residual effects of mutagens, if any.

3.4 Raising the First Mutant (M₁) Generation

Treated and control seeds were thereafter taken to the botanical garden of the department of Biological Science, Ahmadu Bello University, Zaria and sown at 40×75cm intra and inter space on the 15th of July 2015. Each treatment was replicated four times using Completely Randomized Block Design (CRBD). The M₁ was conducted during between July-October 2015.

3.5 Raising the Second Mutant (M₂) Generation

Randomly selected healthy seeds harvested from M₁ generation of each treatment of fast neutron, ethyl methane sulphonate, sodium azide and nitrous acid were planted in Complete Randomized Block Design. (RCBD) with four replications in the botanical garden in 40×75cm intra and inter space to raise the M₂ generation. While M₂ generation were raised during the months of December, 2015- February, 2016

3.6 Morphological Traits Measured

- 3.6.1 Seed Germination percentage: Twenty seeds from each of the treatments were placed on Whatman filter paper in petridish (9cm X 2cm). Each petridish was moistened with 2ml/plate of distilled water and kept cupboard room temperature. The germination percentage was counted on the 7th day.
- 3.6.2 Plant height at Maturity: three plants were randomly selected and determined using a meter rule
- 3.6.3 Number of Leaves: manual counting of leaves present
- 3.6.4 Leaf length: The length of three leaves were measured using a meter rule
- 3.6.5 Leaf Width: The width of top three leaves were measured using a meter rule

- 3.6.6 Fresh Weight: Fresh plants were harvested and weighed using a weighing balance
- 3.6.7 Dry Weight: Fresh plants were harvested and oven dried at 30°c to a constant weight
- 3.6.8 Days to 50% flowering: Numbers of days in which 50% of plants stands in a replication flowered
- 3.6.9 Length of Panicle: Determined using a meter rule
- 3.6.10 Weight of Panicle: Determined using an electric weighing balance
- 3.6.11 1000seed Weight: 1000seeds were counted and weighed using electric weighing balance

Randomly selected M₁ healthy seeds from each treatment of Fast Neutron, Ethyl Methane Sulphonate, Sodium Azide and Nitrous Acid were forwarded to the M₂generation.

Chlorophyll mutations and other quantitative mutants were screened through regular visual observations atseedling stage as well as during their entire period of growth.

3.7 DataAnalysis

One way analysis of variance (ANOVA) was done using SAS (SAS, 2002)version 9.1to compare the means between different dose/concentration of mutagen. Duncan’s multiple range test (DMRT) was used to separate the means where there is significant difference.

Variance component estimate was done using SAS version 9.1was used to determined variance due to environment and variance due to genotype. Broad sense heritability was determined using

the formula by Johnson *et al.* (1955)

$$H_b(\%) = \frac{\sigma_g^2}{\sigma_{ph}^2} \times 100$$

$$\sigma_{ph}^2 = \sigma_g^2 + \sigma_{e/r}^2$$

Where σ_g^2 = variance due to genotype σ_e^2 = variance due to environment, σ_{ph}^2 = phenotypic variance, r = number of replications

And categorized according to Robinson *et al.* (1949) as: 0<30% (low), 30-60% (moderate), >60% (high)

The effectiveness and efficiency of the mutagens in inducing mutations were estimated by adopting the formula suggested by Konzaket *al.*, (1965). Mutation frequency was calculated by the following method.

$$\text{mutagenic frequency} = \frac{\text{number of chlorophyll mutant}}{\text{total number of plants}} \times 100$$

$$\text{mutagenic effectiveness} = \frac{\text{mutation frequency}}{\text{dosage or conc} \times \text{time of treatment}} \times 100$$

$$\text{mutagenic efficiency} = \frac{\text{mutagenic frequency}}{\text{lethality}}$$

CHAPTER FOUR

RESULTS

4.1 Effects of Fast Neutron on Agronomic Traits of Foxtail Millet at M₁ Generation

The results of the effects of different doses of fast neutron on agronomic traits of foxtail millet at M₁ generation is presented in Table 4.1. Plant height, leaf length, leaf weight, panicle length and one thousand seed weight were significantly different ($P < 0.05$). Plant height decreased with treatment as control had the highest values (31.06 cm) and 360 min treatment gave lowest (26.65 cm). Similarly, there was decrease in leaf length with treatment, as the control gave highest mean value of 14.13 cm while 180 min fast neutron treated gave lowest mean of 10.94 cm. Leaf weight also decreased with treatment from 1.3 cm observed control to 1.14 cm observed at 90 min. On the other hand, panicle length was highest at 270 min (4.26 cm) though comparable to 0.0 min (4.24 cm) and 360 min (4.09 cm) and 90 min treatment gave lowest mean value (3.61 cm).

4.2 Effects Fast Neutron on Agronomic Traits of Foxtail Millet at M₂ Generation

There was significant difference ($P \leq 0.05$) observed in the doses of fast neutrons applied on foxtail millet for plant height, leaf length, leaf weight, panicle length and panicle weight (Table 4.2). Plant height increased with increase time of exposure to fast neutrons 180 min was highest (18.47 cm highest) while 90 min was lowest (31.94 cm) but comparable to control (33.08 cm). Similarly, leaf length was highest (18.47 cm) at 180 min and lowest (13.26) at 90 min though comparable to control (16.66 cm). 270 min fast neutron was also highest (1.68 cm) for leaf weight and lowest (1.38 cm) for control. Panicle length was highest (6.82 cm) at 270 min and lowest (5.39 cm) at 90 min. while panicle weight and 1000 seed weight at 270 min fast neutrons was highest (2.51 g and 3.55 g) and control recorded lowest values 1.61 g and 2.86 g respectively.

Table 4.1: Effects Fast Neutrons on Agronomic Traits of Foxtail Millet at M₁ Generation

FN(min)	PGERM(%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.0	81.25 ^a	31.06 ^a	5.25 ^a	14.13 ^a	1.31 ^a	1.25 ^a	0.90 ^a	42.00 ^a	4.24 ^a	0.70 ^a	2.61 ^a
90	81.25 ^a	27.80 ^b	5.00 ^a	12.34 ^b	1.14 ^b	1.23 ^a	0.82 ^a	40.00 ^a	3.61 ^b	0.48 ^a	2.70 ^a
180	81.25 ^a	27.78 ^b	6.00 ^a	10.94 ^b	1.23 ^{ab}	1.05 ^a	0.78 ^a	39.00 ^a	3.74 ^b	0.48 ^a	2.55 ^a
270	85.00 ^a	29.4 ^{ab}	5.50 ^a	11.99 ^b	1.15 ^b	1.05 ^a	0.73 ^a	39.00 ^a	4.26 ^a	0.50 ^a	2.70 ^a
360	83.75 ^a	26.65 ^b	5.25 ^a	11.42 ^b	1.21 ^{ab}	1.05 ^a	0.73 ^a	41.00 ^a	4.09 ^{ab}	0.55 ^a	2.48 ^a
SE±	3.45	0.91	0.39	0.53	0.05	0.11	0.07	1.78	0.18	0.08	0.03

NOTE: Means with the same letter within a column are not significantly different at $P \geq 0.05$

Keys: PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M₁-First mutant generation, SE- Standard error, FN- Fast neutron.

Table 4.2: Effects of Fast Neutrons on Agronomic Traits of Foxtail Millet at M₂ Generation

FN(min)	PGERM (%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.0	81.25 ^a	33.08 ^b	6.58 ^a	16.66 ^{ab}	1.38 ^c	2.62 ^a	1.10 ^a	62.00 ^a	5.42 ^b	1.61 ^b	2.86 ^b
90	86.25 ^a	31.94 ^b	7.42 ^a	13.26 ^b	1.41 ^{bc}	2.75 ^a	0.95 ^a	62.50 ^a	5.39 ^b	2.06 ^{ab}	3.50 ^a
180	85.00 ^a	46.65 ^a	6.94 ^a	18.47 ^a	1.58 ^a	3.85 ^a	1.58 ^a	61.00 ^a	6.70 ^a	2.25 ^a	3.31 ^a
270	85.00 ^a	41.81 ^{ab}	7.67 ^a	17.44 ^a	1.68 ^a	4.20 ^a	1.78 ^a	63.75 ^a	6.82 ^a	2.51 ^a	3.55 ^a
360	87.50 ^a	33.22 ^b	6.94 ^a	16.13 ^{ab}	1.55 ^{ab}	3.28 ^a	1.20 ^a	60.00 ^a	5.57 ^b	2.00 ^b	3.27 ^a
SE±	3.28	3.39	0.44	1.87	0.05	0.61	0.39	1.36	0.29	0.16	0.09

NOTE: Means with the same letter within a column are not significantly different at $P \geq 0.05$

Keys: PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M₂-First mutant generation, SE- Standard error, FN- Fast neutron.

4.3 Effects of EMS on Agronomic Traits of Foxtail Millet at M₁ Generation

The result of the effects of EMS at different concentration recorded significant difference ($P \leq 0.05$) for percentage germination, plant height, leaf length, leaf weight, fresh weight, dry weight, days to fifty percent flowering, panicle length and panicle weight (Table 4.”). Percentage germination increased from 82% in control 95% at 0.1% EMS and then decreased with increase in treatment. Plantheight also increased with increase in concentration from control recording 29.74cm to 34.87cm observed at 0.4% concentration. Leaf length significantly increased to 15.01cm at 0.1% concentration but decreased to 11.40cm further increase to 0.2%. Similarly, leaf width was significantly increased at 0.1% (1.50cm), while 0.0% (1.26) was lowest. There was increase with treatment observed for fresh weight, dry weight and panicle weight with increase in treatment as 0.4% EMS concentration gave highest mean values (2.60g, 1.73g and 1.38g) respectively while lowest (1.25g, 0.80g, and 0.63g) was recorded at the control respectively. Days to 50% flowering was lowest (34 days) at 0.2% EMS while highest (44 days) was at control. On the other hand, panicle length highest mean value (5.67cm) at 0.3% while 0.2% concentration was lowest (4.01cm).

4.4 Effects of EMS on Agronomic Traits of Foxtail Millet at M₂ Generation.

The results recorded significant difference ($P \leq 0.05$) for plant height, number of leaves, leaf length, leaf weight, fresh weight, dry weight, days to 50% flowering, panicle length, panicle weight and 1000 seed weight (Table 4.4). The highest mean value (39.31cm) observed for plant height where at 0.1% concentration then decreased as concentration increased with 0.4% showing lowest plant height (26.29cm). Number of leaves was highest (8.08 leaves) at 0.1% concentration and as the lowest (6.00 leaves) at 0.3% concentration. Similarly, leaf width, fresh weight and dry weight as 0.1% (1.92cm, 4.85g and 1.27 g respectively) were significantly

different as mean value decreased with increase in concentration of ems with 0.4% (1.44cm, 2.00g, 0.70g respectively) showing minimum values. Days to 50% flowering was highest at control (61.25days) and was lowest (52.50days) at 0.2% concentration. While panicle length, panicle weight and 1000seed weight were highest (7.60cm, 2.24g and 3.73g respectively) at 0.1% and lowest 0.0%, 0.40% with 4.57cm each for panicle length, 0.3 % (1.20g) for panicle weight and control with 2.80g.

Table 4.3 Effects of EMS on Agronomic Traits of Foxtail Millet at M₁ Generation

EMS (%)	PGERM	PHT(cm)	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.00	82.50 ^b	29.74 ^c	13.59 ^{ab}	1.26 ^c	1.25 ^b	0.80 ^b	44.00 ^a	4.48 ^{bc}	0.91 ^b	2.63 ^a
0.10	95.00 ^a	32.17 ^b	15.01 ^a	1.50 ^a	2.23 ^a	1.55 ^a	37.00 ^{bc}	4.94 ^{ab}	1.08 ^a	2.71 ^a
0.20	88.75 ^{ab}	31.84 ^{bc}	11.40 ^b	1.36 ^{bc}	2.08 ^a	1.40 ^a	34.00 ^c	4.01 ^c	1.03 ^{ab}	2.70 ^a
0.30	83.75 ^b	33.06 ^{ab}	13.17 ^{ab}	1.40 ^{ab}	2.30 ^a	1.63 ^a	41.00 ^{ab}	5.08 ^a	1.25 ^a	2.69 ^a
0.40	83.75 ^b	34.87 ^a	13.41 ^{ab}	1.36 ^{bc}	2.60 ^a	1.73 ^a	37.25 ^{bc}	5.67 ^a	1.38 ^a	2.64 ^a
SE±	2.36	0.62	0.67	0.03	0.17	0.14	1.36	0.21	0.13	0.33

NOTE: Means with the Same Letter within a Column are not Significantly Different at $P \geq 0.05$

Keys: PGERM- percentage germination,, PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width, FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M₁-First mutant generation, SE- Standard error, EMS-Ethyl Methane Sulphonate.

Table 4.4: Effects of EMS on Agronomic Traits of Foxtail Millet at M₂ Generation

EMS (%)	PGERM (%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.00	83.75 ^a	31.82 ^{ab}	6.75 ^b	16.58 ^{ab}	1.55 ^b	2.73 ^b	0.98 ^b	61.00 ^a	4.57 ^b	1.40 ^b	2.86 ^c
0.10	86.25 ^a	39.31 ^a	8.08 ^a	17.65 ^a	1.92 ^a	4.85 ^a	1.28 ^a	60.00 ^a	7.60 ^a	2.24 ^a	3.73 ^a
0.20	83.75 ^a	27.25 ^b	6.62 ^b	12.71 ^a	1.60 ^b	2.33 ^b	0.83 ^b	52.50 ^b	4.90 ^b	1.38 ^b	3.35 ^b
0.30	81.25 ^a	33.28 ^{ab}	6.00 ^b	17.08 ^{ab}	1.44 ^b	2.33 ^b	0.78 ^b	59.75 ^a	5.46 ^b	1.20 ^b	3.09 ^{bc}
0.40	80.00 ^a	26.29 ^b	6.50 ^b	15.025 ^{ab}	1.44 ^b	2.00 ^b	0.70 ^b	58.50 ^a	4.57 ^b	1.43 ^b	3.25 ^b
SE±	3.67	2.74	0.37	1.34	0.07	0.59	0.23	1.84	0.34	0.19	0.11

NOTE: Means with the Same Letter within a Column are not Significantly Different at $P \geq 0.05$

Keys: PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M₁-First mutant generation, SE- Standard error, EMS-Ethyl Methane Sulphonate

4.5 Effects of Nitrous Acid on Agronomic Traits of Foxtail Millet at M₁ Generation.

There was significant difference ($P \leq 0.05$) difference concentration of nitrous acid on agronomic traits at M₁ in plant height, number of leaves, fresh weight, dry weight, days to 50% flowering, panicle length and panicle weight (Table 4.5). Plant height and panicle length increased with treatment and was highest (37.51cm and 6.08cm) at 0.10% while, the control recorded the lowest mean values (34.18cm and 4.83cm). Number of leaves and fresh weight increased with concentration from 5.00 leaves and 2.10g observed at 0.2% to 6.75 leaves and 3.45g respectively at 0.4% treatment while the control gave lowest values respectively. Dry weight and panicle weight increase with increase in concentration and gave highest values (1.45g and 1.13g) for respectively and at 0.3% concentration while control was lowest (0.78g and 1.13g). Days to 50% flowering decreased to 34.35 days at 0.1% concentration while control was highest at 42.75 days.

4.6 Effects of Nitrous Acid on Agronomic traits of Foxtail Millet at M₂ Generation.

There was significant difference ($P \leq 0.05$) effects of different concentration of nitrous acid on agronomic traits at M₁ in plant height, number of leaves, leaf length, fresh weight, dry weight, panicle length, panicle weight and 1000 seed weight (Table 4.6). While percentage germination, leaf weight and days to 50% flowering were not significant. Plant height, leaf length, fresh weight and dry weight increased with nitrous acid treatment and were highest at 0.1% concentration with 31.95cm, 15.50cm, 2.05g and 1.10g respectively while control recorded lowest (25.10cm, 9.75cm, 1.30g and 0.45g respectively). Panicle length and panicle weight all increased with treatment as 0.4% recorded the highest with 5.05cm and 1.00g while control had the lowest (3.68cm) for panicle length, however, 0.3% had the lowest for panicle weight.

Similarly, 1000seed weight increase with increase in concentration and 0.3% had highest 3.65g was while control had the lowest with 2.84g.

Table 4.5: Effects Nitrous Acid on Agronomic Traits of Foxtail Millet at M₁ generation

NA (%)	PGERM	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.00	83.75 ^a	34.18 ^b	5.70 ^b	14.43 ^a	1.33 ^a	2.10 ^b	0.78 ^b	42.75 ^a	4.83 ^c	0.75 ^b	2.601 ^a
0.10	87.50 ^a	37.51 ^a	5.88 ^b	15.76 ^a	1.42 ^a	3.08 ^a	1.35 ^a	34.35 ^b	6.08 ^a	1.08 ^a	2.60 ^a
0.20	86.25 ^a	34.42 ^a	5.00 ^b	15.59 ^a	1.40 ^a	2.98 ^a	1.43 ^a	34.50 ^b	5.13 ^{bc}	1.10 ^a	2.75 ^a
0.30	82.50 ^a	35.10 ^a	5.75 ^b	15.57 ^a	1.47 ^a	3.13 ^a	1.45 ^a	35.75 ^b	5.69 ^{ab}	1.13 ^a	2.78 ^a
0.40	88.75 ^a	35.91 ^{ab}	6.75 ^a	16.15 ^a	1.48 ^a	3.43 ^a	1.4 ^a	41.75 ^a	5.99 ^a	1.03 ^a	2.94 ^a
SE±	3.17	0.59	0.36	0.76	0.05	0.27	0.15	1.21	0.22	0.08	0.15

NOTE: Means with the Same Letter within a Column are not Significantly Different at $P \geq 0.05$

Keys: PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M1-First mutant generation, SE- Standard error, NA- nitrous acid

Table 4.6: Effects of Nitrous Acid on Agronomic Traits of Foxtail Millet at M₂ generation

NA (%)	PGERM (%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.00	81.25 ^a	25.10 ^b	5.5 ^{bc}	9.75 ^b	1.28 ^a	1.30 ^b	0.45 ^b	57.25 ^a	3.68 ^c	0.86 ^{ab}	2.84 ^c
0.10	78.75 ^a	31.95 ^a	6.00 ^{ab}	15.50 ^a	1.43 ^a	2.05 ^a	1.10 ^a	55.75 ^a	4.75 ^a	0.75 ^{ab}	3.19 ^b
0.20	83.75 ^a	29.73 ^a	5.63 ^{bc}	13.04 ^{ab}	1.37 ^a	1.33 ^b	0.58 ^b	52.25 ^a	4.63 ^{ab}	0.91 ^{ab}	3.23 ^b
0.30	85.5 ^a	29.76 ^a	5.00 ^c	13.26 ^{ab}	1.29 ^a	1.33 ^b	0.68 ^{ab}	54.25 ^a	4.18 ^{bc}	0.69 ^b	3.65 ^a
0.40	82.5 ^a	31.41 ^a	6.63 ^a	14.67 ^{ab}	1.48 ^a	1.35 ^b	0.73 ^{ab}	50.25 ^a	5.05 ^a	1.00 ^a	3.21 ^b
SE±	3.66	1.22	0.39	1.5	0.06	0.2	0.16	2.08	0.17	0.09	0.11

NOTE: Means with the Same Letter within a Column are not Significantly Different at $P \geq 0.05$

Keys: PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M1-First mutant generation, SE- Standard error, NA-nitrous acid

4.7 Effects of Sodium Azide on Agronomic Traits at M₁ Generation of Foxtail Millet

The effects of sodium azide on foxtail millet of m₁ generation is presented in table 4.7. Significant difference ($P \leq 0.05$) was recorded for percentage germination, leaf length, fresh weight, dry weight and panicle length. Percentage germination decreased with increase treatment as control was highest (83.75%) at 0.40% SA concentration was lowest (47.50%). Plant height decreased to 26.40cm at 0.20% then to 32.62cm at 0.30%. The control was highest for (14.13cm) and decreased with treatment and was lowest (10.04cm) at 0.30% which was at par with the other concentrations of SA. At concentration 0.30%, SA was highest (1.55g) for fresh weight but was lowest (0.90g) at 0.20%. While panicle length recorded highest mean (5.07cm) value at 0.10% comparable to control and other concentrations and lowest (3.85) at 0.30%.

4.8 Effects of Sodium Azide on Agronomic Traits of Foxtail Millet at M₂ Generation

The results in Table 4.8 shows the effects of different concentrations of sodium azide on agronomic traits of foxtail millet. Germination percentage plant height Leaf length, leaf width, fresh weight, dry weight, days to 50% flowering, panicle length, panicle weight and 1000seed weight were all significant ($P \leq 0.05$). Germination percentage decreased and was lowest (61.00%) at 0.20% and control highest (80.00%). Plant height, days to 50% flowering and panicle weight shows similar trend as 0.20% concentration was highest (36.42cm, 56days and 2.24g) then decrease and was lowest (26.33cm, 50.00days and 0.93g respectively). Similarly, leaf length, leaf width and panicle length recorded highest (16.71cm, 1.68cm, 5.91cm) and decreased with increase in concentration recording lowest values (12.94cm, 1.19cm and 4.11cm respectively). Fresh weight and dry weight showed similar trend with control being the highest (3.22g and 1.23g) and 0.04% the lowest mean values (1.63g and 0.65g). While 1000seed weight

increased with increase in concentration and was highest (3.59g) at 0.03% while control was lowest with 2.81g.

SA (%)	PGERM	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.00	83.75 ^a	30.20 ^a	5.25 ^a	14.13 ^a	1.31 ^a	1.18 ^{ab}	0.80 ^a	42.00 ^a	4.08 ^{ab}	0.60 ^a	2.64 ^a
0.10	61.25 ^b	31.21 ^a	5.00 ^a	10.90 ^b	1.18 ^a	1.50 ^a	1.00 ^a	37.67 ^a	4.93 ^a	0.73 ^a	2.55 ^a
0.20	61.25 ^b	26.40 ^b	4.75 ^a	10.52 ^b	1.13 ^a	0.90 ^b	0.50 ^a	38.82 ^a	3.85 ^b	0.50 ^a	2.94 ^a
0.30	60.00 ^b	32.62 ^a	6.00 ^a	10.04 ^b	1.30 ^a	1.55 ^a	1.13 ^a	39.25 ^a	4.65 ^a	0.90 ^a	2.64 ^a
0.40	47.50 ^b	28.92 ^a	5.50 ^a	10.84 ^b	1.15 ^a	1.13 ^{ab}	0.88 ^a	38.50 ^a	4.50 ^a	0.65 ^a	2.44 ^a
SE±	7.05	2.45	0.38	1.25	0.05	0.12	4.48	6.58	0.44	0.27	0.17

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Azide on Agronomic Traits of Foxtail Millet M₁ Generation

NOTE: Means with the Same Letter within a Column are not Significantly Different at $P \geq 0.05$

Keys: PGERM- percentage germination, PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length, PW- panicle weight, TWS- one thousand seed weigh, M1-First mutant generation, SE- Standard error, SA-sodium azide

Table 4.8 Effects of Sodium Azide on Agronomic of Foxtail Millet traits at M₂ Generation

SA (%)	PGERM (%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
0.00	80.00 ^a	29.80 ^{ab}	6.16 ^a	15.41 ^{ab}	1.56 ^{ab}	3.22 ^a	1.23 ^a	55.5 ^a	5.68 ^a	1.5 ^b	2.81 ^b
0.10	70.00 ^{ab}	31.99 ^{ab}	6.16 ^a	16.71 ^a	1.68 ^a	2.55 ^a	0.93 ^{ab}	54.5 ^a	5.91 ^a	2.22 ^a	2.84 ^b
0.20	61.00 ^b	36.42 ^a	6.25 ^a	16.9 ^{ab}	1.45 ^b	2.93 ^a	1.08 ^a	56.00 ^a	5.38 ^a	2.24 ^a	3.47 ^a
0.30	80.00 ^a	28.75 ^{ab}	5.68 ^a	14.86 ^{ab}	1.46 ^b	3.00 ^a	0.95 ^{ab}	54.00 ^a	5.21 ^a	1.89 ^{ab}	3.59 ^a
0.40	77.5 ^a	26.33 ^b	5.75 ^a	12.94 ^b	1.19 ^c	1.63 ^b	0.65 ^b	50.00 ^b	4.11 ^b	0.93 ^c	3.17 ^{ab}
SE±	5.72	2.52	0.36	1.04	0.05	0.29	0.09	1.41	0.31	0.14	0.14

NOTE: Means with the Same Letter within a Column are not Significantly Different at $P \geq 0.05$

Keys: PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M1-First mutant generation, SE- Standard error, SA-sodium azide

4.9 Heritability Estimate of Foxtail Millet Traits Treated with Fast Neutron

High broad sense heritability was recorded in plant height, leaf length, leaf weight, panicle length, panicle weight and 1000 seed weight with (64.89, 69.87, 100, 82.69, 72.72 and 75.00) % respectively (Table 4.9). Percentage germination Moderate heritability had in while number of leaves, fresh weight, dry weight and days to fifty 50% flowering showed low heritability values.

4.10 Heritability Estimate of Foxtail Millet Traits Treated with EMS

High genetic heritability recorded for percentage germination (71.22%), plant height (72.51%), number of leaves (76.67%), leaf width (97.56%), fresh weight (73.48%), dry weight (60.00%), days to 50% flowering (71.60), panicle length (92.11%), panicle weight (76.47%) one thousand seed weight (62.29%) while moderate heritability of 54.68 was recorded for leaf length (Table 4.10)

4.11 Heritability Estimate of Foxtail Millet Traits Treated with Nitrous Acid

The result in Table 4.11 shows high heritability for Plant height (99.71%) Number of leaves (75.68%), fresh weight (80.95%) Panicle length (99.71%) and one thousand seed weight (77.78%) While leaf length (51.61%), leaf width (40.00%), dry weight (57.14%), days to 50% flowering (43.56%), and panicle weight (50.00 %) showed moderate heritability values and percentage germination (17.90%) low heritability.

4.12 Heritability Estimate of Foxtail Millet Traits Treated with Sodium Azide

Table 4.12 shows heritability of traits of foxtail millet treated with sodium azide. High heritability values were obtained in leaf width (75 %), fresh weight (77.50%) and dry weight (80.00%), days to 50% flowering (65.16). Panicle length (79.59%), Panicle weight (80.56 %) and one thousand seed weight (84.62%). Percentage germination, plant height, leaf length

number of leaves with(50.16, 56.13 and 49.30)% respectively were moderate and low (0.46%) in
number of leaves

Table 4.9: Heritability estimate of foxtail millet traits treated with Fast Neutron

Traits	σ_g^2	σ_e^2	$\sigma_{e/r}^2$	σ_{ph}^2	H (%)
PGERM (%)	4.79	41.04	10.26	15.05	31.83
PHT(cm)	21.29	46.06	11.52	32.81	64.89
NL	0.00	0.75	0.19	0.19	0.00
LL(cm)	2.69	4.62	1.16	3.85	69.87
LW(cm)	0.01	0.01	0.00	0.01	100
FW(g)	0.09	1.5	0.38	0.47	19.15
DW(g)	0.02	0.37	0.09	0.11	18.18
DPFL(days)	0.23	7.3	1.83	2.06	11.17
PL(cm)	0.43	0.34	0.09	0.52	82.69
PW(g)	0.08	0.1	0.03	0.11	72.72
TSW(g)	0.06	0.03	0.01	0.08	75.00

Note: heritability category 0<30% (low), 30-60% (moderate), >60% (high)

σ_g^2 - genetic variance, σ_e^2 - environmental variance, σ_{ph}^2 - phenotypic variance, h^2 - heritability, PGERM- percentage germination, PHT- plant height at maturity, NL- number of leaves, LL- leaf length, LW-leaf width, FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length, PW- panicle weight, TWS- one thousand seed weight.

Table 4.10: Heritability estimate of foxtail millet traits treated with EMS

Traits	σ_g^2	σ_e^2	$\sigma_{e/r}^2$	σ_{ph}^2	H (%)
PGERM (%)	53.75	86.88	21.72	75.47	71.22
PHT(cm)	19.84	30.08	7.52	27.36	72.51
NL	0.46	0.54	0.14	0.60	76.67
LL(cm)	2.16	7.15	1.79	3.95	54.68
LW(cm)	0.04	0.02	0.01	0.41	97.56
FW(g)	0.97	1.39	0.35	1.32	73.48
DW(g)	0.09	0.22	0.06	0.15	60.00
DPFL(days)	8.64	13.61	3.40	12.04	71.76
PL(cm)	1.4	0.47	0.12	1.52	92.11
PW(g)	0.13	0.15	0.04	0.17	76.47
TSW(g)	0.09	0.05	0.01	0.10	90.00

Note: heritability category 0<30% (low), 30-60% (moderate), >60% (high)

σ_g^2 - genetic variance, σ_e^2 - environmental variance, σ_{ph}^2 - phenotypic variance, h^2 - heritability, PGERM- percentage germination, PHT- plant height at maturity, NL- number of leaves, LL- leaf length, LW-leaf width, FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length, PW- panicle weight, TWS- one thousand seed weight.

Table 4.11: Heritability estimate of foxtail millet traits treated with Nitrous Acid

Traits	σ_g^2	σ_e^2	$\sigma_{e/r}^2$	σ_{ph}^2	H (%)
PGERM (%)	2.92	53.54	13.39	16.31	17.90
PHT(cm)	6.01	5.04	1.26	7.27	82.67
NL	0.28	0.36	0.07	0.37	75.68
LL(cm)	2.39	8.97	2.24	4.63	51.61
LW(cm)	0.004	0.02	0.01	0.01	40.00
FW(g)	0.17	0.17	0.04	0.21	80.95
DW(g)	0.04	0.10	0.03	0.07	57.14
DPFL(days)	3.35	17.37	4.34	7.69	43.56
PL(cm)	10.26	0.12	0.03	10.29	99.71
PW(g)	0.01	0.03	0.01	0.02	50.00
TSW(g)	0.07	0.06	0.02	0.09	77.78

Note: heritability category 0<30% (low), 30-60% (moderate), >60% (high)

σ_g^2 - genetic variance, σ_e^2 - environmental variance, σ_{ph}^2 - phenotypic variance, h^2 - heritability, PGERM- percentage germination, PHT- plant height at maturity, NL- number of leaves, LL- leaf length, LW-leaf width, FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length, PW- panicle weight, TWS- one thousand seed weight.

Table 4.12: Heritability estimate of foxtail millet traits treated with Sodium Azide

Traits	σ_g^2	σ_e^2	$\sigma_{e/r}^2$	σ_{ph}^2	H (%)
PGERM	32.92	130.83	32.71	65.63	50.16
PHT	8.15	25.48	6.37	14.52	56.13
NL	0.06	52.00	13.00	13.06	0.46
LL	1.05	4.33	1.08	2.13	49.30
LW	0.03	0.01	00	0.04	75.00
FW	0.31	0.35	0.09	0.40	77.50
DW	0.04	0.04	0.01	0.05	80.00
DPFL	3.74	7.98	2.00	5.74	65.16
PL	0.39	0.39	0.10	0.49	79.59
PW	0.29	0.07	0.02	0.31	93.54
TSW	0.11	0.08	0.02	0.13	84.62

Note: heritability category 0<30% (low), 30-60% (moderate), >60% (high)

σ_g^2 - genetic variance, σ_e^2 - environmental variance, σ_{ph}^2 - phenotypic variance, h^2 - heritability, PGERM- percentage germination, PHT- plant height at maturity, NL- number of leaves, LL- leaf length, LW-leaf width, FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length, PW- panicle weight, TWS- one thousand seed weight.

4.13 Effectsof Mutagens and their Concentrations for M₁ Generation

The results presented in Table 4.13 shows significant difference of all traits considered. Germination percentage increased to 95% at EMS 0.1% and SA at 0.4% was lowest with 47.50%. Plantheightincreased from 26.40cm sodium azide at 0.2% concentration to 37.51cm at nitrous acid 0.1%. Number of leaves and leaf length were highest (6.75leaves and 16.15cm) at Nitrous acid 0.4 % and lowest at SA 0.4% with 4.5leaves at 0.02 SA concentration for number of leaves and 0.3% with 10.04cmfor leaf length. Leaf width was highest (1.50cm) at EMS 0.1% concentration. Fresh weight was highest at 0.3% nitrous acid concentration and lowest at SA 0.02%. Dry weight was highest (1.78g) at 0.4% EMS and lowest (0.50g) at 0.2% sodium azide concentration. Days to 50% flowering was highest (42.69days) while lowest (34.25) at EMS 0.02% concentration. Panicle length was highest (6.08cm)at 0.1% nitrous acid while fast neutrons 90min exposure time gave lowest (3.6cm) whereas panicle weight was highest (1.38g) at 0.4% EMS.

4.14 Effectsof Mutagens and their Concentrations for M₂ Generation

The results presented in Table 4.14 shows significant difference of all traits considered. Fast neutrons at 360min time of exposure gave the highest (87.5%) germination percentage and SA at 0.2% was lowest with 61.25%. For plant height, Fast neutrons at 180min time of exposure was found to induce the highest (46.65cm, 18.47cm) plant height and leaf length respectively while sodium azideat 0.4% concentration gave the shortest (26.33cm and 12.94cm) plant. Number of leaves, leaf width, fresh weight, panicle length and 1000seed weight were highest (8.00leaves, 1.92cm, 4.85g, 7.60cm, 3.73g) at EMS 0.1% while number of leaves, fresh weight and panicle length were lowest (5leaves, 1.33g, 0.69g) at NA (0.3%) concentration, leaf width was lowest at 0.4% SA concentration while 0.4% and was lowest for 1000seed weight. Fast neutron at 360min

exposure time recorded highest mean values for dry weight, days to 50% flowering and panicle weight which were all comparable to 90min and 0.1% EMS treatment.

Table 4.13 Effectsof Mutagens and their Concentrations for M₁ Generation

TRT	PGERM(%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
CNTRL	82.50 ^{ab}	31.28 ^{efg}	5.56 ^{abcd}	14.07 ^{bcd}	1.30 ^{cde}	1.44 ^{ef}	0.82 ^{def}	42.69 ^a	4.40 ^{defgh}	0.63 ^{fg}	2.78 ^a
FN 90	81.25 ^b	27.80 ^{hi}	5.00 ^{dc}	12.34 ^{defg}	1.14 ^f	1.23 ^{ef}	0.83 ^{def}	40.25 ^{abc}	3.61 ⁱ	0.48 ^{gh}	2.70 ^a
FN 180	81.25 ^b	27.78 ^{hi}	6.00 ^{abc}	10.94 ^{gh}	1.23 ^{def}	1.05 ^{ef}	0.78 ^{ef}	39.25 ^{abc}	3.74 ^{hi}	0.48 ^{gh}	2.55 ^a
FN 270	85.00 ^{ab}	29.40 ^{fgh}	5.50 ^{abcd}	12.00 ^{efg}	1.15 ^f	1.05 ^{ef}	0.73 ^{ef}	39.00 ^{abc}	4.26 ^{efghi}	0.50 ^{gh}	2.70 ^a
FN 360	83.75 ^{ab}	26.65 ^{hi}	5.25 ^{bcd}	11.40 ^{fgh}	1.2 ^{ef}	1.05 ^{ef}	0.73 ^{ef}	41.50 ^{ab}	4.09 ^{fghi}	0.55 ^{fgh}	2.48 ^a
EMS 0.1%	95.00 ^a	32.17 ^{cdef}	6.25 ^{abc}	15.01 ^{abc}	1.50 ^a	2.23 ^c	1.55 ^{ab}	37.00 ^{bcd}	4.94 ^{cde}	1.08 ^{bc}	2.72 ^a
EMS 0.2%	88.75 ^{ab}	31.84 ^{def}	5.50 ^{abcd}	11.4 ^{fgh}	1.36 ^{bcd}	2.08 ^{cd}	1.40 ^{abc}	34.25 ^d	3.85 ^{ghi}	1.03 ^{bcd}	2.69 ^a
EMS 0.3%	83.75 ^{ab}	33.06 ^{bcd}	5.50 ^{abcd}	13.17 ^{edf}	1.40 ^{abc}	2.30 ^c	1.63 ^{ab}	41.00 ^{ab}	5.08 ^{bc}	1.25 ^{ba}	2.64 ^a
EMS 0.4%	83.75 ^{ab}	34.10 ^{abc}	6.5 ^{ab}	13.41 ^{cde}	1.36 ^{bcd}	2.60 ^{bc}	1.73 ^a	37.25 ^{bcd}	5.67 ^{ab}	1.38 ^a	2.70 ^a
NA 0.1%	87.50 ^{ab}	37.51 ^a	5.88 ^{abc}	15.76 ^{ab}	1.42 ^{abc}	3.08 ^{ab}	1.35 ^{bc}	34.25 ^d	6.08 ^a	1.07 ^{bc}	2.60 ^a
NA 0.2%	86.25 ^{ab}	34.42 ^{bcd}	5.00 ^{dc}	15.59 ^{ab}	1.40 ^{abc}	2.98 ^{ab}	1.43 ^{abc}	34.5 ^d	5.13 ^{abc}	1.10 ^{bc}	2.75 ^a
NA 0.3%	82.50 ^{ab}	35.10 ^{abc}	5.75 ^{abcd}	15.57 ^{ab}	1.47 ^{ab}	3.43 ^a	1.45 ^{abc}	35.75 ^{dc}	5.69 ^{ab}	1.13 ^{abc}	2.78 ^a
NA 0.4%	88.75 ^{ab}	35.91 ^{ab}	6.75 ^a	16.15 ^a	1.48 ^{ab}	3.13 ^{ab}	1.40 ^{abc}	41.00 ^{ab}	5.99 ^a	1.03 ^{bcd}	2.94 ^a
SA 0.1%	61.25 ^c	31.29 ^{efg}	5.00 ^{dc}	10.90 ^{gh}	1.21 ^{ef}	1.5 ^{fe}	0.98 ^{de}	37.50 ^{bcd}	4.93 ^{cde}	0.78 ^{def}	2.57 ^a
SA 0.2%	61.25 ^c	26.40 ⁱ	4.50 ^d	10.52 ^{gh}	1.2 ^{ef}	0.90 ^f	0.50 ^f	38.50 ^{abcd}	3.85 ^{ghi}	0.3 ^h	2.59 ^a
SA 0.3%	60.00 ^c	32.62 ^{cde}	6.0 ^{abc}	10.04 ^h	1.3 ^{cde}	1.55 ^{de}	1.13 ^{cd}	39.25 ^{abc}	4.65 ^{cdef}	0.9 ^{cde}	2.64 ^a
SA 0.4%	47.50 ^d	28.92 ^{ghi}	5.50 ^{abcd}	10.834 ^{gh}	1.15 ^f	1.13 ^{fe}	0.88 ^{de}	38.50 ^{abcd}	4.50 ^{cdefg}	0.65 ^{fgh}	2.44 ^a
SE±	3.88	0.94	0.4	0.58	0.04	0.2	0.11	1.4	0.22	0.09	0.2

NOTE: Means with the Same Letter within a Column are not Significantly Different at P≥0.05

Keys:TRT-treatment, PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M1-First mutant generation, SE- Standard error, FN- fast neutron, EMS- ethyl methane sulphonate, NA- nitrous acid, SA-sodium azide.

Table 4.14: Effectsof Mutagens and their Concentrations for M₂ Generation

TRTS	PGERM (%)	PHT(cm)	NL	LL(cm)	LW(cm)	FW(g)	DW(g)	DPFL(days)	PL(cm)	PW(g)	TSW(g)
CTRL	81.25 ^{ab}	31.17 ^{cde}	6.25 ^{cdef}	14.60 ^{abc}	1.44 ^{cde}	2.47 ^{cdef}	0.94 ^{bcd}	59.00 ^{abcd}	4.90 ^{cde}	1.34 ^c	2.84 ^g
FN 90	86.25 ^a	31.41 ^{cde}	7.42 ^{abc}	13.26 ^{bc}	1.41 ^{cde}	2.75 ^{bcde}	0.95 ^{bcd}	62.5 ^a	5.39 ^{cd}	2.06 ^{ab}	3.47 ^{abcd}
FN 180	85.00 ^a	46.65 ^a	7.42 ^{abc}	18.47 ^a	1.58 ^{bc}	3.85 ^{abc}	1.58 ^{ab}	61.00 ^{ab}	6.70 ^{ab}	2.25 ^{ab}	3.31 ^{cde}
FN 270	85.00 ^a	41.81 ^{ab}	7.67 ^{ab}	17.44 ^{abc}	1.68 ^b	4.2 ^{ab}	1.78 ^a	63.75 ^a	6.82 ^{ab}	2.51 ^a	3.55 ^{abc}
FN 360	87.50 ^a	35.22 ^{bcde}	6.94 ^{abcd}	16.13 ^{abc}	1.55 ^{bcd}	3.28 ^{bcd}	1.2 ^{abcd}	60.00 ^{abc}	5.57 ^{dc}	2.00 ^{ab}	3.27 ^{cdef}
EMS 0.1%	86.25 ^a	39.31 ^{abc}	8.08 ^a	17.65 ^{ab}	1.92 ^a	4.85 ^a	1.28 ^{abc}	60.25 ^{abc}	7.60 ^a	2.24 ^{ab}	3.73 ^a
EMS 0.2%	82.50 ^{ab}	27.25 ^e	6.62 ^{bcde}	12.71 ^c	1.6b ^c	2.33 ^{cdef}	0.78 ^{cd}	59.75 ^{abcd}	4.90 ^{cde}	1.39 ^c	3.35 ^{cde}
EMS 0.3%	81.25 ^{ab}	33.28 ^{bcde}	6.00 ^{def}	17.08 ^{abc}	1.43 ^{cde}	2.32 ^{cdef}	0.83 ^{cd}	52.50 ^{fgh}	5.46 ^{dc}	1.2 ^{cd}	3.09 ^{fg}
EMS 0.4%	80.00 ^{ab}	26.29 ^e	6.53 ^{bcde}	15.03 ^{abc}	1.44 ^{cde}	2.00 ^{def}	0.7 ^{cd}	58.50 ^{bcde}	4.57 ^{de}	1.42 ^c	3.25 ^{cdef}
NA 0.1%	78.75 ^{ab}	31.95 ^{cde}	6.00 ^{def}	15.30 ^{abc}	1.43 ^{cde}	2.35 ^{cdef}	1.10 ^{bcd}	55.75 ^{cdef}	4.75 ^{cde}	0.75 ^d	3.19 ^{ef}
NA 0.2%	83.75 ^a	29.73 ^{de}	5.63 ^{ef}	13.04 ^{bc}	1.37 ^{def}	1.33 ^f	0.58 ^d	52.25 ^{fgh}	4.63 ^{de}	0.93 ^{cd}	3.23 ^{def}
NA 0.3%	87.50 ^a	29.65 ^{de}	5.00 ^f	13.26 ^{bc}	1.28 ^{ef}	1.33 ^f	0.68 ^{cd}	54.25 ^{fgh}	4.18 ^e	0.69 ^d	3.65 ^{ab}
NA 0.4%	82.50 ^{ab}	31.41 ^{cde}	6.63 ^{bcde}	14.67 ^{abc}	1.48 ^{cde}	1.35 ^f	0.73 ^{cd}	50.25 ^{gh}	5.05 ^{cde}	1.00 ^{cd}	3.21 ^{def}
SA 0.1%	70.00 ^{bc}	31.99 ^{cde}	6.16 ^{def}	16.71 ^{abc}	1.68 ^b	2.55 ^{cdef}	0.93 ^{bcd}	54.50 ^{defg}	5.91 ^{bc}	2.22 ^{ab}	2.84 ^g
SA 0.2%	61.25 ^c	36.42 ^{bcd}	6.25 ^{cdef}	16.19 ^{abc}	1.45 ^{cde}	2.93 ^{bcde}	1.08 ^{bcd}	56.00 ^{cdef}	5.38 ^{dc}	2.25 ^{ab}	3.47 ^{bcde}
SA 0.3%	80.00 ^{ab}	28.75 ^{de}	5.69 ^{ef}	14.86 ^{abc}	1.46 ^{cde}	3.00 ^{bcd}	0.95 ^{bcd}	54.75 ^{defg}	5.21 ^{cde}	1.89 ^b	3.59 ^{abc}
SA 0.4%	77.50 ^{ab}	26.33 ^e	5.75 ^{def}	12.94 ^{bc}	1.19 ^f	1.63 ^{ef}	0.65 ^{cd}	50.00 ^h	4.11 ^e	0.93 ^{cd}	3.17 ^{ef}
SE±	4.18	2.78	0.39	1.43	0.06	0.49	0.2	1.75	0.36	0.17	0.11

NOTE: Means with the Same Letter within a Column are not Significantly Different at P≥0.05

Keys: TRT- treatment,PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves, LL- leaf length LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed weigh, M1-First mutant generation, SE- Standard error, FN- fast neutron, EMS- ethyl methane sulphonate, NA- nitrous acid, SA-sodium azide

4.15 Effects of Dosage on two Generations of Fast Neutron

The result in table 4.15 compares the M_1 and M_2 for all the trait considered across the treatments. There was no significant difference ($P \leq 0.05$) in the germination percentage among all the doses in both M_1 and M_2 generations. For plant height, there was no significant difference at 0min of the two generations (31.06 and 33.08cm) at m_1 and m_2 respectively, however, significant difference ($P \leq 0.05$) was observed between the two generations, 90min (27.80 and 31.94cm), 180min (27.78 and 46.65cm), 270min (29.40 and 41.81cm) and 360min (26.65 and 35.22cm). The number of leaves shows significant difference ($P \leq 0.05$) between the two generations as m_2 was highest for all the doses including the control (5.25 and 6.58) for m_1 and m_2 respectively, 90 min (5.00 and 6.58), 180min (6.00 and 6.94), 270min (5.50 and 7.66) and 360min (5.25 and 6.96) for m_1 and m_2 respectively, with 270min m_2 (7.66) being the highest and 0min and 360min m_1 (5.25) lowest.

Similarly, there was significant difference ($P \leq 0.05$) in the leaf length, fresh weight, days to 50% flowering, panicle length and panicle weight for two generations including the control as M_2 recorded the highest mean values all treatments. While The leaf width, dry weight and 1000seed weight were not significant ($P \leq 0.05$) at control for the two generations while treatment with fast neutrons were significant ($P \leq 0.05$) at all time of exposure with M_2 recording highest mean values.

4.16 Effects of Concentrations on two Generations of EMS

The result in table 4.16 compares the M_1 and M_2 for all the trait considered across the treatments. Percentage germination shows no significant difference ($P \leq 0.05$) at control for the two generations but significant difference ($P \leq 0.05$) whereas, in the other concentrations of the two generations, 0.10%, 0.20%, 0.30% and 0.40% were significant were M_1 recorded higher

germinations for all treatments. However, 0.10% at M₁ (95.0%) gave the highest percentage and the same concentration and 0.20% at m₂ (70%) gave the least percentage.

Significant differences ($P \leq 0.05$) were observed for plant height at 0.10%, 0.20% and 0.40% EMS conc. were 0.10% of M₂ (39.31cm) induced the tallest plant and 0.40% (26.29cm) of M₂ also gave the shortest plant. There was significant difference ($P \leq 0.05$) in the number of leaves of the two generations 0.00%, 0.10% and 0.20%. Were M₂ generally showed higher leave number and the concentration with the highest number of leaves was 0.10% of m₂ (8.08) and the least was 0.20% and 0.30% of M₁(5.50). The leaf length and leaf width showed significant difference ($P \leq 0.05$) in the two generation for all treatments with M₂ recording the highest values except 0.20% were there was no significant difference ($P \leq 0.05$). The longest leaf was induced at 0.10% of M₂ (17.65cm and 1.92cm) and the shortest leaf length was 0.20% of M₁ (11.40cm) while smallest (1.26) leaf width was control. Fresh weight showed significant difference ($P \leq 0.05$) in the two generations at 0.00%, 0.10% and 0.4% conc. Similarly, dry weight was different ($P \leq 0.05$) for M₁ and M₂ for only 0.2%, 0.3% and 0.4% EMS concentrations. The days to 50% flowering shows significance ($P \leq 0.05$) for concentrations in the two generations. Earliest days to flowering was observed in 0.20% of M₁ (34.00 days) and the latest being 0.00% of M₂ (61.00 days)

The panicle length showed significance ($P \leq 0.05$) for two generations at 0.10%, 0.20% and 0.40%. The longest panicle being 0.10% of M₂ (7.60cm) and the shortest, 0.20% of M₁ (4.01cm). There was significant difference in the panicle weight in the two generations at 0.00% (0.91 and 1.40g), 0.10% (1.08 and 2.24g) and 0.20% (1.03 and 1.39g) all at M₁ and M₂ respectively. As, 0.10% of M₂ (2.24g) gave the highest weight and 0.00% of M₁ (0.91g) gave the least. The one thousand seed weights recorded significant difference ($P \leq 0.05$) at 0.10% (2.71 and 3.73g),

0.20% (2.69 and 3.35g), 0.30% (2.64 and 3.09g) and 0.40% (2.69 and 3.25g) all at M_1 and M_2 respectively. But 0.10% of M_2 (3.73g) is the highest and the least is 0.00% of M_1 (2.62g).

Table 4.15: Effects of Dosageon Generations of Fast Neutron

TRAITS	GEN	0min	90min	180min	270min	360min
PGERM (%)	M ₁	81.25 ^a	81.25 ^a	81.25 ^a	85.00 ^a	83.75 ^a
	M ₂	81.25 ^a	86.25 ^a	85.00 ^a	85.00 ^a	87.50 ^a
	SE±	2.24	2.24	2.24	2.24	2.24
PHT(cm)	M ₁	31.06 ^a	27.8 ^b	27.78 ^b	29.4 ^b	26.65 ^b
	M ₂	33.08 ^a	31.94 ^a	46.65 ^a	41.81 ^a	35.22 ^a
	SE±	1.7	1.7	1.7	1.7	1.7
NL	M ₁	5.25 ^b	5.00 ^b	6.00 ^b	5.50 ^b	5.25 ^b
	M ₂	6.58 ^a	7.42 ^a	6.94 ^a	7.66 ^a	6.96 ^a
	SE±	0.28	0.28	0.28	0.28	0.28
LL(cm)	M ₁	14.13 ^b	12.34 ^b	10.94 ^b	11.99 ^b	11.42 ^b
	M ₂	16.66 ^a	13.26 ^a	18.47 ^a	17.44 ^a	16.13 ^a
	SE±	0.91	0.91	0.91	0.91	0.91
LW(cm)	M ₁	1.31 ^a	1.14 ^b	1.23 ^b	1.16 ^b	1.21 ^b
	M ₂	1.38 ^a	1.41 ^a	1.58 ^a	1.68 ^a	1.55 ^a
	SE±	0.06	0.06	0.06	0.06	0.06
FW(g)	M ₁	1.25 ^b	1.23 ^b	1.05 ^b	1.05 ^b	1.05 ^b
	M ₂	2.63 ^a	2.75 ^a	3.38 ^a	4.20 ^a	3.28 ^a
	SE±	0.3	0.3	0.3	0.3	0.3
DW(g)	M ₁	0.90 ^a	0.83 ^a	0.78 ^b	0.73 ^b	0.73 ^b
	M ₂	1.10 ^a	0.95 ^a	1.58 ^a	1.78 ^a	1.20 ^a
	SE±	0.12	0.12	0.12	0.12	0.12
DPFL(days)	M ₁	42.00 ^b	40.00 ^b	39.00 ^b	39.00 ^b	41.00 ^b
	M ₂	62.00 ^a	62.50 ^a	61.00 ^a	63.75 ^a	60.00 ^a
	SE±	1.69	1.69	1.69	1.69	1.69
PL(cm)	M ₁	4.24 ^b	3.61 ^b	3.74 ^b	4.26 ^b	4.09 ^b
	M ₂	5.42 ^a	5.39 ^a	6.70 ^a	6.82 ^a	5.57 ^a
	SE±	0.12	0.12	0.12	0.12	0.12
PW(g)	M ₁	0.70 ^b	0.48 ^b	0.48 ^b	0.50 ^b	0.55 ^b
	M ₂	1.61 ^a	2.06 ^a	2.25 ^a	2.51 ^a	2.00 ^a
	SE±	0.07	0.07	0.07	0.07	0.07
TSW(g)	M ₁	2.60 ^a	2.70 ^b	2.55 ^b	2.70 ^b	2.48 ^b
	M ₂	2.86 ^a	3.50 ^a	3.31 ^a	3.55 ^a	3.26 ^b
	SE±	0.18	0.18	0.18	0.18	0.18

NOTE: Means with the Same Letter within a Column are not Significantly Different at P≤0.05

PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves , LL- leaf length, LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed Weigh, M₁- first mutant generation, M₂- second mutant generation

Table 4.16: Effects of Concentration on Generations of EMS

TRAITS	GEN	0.00%	0.10%	0.20%	0.30%	0.40%
PGERM (%)	M ₁	82.50 ^a	95.00 ^a	88.75 ^a	83.75 ^a	83.75 ^a
	M ₂	83.75 ^a	70.00 ^b	70.00 ^b	78.75 ^b	78.75 ^b
	SE±	1.67	1.67	1.67	1.67	1.67
PHT(cm)	M ₁	29.74 ^a	32.18 ^b	31.84 ^a	33.06 ^a	34.87 ^a
	M ₂	31.82 ^a	39.31 ^a	27.25 ^b	33.27 ^a	26.29 ^b
	SE±	1.08	1.08	1.08	1.08	1.08
NL	M ₁	6.00 ^b	6.25 ^b	5.50 ^b	5.50 ^a	6.50 ^a
	M ₂	6.75 ^a	8.08 ^a	6.62 ^a	6.00 ^a	6.53 ^a
	SE±	0.32	0.32	0.32	0.32	0.32
LL(cm)	M ₁	13.59 ^b	15.01 ^b	11.40 ^a	13.17 ^b	13.41 ^b
	M ₂	16.58 ^a	17.65 ^a	12.71 ^a	17.07 ^a	15.02 ^a
	SE±	0.77	0.77	0.77	0.77	0.77
LW(cm)	M ₁	1.26 ^b	1.50 ^b	1.36 ^b	1.40 ^a	1.36 ^a
	M ₂	1.55 ^a	1.92 ^a	1.60 ^a	1.44 ^a	1.44 ^a
	SE±	0.09	0.09	0.09	0.09	0.09
FW(g)	M ₁	1.25 ^b	2.23 ^b	2.07 ^a	2.30 ^a	2.60 ^a
	M ₂	2.73 ^a	4.85 ^a	2.33 ^a	2.33 ^a	2.00 ^b
	SE±	0.2	0.2	0.2	0.2	0.2
DW(g)	M ₁	0.80 ^a	1.55 ^a	1.40 ^a	1.63 ^a	1.73 ^a
	M ₂	0.98 ^a	1.28 ^a	0.78 ^b	0.83 ^b	0.70 ^b
	SE±	0.08	0.08	0.08	0.08	0.08
DPFL(days)	M ₁	44.00 ^b	37.00 ^b	34.00 ^b	41.00 ^b	37.00 ^b
	M ₂	61.00 ^a	60.25 ^a	59.75 ^a	52.50 ^a	58.50 ^a
	SE±	1.7	1.7	1.7	1.7	1.7
PL(cm)	M ₁	4.48 ^a	4.94 ^b	4.01 ^b	5.08 ^a	5.67 ^a
	M ₂	4.83 ^a	7.60 ^a	4.90 ^a	5.46 ^a	4.57 ^b
	SE±	0.26	0.26	0.26	0.26	0.26
PW(g)	M ₁	0.91 ^b	1.08 ^b	1.03 ^b	1.25 ^a	1.38 ^a
	M ₂	1.40 ^a	2.24 ^a	1.39 ^a	1.20 ^a	1.43 ^a
	SE±	0.19	0.19	0.19	0.19	0.19
TSW(g)	M ₁	2.62 ^a	2.71 ^b	2.69 ^b	2.64 ^b	2.69 ^b
	M ₂	2.86 ^a	3.73 ^a	3.35 ^a	3.09 ^a	3.25 ^a
	SE±	0.14	0.14	0.14	0.14	0.14

NOTE: Means with the Same Letter within a Column are not Significantly Different at $p \leq 0.05$

PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves , LL- leaf length, LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed Weigh, M₁- first mutant generation, M₂- second mutant generation

4.17 Effects of Concentrations on two Generations of Nitrous Acid

The result in Table 4.17 compares M₁ and M₂ generation at all the concentrations of Nitrous acid. There was a significant difference in plant height, fresh weight, dry weight, days to 50% flowering and panicle length as all M₁ concentration were higher than M₂ and 0.1% M₁ was highest (37.51cm, 34days and 6.08cm) for plant height, days to 50% flowering and panicle length while m₂ generation progenies 0.0% at was lowest (25.10cm, 57days and 3.68cm), fresh weight and dry weight were highest (3.43g and 1.45g) at m₁ 0.3% and lowest (0.13g) at 0.3% for dry weight and control for dry weight. Significant difference ($P \leq 0.05$) was also observed in leaf length as a decrease was observed at m₂ generation for all treatments with M₂ control lowest (9.75cm) and 0.4% conc. Highest (16.15cm). Significant difference ($P \leq 0.05$) was observed for M₁ and M₂ generations only at 0.30% (1.40cm and 1.29cm respectively) for leaf width. There was also significant difference ($P \leq 0.05$) in panicle weight of 0.00%(0.68g and 0.86g), 0.10%(1.08g and 0.75g), 0.4%(1.03g and 1.00g). There was significant ($P \leq 0.05$) increase weight of one thousand seed for 0.10%(2.60g and 3.19g), 0.20% (2.75g and 3.22g) and 0.30%(2.78g and 3.65g) for M₁ and M₂ generations respectively.

4.18 Effects of Concentrations on two Generations of Sodium Azide

The result in Table 4.18 compares the M₁ and M₂ for all the trait considered across the treatments. There is significant difference ($P \leq 0.05$) at 0.10% (61.25% and 70.00%), 0.30% (60.00% and 80.00%), 0.40% (47.50 and 77.50) between the M₁ and M₂ generation. There was significant difference ($P \leq 0.05$) in plant height between M₁ and M₂ generation for only 0.20% (32.62cm and 36.42cm). Significant difference in number of leaves at 0.00% (5.25 and 6.16), 0.10% (5.00 and 6.16), and 0.20% (4.50 and 6.25). There was significant difference ($P \leq 0.05$) observed between m₁ and m₂ generations for 0.10% (10.90cm and 16.71cm), 0.20%(10.52cm

and 16.19cm), 0.30% (10.04cm and 14.86cm) and 0.40% (10.84cm and 12.94cm) with M₂ having increased values. There was significant difference in leaf width between M₁ and M₂ generations for 0.00% (1.31cm and 1.56cm), 0.10% (1.21cm and 1.68cm), 0.20% (1.20cm and 1.45cm), 0.30% (1.30cm and 1.46cm) respectively. M₂ generation was higher. There was significant difference in fresh weight and dry weight between M₁ and M₂ generations for all treatments with M₂ control as highest (3.22g and 1.23g) while 0.02% M₁ conc. of sodium azide was lowest (0.90g and 0.50g). There was also significant difference in M₁ and M₂ generations for all treatments: 0.00% (42.00days and 55.50days), 0.10% (37.50days and 54.50days), 0.20% (38.50days and 56.00days), 0.30% (39.25days and 54.75days) and 0.40% (38.50days and 50.00days) respectively for M₁ and M₂ generations. There was significant difference in panicle length between M₁ and M₂ generations for all treatments; 0.10% M₂ was highest (5.91cm) and 0.20% M₁ (3.85cm). Significant (P≤0.05) increase in all M₂ generation for all treatments; 0.20% M₂ conc. Highest (2.24g) and M₁ lowest (0.30g). There was significant increase weight of 1000seed for 0.20% (2.59g and 3.47g), 0.30% (2.64g and 3.59g) and 0.40% (2.44g and 3.17g) for M₁ and M₂ generations respectively.

4.19 Mutagenic Frequency, Efficiency and Effectiveness of EMS and Sodium Azide

The result shows highest mutation frequency (7.5%), mutagenic effectiveness (18.75%), mutagenic efficiency (7.5%) at 0.1% concentration of EMS and decreased with increase in concentration. Lethality, effectiveness efficiency also increased with concentration. While for sodium azide, mutagenic frequency (7.00%), and mutagenic effectiveness (8.75%) were highest at 0.02% concentration and then decrease. Lethality increased with increase in concentration with 0.04% with highest lethality. While mutagenic efficiency decrease with increase concentration with 0.01% and 0.02% with highest values (Table 4.19). In general, EMS shows

higher mutagenic frequency (7.5%), effectiveness (18.75%) and efficiency (7.5%) than sodium azide. While Sodium azide showed higher lethality (15%)

Table 4.17: Effectsof Concentrations on Generations of Nitrous Acid

TRAITS	GEN	0.00%	0.10%	0.20%	0.30%	0.40%
PGERM (%)	M ₁	83.75 ^a	87.50 ^a	86.25 ^a	82.50 ^a	88.75 ^a
	M ₂	81.25 ^a	79.75 ^a	83.75 ^a	87.50 ^a	82.50 ^a
	SE±	4.78	4.78	4.78	4.78	4.78
PHT(cm)	M ₁	34.18 ^a	37.51 ^a	34.42 ^a	35.10 ^a	35.91 ^a
	M ₂	25.10 ^b	31.95 ^b	29.65 ^b	29.65 ^b	31.41 ^b
	SE±	0.74	0.74	0.74	0.74	0.74
NL	M ₁	5.75 ^a	5.88 ^a	5.00 ^a	5.75 ^a	6.75 ^a
	M ₂	5.50 ^a	6.00 ^a	5.63 ^a	5.00 ^b	6.63 ^a
	SE±	0.34	0.34	0.34	0.34	0.34
LL(cm)	M ₁	14.43 ^a	15.76 ^b	15.59 ^a	15.57 ^a	16.15 ^a
	M ₂	9.75 ^b	15.30 ^b	13.04 ^b	13.26 ^b	14.67 ^a
	SE±	1.4	1.4	1.4	1.4	1.02
LW(cm)	M ₁	1.33 ^a	1.42 ^a	1.40 ^a	1.47 ^a	1.48 ^a
	M ₂	1.28 ^a	1.43 ^a	1.37 ^b	1.29 ^b	1.48 ^a
	SE±	0.06	0.06	0.06	0.06	0.06
FW(g)	M ₁	2.10 ^a	3.08 ^a	2.98 ^a	3.43 ^a	3.13 ^a
	M ₂	0.36 ^b	0.34 ^b	0.54 ^b	0.13 ^b	0.48 ^b
	SE±	0.2	0.2	0.2	0.2	0.2
DW(g)	M ₁	0.78 ^a	1.35 ^a	1.43 ^a	1.45 ^a	1.40 ^a
	M ₂	0.45 ^b	1.10 ^b	0.58 ^b	0.68 ^b	0.73 ^b
	SE±	0.14	0.14	0.14	0.14	0.14
DPFL(days)	M ₁	42.75 ^b	34.25 ^b	34.50 ^b	35.75 ^b	41.00 ^b
	M ₂	57.25 ^a	55.75 ^a	52.25 ^a	54.25 ^a	50.25 ^a
	SE±	2.42	2.42	2.42	2.42	2.42
PL(cm)	M ₁	4.83 ^a	6.08 ^a	5.13 ^a	5.69 ^a	5.99 ^a
	M ₂	3.68 ^b	4.75 ^b	4.63 ^b	4.18 ^b	5.05 ^b
	SE±	0.19	0.19	0.19	0.19	0.19
PW(g)	M ₁	0.68 ^b	1.08 ^a	1.10 ^a	1.13 ^a	1.03 ^a
	M ₂	0.86 ^a	0.75 ^b	0.91 ^a	0.69 ^b	1.00 ^a
	SE±	0.08	0.08	0.08	0.08	0.08
TSW(g)	M ₁	2.61 ^a	2.60 ^b	2.75 ^a	2.78 ^a	3.03 ^a
	M ₂	2.84 ^a	3.19 ^a	3.22 ^b	3.65 ^b	3.21 ^a
	SE±	0.12	0.12	0.12	0.12	0.12

NOTE: Means with the Same Letter within a Column are not Significantly Different at p≤0.05

PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves , LL- leaf length, LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed Weigh, M₁- first mutant generation, M₂- second mutant generation

Table 4.18: Effects of Concentrations on Generations of Sodium Azide

TRAITS	GEN	0.00%	0.10%	0.20%	0.30%	0.40%
PGERM (%)	M ₁	83.75 ^a	61.25 ^b	61.25 ^a	60.00 ^b	47.50 ^b
	M ₂	80.00 ^a	70.00 ^a	61.25 ^a	80.00 ^a	77.50 ^a
	SE±	2.76	2.76	2.76	2.76	2.76
PHT(cm)	M ₁	30.20 ^a	31.29 ^a	32.62 ^b	26.84 ^a	28.92 ^a
	M ₂	29.80 ^a	31.99 ^a	36.42 ^a	28.75 ^a	26.33 ^a
	SE±	0.9	0.9	0.9	0.9	0.9
NL	M ₁	5.25 ^b	5.00 ^b	4.50 ^b	6.00 ^a	5.50 ^a
	M ₂	6.16 ^a	6.16 ^a	6.25 ^a	5.69 ^a	5.75 ^a
	SE±	0.18	0.18	0.18	0.18	0.18
LL(cm)	M ₁	14.13 ^a	10.90 ^b	10.52 ^b	10.04 ^b	10.84 ^b
	M ₂	15.41 ^a	16.71 ^a	16.19 ^a	14.86 ^a	12.94 ^a
	SE±	0.36	0.36	0.36	0.36	0.36
LW(cm)	M ₁	1.31 ^b	1.21 ^b	1.20 ^b	1.30 ^a	1.15 ^a
	M ₂	1.56 ^a	1.68 ^a	1.45 ^a	1.46 ^b	1.19 ^a
	SE±	0.04	0.04	0.04	0.04	0.04
FW(g)	M ₁	1.17 ^b	1.50 ^b	0.90 ^b	1.55 ^b	1.13 ^b
	M ₂	3.22 ^a	2.55 ^a	2.93 ^a	3.00 ^a	1.63 ^a
	SE±	0.2	0.2	0.2	0.2	0.2
DW(g)	M ₁	0.80 ^b	0.98 ^b	0.50 ^b	1.13 ^a	0.88 ^a
	M ₂	1.23 ^a	0.93 ^b	1.07 ^a	0.95 ^a	0.65 ^b
	SE±	0.08	0.08	0.08	0.08	0.08
DPFL(days)	M ₁	42.00 ^b	37.50 ^b	38.50 ^b	39.25 ^b	38.50 ^b
	M ₂	55.50 ^a	54.50 ^a	56.00 ^a	54.75 ^a	50.00 ^a
	SE±	1.22	1.22	1.22	1.22	1.22
PL(cm)	M ₁	4.08 ^b	4.93 ^b	3.85 ^b	4.65 ^a	4.50 ^a
	M ₂	5.68 ^a	5.91 ^a	5.38 ^a	5.21 ^a	4.11 ^a
	SE±	0.28	0.28	0.28	0.28	0.28
PW(g)	M ₁	0.60 ^b	0.78 ^b	0.30 ^b	0.90 ^b	0.65 ^b
	M ₂	1.50 ^a	2.22 ^a	2.24 ^a	1.89 ^a	0.93 ^a
	SE±	0.12	0.12	0.12	0.12	0.12
TSW(g)	M ₁	2.64 ^a	2.57 ^a	2.59 ^b	2.64 ^a	2.44 ^b
	M ₂	2.80 ^a	2.84 ^a	3.47 ^a	3.59 ^b	3.17 ^a
	SE±	0.14	0.14	0.14	0.14	0.14

NOTE: Means with the Same Letter within a Column are not Significantly Different at $p \leq 0.05$

PGERM- percentage germination , PHT- plant height at maturity, NL- number of leaves , LL- leaf length, LW-leaf width , FW- fresh weight, DW-dry weight, DPFL- days to 50% flowering, PL- panicle length PW- panicle weight, TWS- one thousand seed Weigh, M₁- first mutant generation, M₂- second mutant generation.

Table 4.19: Mutagenic Frequency, Efficiency and Effectiveness of EMS and Sodium Azide

Treatment	Mutagenic frequency %	Lethality %	Mutagenic effectiveness %	Mutagenic efficiency %
Ems %				
0.1	7.50	0.00	18.75	7.50
0.2	5.00	0.00	6.25	5.00
0.3	2.50	2.50	2.08	1.00
0.4	5.50	2.50	3.44	2.20
SA %				
0.1	2.50	2.50	6.25	1.00
0.2	7.00	7.00	8.75	1.00
0.3	2.50	10.50	2.08	0.24
0.4	2.50	15.00	1.56	0.17

Note: 0.1%-10mM, 0.2%-20mM, 0.3%-30mM. 0.4%-40mM,

4.20 Chlorophyll Mutants Induced by EMS

Some spectrum of chlorophyll mutations obtained as a result of mutation induced by EMS are shown in Plate. I. Straita, Tigrina and a mutant that has not been characterized was observed.

4.21 Chlorophyll Mutants, Panicle and Dwarf Mutants Induced by Sodium Azide

Plate II. Presents some chlorophyll, panicle and some dwarf mutants induced by Sodium azide.

Viridis: having a yellow mosaic pattern on the leaves. While panicle colour showed changes with treatment.



Plate I: Showing Some Chlorophyll Mutant as a Result of Treatment with EMS

A- Control, B- Striata, C-striata, D-striata, E-Tigrina, F- unknown.



A

D

Plate II: Showing Some Chlorophyll and Panicle Mutant as a Result of Treatment with Sodium Azide

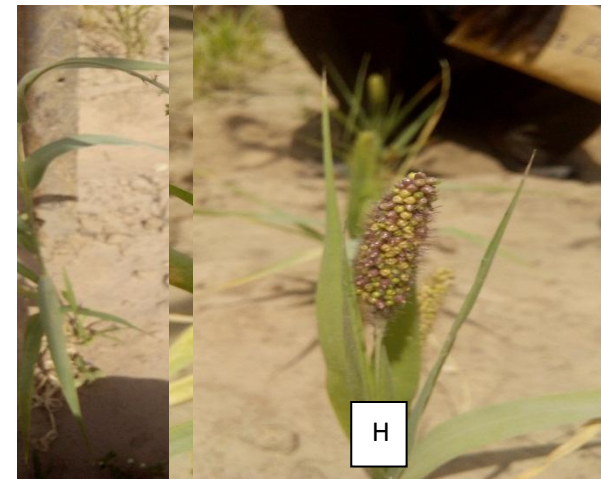
A-normal leaf and height for control, B and C- Sodium Azide treated dwarf mutants, D-Chlorophyll Mutant (Viridis)

E-normal panicle colour (control), F, G and H-mutant panicle colour (treated with Sodium Azide)

E

F

G



H

CHAPTER FIVE

5.0

DISCUSSION

5.1 Effects of Fast Neutron on Agronomic Traits of Foxtail Millet

The decrease in plant height, leaf length and leaf width M_1 generations with increase in exposure to fast neutrons may be due to the induction of oxidative damage in some cells by production of the free radical oxygen that lead to higher frequency of chromosomal aberrations and DNA damage due to the accumulative genotoxicity and chromosomal aberrations (Uhl *et al.*, 2003). Similar result was reported on Niger (*Guizzotia abyssinica*) treated with gamma rays (Poornananda and Hosakate, 2009) and on (pop-corn maize) as the exposure period of thermal neutron and gamma irradiation increased (Adamu, 2004). However, contrary result was reported in *Capsicum annum* (Dauda *et al.*, 2012). There was no increase in percentage germination, number leaves, fresh weight, dry weight days to 50% flowering, panicle weight, one thousand weight with increase in the dosage of fast neutron on foxtail millet. These may be that the gene that confer this characters were not affect by fast neutrons thus showing no effect morphologically or the mutations on these loci are recessive or the dosage was not enough to induce mutations in this traits.

The increase observed in plant height, leaf length, leaf width, fresh weight, panicle length, panicle weight and 1000 seed weight M_2 generation compared to the control could have been as a result of irradiation doses having biopositive effects on the gene resulting to a new altered foxtail germplasms. Fast Neutrons irradiation had bio-positive effects at specific dose probably by increasing number of amino acid produced in a protein (Haliem *et al.*, 2013). FN may cause special interference with DNA leading to induction of structural changes in DNA, such as chromosomal rearrangement, strand breaks, base deletions, pyrimidine dimers, cross-links and base modifications, and other effects (Gill and Tuteja, 2010). These DNA structural

changes(breaks, transpositions, deletion, etc.) which led to change in amino acids and consequently protein formed (Mondini *et al.*, 2009) may influence the expression of a number of genes leading to alteration in proteins that control many metabolic processes like plant development, cell cycle, and other processes in plant (Agrawal *et al.*, 2009). Similar findings were reported in *Capsicum annum*, *Capsicum frutescens* treated with fast neutrons that an increase in irradiation exposure period led to increase in morphological traits (Falusi *et al.*, 2012), *Vicia faba* (Haliem *et al.*, 2013). The not significant difference percentage germination, number of leaves, fresh weight, dry weight and days to 50% flowering could be because the dosage of fast neutron were not enough to induce missense mutation. Highest means values observed at 180 min and 270 min could be used for improvement of this agronomic traits in foxtail millet.

It is noteworthy that plant height, leaf length, leaf width, fresh weight, dry weight, panicle length, panicle weight, and one thousand seed weight did not show any significant increase in the first mutant generation but did so in the second generation. This finding is similar to report on soybean (Bolon *et al.*, 2011) and peanut (Wang *et al.*, 2015) with M₂ generation showing variation not observed in the M₁ generation. This is expected as M₂ phenotype are as a result of heritable effects (mutation that affected meiosis) while M₁ may be due to fast neutrons effects on somatic cells. This may also be possible because most mutations are recessive therefore variations can only be expressed in the M₂ generation after segregation has occurred during meiosis in the M₁ generation.

5.2 Heritability in Foxtail Treated With Fast Neutrons

Estimation of heritability in broad sense gives the indication of heritable component of variation. It favours effective selection on single plant basis. The high heritability estimates for one 1000 seed weight, plant height, leaf length, leaf width, panicle length and panicle weight, and low heritability for percentage germination, leaf number of leaves, fresh weight, dry weight and one

thousand seed weight were similar to the high heritability of many characters in mutant mungbean (Khan and Goyal, 2009). The High heritability found in some characters, indicates that the induce variability in mutant population were fixed and this characters can be selected for improved in subsequent generations.

5.3 Effects of Ethyl Methane Sulphonate on Agronomic Traits of Foxtail Millet

The result at M₁ showed a significant increase germination at 0.1% concentration. But further increase in concentration did not differ significantly with the control. While M₂ generation showed no significant difference. This could be due to promotion of physiological and biological processes necessary for seed germination which include enzyme activity (Kurobane *et al.*, 1979). Promoting effects EMS at low doses EMS on biological traits has been reported in cowpea (Bind *et al.*, 2016), Mungbean (Khan and Goyal, 2009) *Vicia faba* (Vandana and Dubey, 1988) and contrary effects on seed germination by the various mutagenic treatments were reported earlier in *Vinca rosea* (Murugan and Dhanvel, 2015), Onion (Joshi *et al.*, 2011), Grasspea (Ramezani and More, 2013).

Variation was observed in plant height from 29.74cm to 34.87cm, fresh weight from 1.25g to 2.60g, dry weight 0.8g to 1.73g, panicle length from 4.48g to 5.67g, and panicle weight from 0.91g to 1.38g where significant increase was recorded with increase in concentration as 0.4% gave best values and leaf length and leaf width showed significant variation which are not consistent with increases in concentration. This is similar to the findings reported in finger millet (Eswari *et al.*, 2014), on soybean (Khan and Goyal, 2009), on cowpea treated with EMS (Girija *et al.*, 2013) and contrary reported decrease in some quantitative traits of garden bean (Monica and Seetharaman 2016). The variations observed in the M₁ may be because of physiological disturbance induced by the EMS.

The second mutant generation showed highest mean value at 0.1% while further increase in concentration. There was decrease in mean value for plant height from 39.31cm to 26.29cm, number of leaves from 8.08leaves to 6leaves, leaf length from 17.65cm to 12.71cm, leaf width from 1.92g to 1.44g, fresh weight 4.00g to 2.00g, dry weight 1.28g to 0.78g, panicle length 7.60g to 4.57g, panicle weight 3.73g to 3.09g. While one thousand seed weight increased significantly different from the control although further increase had no effect on seed weight. This increase in mean values is probably due to the occurrence of polygenic mutations with cumulative effects (Imran *et al.*, 2011). EMS modifies the DNA mainly by causing chemical alteration of nucleotide by reacting with guanine or thymine by adding an ethyl group which causes the DNA replication machinery to recognize the modified base as an adenine or cytosine, respectively. This base substitution typically does not result in reading frame shifts, but instead causes altered forms of a triplet sequence. Changing a single base within a coding region causes either a nonsense codon which stops transcription or an altered codon which changes the amino acid transcribed, which can de-activate, reduce efficiency of, or produce a new protein (Kim *et al.*, 2004). This changes may occur randomly throughout the entire genome Greene *et al.* (2003). Similar result was reported on finger millet (Muduli and Misra 2008), in chickpea (*Cicer arietinum* L.) (Wani *et al.* (2012), in cowpea (Girija *et al.*, 2013), in Isabgol (*Plantago ovata* Forsk.) treated with EMS (Mishra and Khan 2014). But contrary to the work of Hridhya and Remesh, 2014 on *Amaranthus tricolor* L. which showed decrease in all traits at all concentrations.

5.4 Heritability in Foxtail Millet Treated With Ethyl Methane Sulphunate

Estimation of heritability in broad sense gives the indication of heritable component of variation i.e variations that are due to environment and those due to genotype. The high heritability value observed in leaf width, panicle length, and one thousand seed weight, number of leaves, fresh

weight, panicle weight indicates that the variation observed in these characters are mostly due to genotype hence plants treated with EMS can be used to increase leaf width, panicle length, and one thousand seed weight. Characters with high heritability can therefore be improved rapidly through selection than those with moderate heritability (leaf length) since the latter are influenced by environmental factors (Mensah and Obadoni, 2007). This result is similar with the findings of Arulbalachandran *et al.*, (2010) working on black gram (*Vigna mungo*) treated with gamma ray and on sesame (Begum and Dasgupta, 2014) who found high heritability value for most traits.

5.5 Effects of Nitrous Acid on Agronomic Traits of Foxtail Millet

There was an increase observed in plant height for treatment with nitrous acid with 0.1% giving maximum value for both M₁ and M₂ generation. This increase may be due to the mean increase in plant height at maturity in the present study might be due to the alteration of their genome integrated by environmental signals as reported by Uno *et al.* (2001) or probably by increasing the rate of cellular division and expansion at their meristematic regions. This is also in agreement with the findings of Hoballah (1999) who reported an increase in plant height of Sesame due to radiation mutagenesis and in Fonio millet (Animasaun *et al.* 2014).

Number of leaves increased significantly at 0.4% nitrous acid concentration both M₁ and M₂ generation even though it was not a linear increase. Similar observation was reported in Soya beans using ionizing radiation, (Khan and Goyal, 2009). Such unpredictable results may have been due to some random mutations at various loci. Fresh weight and dry weight increased significantly from the control although increase in concentration did not affect this character. This is probably because concentrations of nitrous acid might be due to chromosomal aberrations that tend to produce an increase in certain morphological traits such as leaf number. The stimulatory effect might be due to an activation of growth hormones (auxin) (Zaka *et al.*, 2004).

The significant decrease in days to 50% flowering may be as a result of nitrous acid deamination of DNA resulting mutation which causing variability in various characters. Similar findings was reported in fonio millet (Animasaun *et al.*, 2014).

Panicle length, panicle weight, 1000 seed weight showed similar trend of increase with increase in concentration of nitrous acid where 0.4% maximum value for panicle length and panicle weight while 0.3% maximum value for one thousand seed weight. Similar dose dependant variation in quantitative traits was reported sunflower treated (Selvaraj and Jaykumar 2004), on *Trigonella foenum-graecum* (Basu *et al.*, 2008). This may be as a result of nitrous acid acts as deaminating agent, removes the amine group from adenine or cytosine. When the cell replicates this altered area, it matches adenine to the deaminated cytosine, and cytosine to the deaminated adenine causing alteration in amino acid and consequently protein produced (Thomas *et al.*, 1979).

5.6 Heritability in Foxtail Treated With Nitrous Acid

Heritability helps the breeder to make selection for a character with desirable mean value and high heritability (Unche *et al.*, 2008). The high heritability recorded panicle length, plant height, number of leaves, fresh weight and 1000seed weight, signifies that phenotype was as a result of variation on the genotype of the plant and similar to the findings of Kumar, (2008) who observed high heritability pea plant. While percentage germination, leaf weight, leaf width, dry weight, days to fifty 50% flowering and panicle length recorded low heritability values. This indicates that selection will be rewarding for improvement of panicle length, plant height, number of leaves, fresh weight and 1000seed weight

5.7 Effects of Sodium Azide on Agronomic Traitsof Foxtail Millet

Sodium azide showed significant decrease in percentage germination with increase in concentration for both M₁ and M₂ generation. Similar inhibitory effect on seed germination by the various mutagenic treatments were reported earlier in Coriander (Sarada *et al.*, 2015) and Cluster bean (Deepika *et al.*, 2016) and *Capsicum annum* (Umar *et al.*, 2012). Although contrary to the findings on *Browellia speciosa* (El-mokadem and Mostafa, 2014). This may be as a result of hormonal imbalance (Chrispeels and Varner, 1967) and inhibition of mitotic process (Ananthaswamy *et al.*, 1971). Sodium azide may interfere with the synthesis of enzymes and at the same time accelerated the degradation of existing enzymes involved in the formation of auxins and thus reduces the germination of seeds. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity.

There was a decrease in plant height, leaf length, fresh weight, dry weight, and panicle length with sodium azide treatment with 0.02% concentration having low values for M₁ generation. While maximum mean value for plant height for M₂ generation was still at 0.02% concentration of sodium azide. These findings agree with Poornananda and Hosakate 2009, who reported a decrease in plant height of *Guizotia abyssinica* treated with sodium azide and gamma rays, report on musk okra (*Abelmoschus moschatus*) showing decreasing percentage germination, survival, average plant height, number of buds and number of branches but increasing total leaf area, number of flower and number of fruits/plants (Ashish *et al.*, 2011). Similarly, negative effects of Sodium Azide and Colchicine were observed on growth traits of *Sesamum indicum* (Menasha *et al.*, 2007). This reduction in some agronomic traits at M₁ may be due to the effect of mutagen on meristematic tissues of the plumule (Deepika *et al.*, 2016).

The result of the M₂ on the other hand showed increase in plant height, leaf length, panicle weight which decrease below the control at higher concentrations of sodium azide. THIS agrees with the

work on *Browallia speciosa* (El-mokadem and Mostafa, 2014), on groundnut (Animasaun *et al.*, 2014) and in rice (Ikhajiagbe *et al.*, 2014). This may be as a result of segregation which occurs during meiosis as recessive genes may find expression in the M_2 generation. One thousand seed weight significantly increased with increase in concentration and was maximum at 0.03%. Similar to the report of increase in some yield traits by Srivastava *et al.*, 2011 and on groundnut (Animasaun *et al.*, 2014). The significant decrease of plant height, number leaves, leaf length, leaf width, fresh weight, dry weight and panicle length at 0.04% Sodium azide concentration of M_2 may be as a result of higher frequency of chromosomal aberrations and DNA damage which in turn can affect the vigor, agronomic characters and likely to persist in seeds yield or even longer due to the accumulative genotoxicity and chromosomal aberrations (Uhl *et al.*, 2003).

5.8 Heritability in Foxtail Treated with Sodium Azide

High heritability values were recorded for panicle weight and leaf width, fresh weight, dry weight, panicle length, one thousand seed weight this maybe that growth traits are more influenced by environment than yield parameters. This findings is similar to the report on black gram treated with EMS and colchicines (Selvam *et al.*, 2010) and contrary to that of Kaul and Kumar (1983) who obtained low heritability values for grain yield in rice. This may be because heritability is a property not only of a character but also of the population and the environment to which the genotypes are subjected. Hence can these traits if desirable can be used in breeding programme.

5.9 Effect of Mutagens and their Concentrations

The result generally showed maximum values for agronomic traits with treatment with fast neutrons (esp. 180min and 270min) and EMS (esp. 0.1% showing maximum values for number of leaves, leaf width, fresh weight, panicle length, panicle weight and one thousand seed weight)

this may be as a result of mutation at single nucleotide pairs. EMS specifically creates a large proportion of non-sense mutations, involving the introduction of novel stop codons than other mutagens as it create mainly G-A and C-T transition, and any individual mutation is therefore more likely to express phenotypically (Suzuki *et al.*, 1997). The decrease usually below the control generally shown by higher treatments of sodium azide and nitrous acid may be as a result of large scale structural changes (breaks, transpositions, deletion, etc.) which will lead to change in amino acids and consequently protein formed lead to decrease in agronomic characters of the plant(Mondini *et al.*, 2009). Meanwhile the general decrease observed at 0.04% sodium azide may be as a result of sodium azide induces chromosomal aberrations only at a very low rate compared to other mutagenic treatments. Mutagenic activity of this compound was attenuated by a deficiency in the excision of UV-like DNA damage in both plants and bacteria, therefore it seems that a lesion recognizable by the excision-repair mechanisms must be formed to evoke the effect. Mutagenesis proceeds from this by ‘direct mispairing’ (Owais and Kleinhofs, 1988).

5.10 Effects of Dosage on Generations of Fast Neutron

The general, increase observed in all the treatments including control for number of leaves, leaf length, fresh weight, days to 50% flowering, panicle length and panicle weight maybe be as a result of the effect of environment as M₁ (planted during the raining season; August to October) was rain fed while M₂ was irrigated (planted during the dry season; December to February). This suggest that foxtail millet this characters are largely affected by environmental conditions as the control showed varied in the M₂ generation. Meanwhile plant height, dry weight, leaf width and one thousand seed weight which did not differ in the control but showed difference in other treatments may be as a result of segregation that occurred in the M₁ giving rise to expression of mutant alleles that were recessive in the M₁ generation (Parry *et al.*, 2009).

5.11 Effects of Concentration on two Generations of EMS

The similar response in percentage germination, plant height, dry weight, panicle length and 1000 seed weight in control from M_1 and M_2 indicated that environment does not affect variation seen in the other treatments rather it may be genetic effect expressed in the M_2 generation. While the variation observed in number of leaves, leaf length, leaf width, fresh weight, days to fifty 50% flowering and Panicle weight may be affected large by environment since difference was also observed in the control.

5.12 Effect of Concentration on two Generations of Nitrous Acid

Variation in Plant height, leaf length, fresh weight, dry weight, days to fifty percent flowering panicle length and panicle weight in M_1 and M_2 generation including control maybe because of environmental effects on characters as change in environment may be responsible for large part of variation observed in M_1 and M_2 generation. However, number of leaves, leaf width and one thousand seed weight which showed consistency in the value of control maybe be because of segregation in M_1 plants producing seeds that a genetically variant from their M_1 progenitors as a result of mutation.

5.13 Effects of Concentration on two Generations of Sodium Azide

The results which shows increase in values for all treatment in the M_2 generation may be as a result of environmental condition in which the M_2 was planted was more favourable than the one which the M_1 was planted.

5.14 Mutagenic Frequency, Efficiency and Effectiveness of EMS and Sodium Azide

A mutagen may effectively bring about mutations but the accompanying undesirable traits like lethality, may decrease its efficiency. Thus, in order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop are necessary to recover high frequency of desirable mutations (Badere and Chaudhary, 2007). Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, whereas mutagenic efficiency indicates the extent of genetic damage recorded (Wani, 2009). In the present study, the degree of effectiveness and efficiency varied among different mutagens. The mutagenic effectiveness decreased with the increase in concentration of mutagen in both EMS and sodium azide, indicating that negative relationship between effectiveness and dose of mutagen. While Mutagenic efficiency (mutation rate in relation to damage) in both mutagens, highest at the lowest dose and it decreased with the increase in dose. In general lower (0.1% for EMS and 0.02% for Sodium azide) were most effective in inducing mutations. The decrease in effectiveness at higher concentrations may be attributed to the failure in proportional increase of mutation frequency induced at higher treatments (Bashir *et al.*, 2013). Similar result was reported in of chickpea(Wani, 2009), sunflower (Kumar and Ratman, 2010)and little millet(Ganapathy *et al.*, 2008).In EMS, mutagenic effectiveness and efficiency were highest (18.75% and 7.50%) at 0.1% EMS concentration and decreased with increase concentration. Therefore could be said to be more effective and efficient than the latter doses. Similarly mutagenic effectiveness and efficiency were highest (8.75% and 1%) at 0.2% sodium azide concentration and decreased with increase concentration. This corroborated with the results in groundnut (Mensah and Obadoni, 2007) and (Dhanavel *et al.*, 2008) in cowpea. Generally, EMS proved to be the most effective and efficient than Sodium Azide. This may be because ethylating agents, being less toxic, can be applied at relatively higher concentrations to yield more mutations(Bashir *et al.*, 2013). Thilagavathi and Mullainathan (2009) in blackgram

and Velu *et al.* (2008) in cluster bean also reported the greater efficiency and effectiveness of EMS

5.15 Chlorophyll and Some Morphological Mutant

Leaf color mutations are one kind of most frequently observed mutation in both spontaneous and induced mutant populations, and often used as an indicator of mutagenic effects and efficiency of various mutagens. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes (Swaminathan, 1964). Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments (Gautam *et al.* 1992).

Spectrum of chlorophyll mutations suggest that the chemical mutagen EMS and Sodium azide were efficient in inducing mutations of genes needed for chlorophyll development. Higher frequency and a wide spectrum of chlorophyll mutants in chemical mutagen EMS have been reported in grasspea (Ramezani and More, 2014), in carnation (Bhattacharya, 2003), in lentil (Sharma and Sharma 1984), in okra treated with sodium azide (Ashish *et al.*, 2011), in *Delphinium malabaricum* (Kolar *et al.*, 2011). Different shades of purple in panicle colour observed on treatment with sodium azide may be as a result of mutation in some genes that control panicle colour. Similar results were observed in cowpea (Girija *et al.*, 2013), pepper (Falusi and Dauda, 2012). The Dwarf mutants observed in different Sodium azide treatments in foxtail millet is similar mutants were observed *Catharanthus roseus*(Ramezani and More, 2014), in *Lathyrus sativus* (Kumar and Dubey, 1998), in rice (Ramesh and Reddi, 2002).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This study was conducted to ascertain the effects of fast neutrons, EMS, nitrous acid and sodium azide at various levels of treatments on agronomic and morphological traits of foxtail millet in the M_1 and M_2 generations. These mutagens showed significant effects on the traits evaluated as great variation was observed as a result of the treatments. The result obtained from treatment with fast neutron, showed increase in some agronomic traits even though increase in dosage did not lead to increase in this character instead a decrease as 180min and 270min gave maximum values in most parameter measured for the M_2 generation. Similar result were recorded in foxtail millet treated with EMS as increase was observed in low dosage (0.1%) for many traits measured M_2 generation. Further increase in concentration showed increase in some traits and decrease in others. Nitrous acid also showed increase at low concentration with no decrease in some traits while other remain the same as concentration increased. Sodium acid showed significant

increase at low concentration while increase in concentration led to decrease in most agronomic parameters.

Heritability values were high only for one thousand seed weight for foxtail millet treated with fast neutrons. EMS treated foxtail millet showed high heritability for leaf width, panicle length, and 1000 seed weight. Nitrous acid treated foxtail millet showed high heritability for panicle length. While sodium azide recorded high heritability for panicle weight and leaf width.

Comparing the treatments together, it was noted that EMS at 0.1% concentration performed better.

6.2 Conclusions

- 1 Fast neutrons, ethyl methane sulphonate, nitrous acid and sodium azide affected most agronomic traits such as germination, plant height, number of leaves, leaf length, leaf width, fresh weight, dry weight, panicle length, panicle weight and one thousand seed weight of foxtail millet.
- 2 The optimum dosage that created useful variability were 270min for fast neutrons, 0.1% for Ethyl Methane Sulphonate, 0.4% for nitrous acid and 0.2% for sodium azide. The results that showed at EMS at 0.1% concentration was the best treatment for most of the traits evaluated.
- 3 Heritability values were high for plant height, leaf length, leaf weight, panicle length, panicle weight and 1000 seed weight for foxtail millet treated with fast neutrons. EMS treated foxtail millet showed high heritability for percentage germination, plant height, number of leaves, leaf width, fresh weight, dry weight, days to 50% flowering, panicle length, panicle weight,

1000seed weight. Nitrous acid treated foxtail millet showed high heritability for plant height, number of leaves, fresh weight, Panicle length and 1000seed weight. While sodium azide recorded high heritability for leaf width, fresh weight, dry weight, days to 50% flowering, panicle length, panicle weight and 1000 seed weight. The showed that variations created by the mutagens on various traits are heritable.

- 4 The results from this study also indicated that EMS and sodium azide were most effective and efficient at lower concentrations(EMS esp. 0.1%, effectiveness 18.75%, efficiency 7.50%and Sodium azide 0.2% effectiveness 1.84% and efficiency 1%).EMS was more effective and effiecient in inducing variability in foxtail millet than sodium azide.

6.3 Recommendations

The overall results showed that fast neutrons, ethyl methane sulphonate, nitrous acid and sodium azide created high variability in agronomic traits hence the following recommendations

- 1 Further studies should be carried to determine the heterotic value of crossing some of these mutants.
- 2 Further trials for disease resistance, water tolerance, drought tolerance and so on could be carried out in subsequent generations to determine mutantsthat can adapt and produce maximally under such conditions.
- 3 Further research could be carried out to determine the biochemical and molecular effects of these mutagens on foxtail millet.

CONTRIBUTION TO KNOWLEDGE

1. Treatment of foxtail millet with Fast neutrons, ethyl methane sulphonate, nitrous acid and sodium azide could induce variations in agronomic traits (germination, plant height, number of leaves, leaf length, leaf width, fresh weight, dry weight, panicle length, panicle weight and one thousand seed weight) of foxtail millet and some quantitative traits.
2. The optimum dosage for inducing variability in agronomic traits were 270min for fast neutrons, 0.1% for ethyl methane sulphunate, 0.4% for nitrous acid and 0.1% for sodium azide.
3. EMS (0.1% with effectiveness of 18.75% and efficiency of 7.5%) was more effective and efficient than sodium azide in inducing mutation in foxtail millet.

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