GENETIC DIVERSITY OF M_2 MUTANTS OF SUNFLOWER (HELIANTHUS ANNUS L.) INDUCED BY ETHYL- METHANESULPHONATE (EMS)

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DECLARATION

I declare that the work in this dissertation entitled **Genetic Diversity of M_2 mutants of Sunflower (*Helianthus annus* L.) induced by Ethyl-Methanesulfonate (EMS)** has been carried out by me in the Department of Plant Science. The information derived from 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 at this or any other institution.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled GENETIC DIVERSITY OF M_2 MUTANTS OF SUNFLOWER (*HELIANTHUS ANNUS* L.) INDUCED BY ETHYL-METHANESULFONATE (EMS) by YEKINI AISHA EPEMIRE meets the regulations governing the award of the Master's degree of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to Almighty Allah. With You, all things are possible. Also, to my beloved father, Late Gen. Suleiman Yekini (Rtd). May Allah grant you an eternal bliss.

ABSTRACT

Sunflower is an important oilseed crop due to its health related values. Induced mutations are often used to create new genetic variability where sufficient variation does not exist naturally as in the case of sunflower. This study was carried out at the laboratory and research farm of the Institute for Agricultural Research (IAR), Samaru, Zaria to assess the response of the sunflower seeds to different EMS doses and determine optimal doses for successful mutation; estimate genetic variability for agronomic traits among the M_2 mutant lines and identify some desirable mutants for oil content and other agronomic traits; assess the degree of association among oil content, grain yield and other agronomic traits and assess the degree of similarity among the selected mutants. Two sunflower varieties (SAMSUN2 and SAMSUN4) obtained from Institute of Agricultural Research were treated with EMS concentrations of 0.1, 0.2, 0.3, 1.0 and 1.5% for 2, 4, 6 and 8 hrs with controls to ascertain optimum EMS concentration and exposure time to bring about desirable mutation. The laboratory study showed progressive decrease in seed germination as EMS concentration and exposure durations were increased. Optimum doses were observed at 0.1% EMS for 6 hrs, 0.2% EMS for 4 hrs, 0.2% EMS for 6 hrs, 0.3% EMS for 4 hrs and 0.3% EMS for 6 hrs for the two varieties out of which 0.2% EMS for 4 hrs, 0.2% EMS for 6 hrs, 0.3% EMS for 4 hrs and 0.3% EMS for 6 hrs were chosen to generate M_1 population in the research farm. A total of 78 M_1 plants with substantial seed set were used to raise M_2 population in progeny rows. Surviving 418 mutant progenies as well as two checks were evaluated for desired agronomic traits in M_2 generation using 16 x 5 lattices design with two replications. The analysis of variance for agronomic traits revealed significant ($P<0.05$) differences amongst the mutants progenies. Sixty (60) mutants professing higher seed yield per plant (11 - 23 g) were evaluated for oil content. The correlation study showed that grain yield per plant was

significantly correlated with plant height (r = 0.68), stem diameter (r = 0.61), capitulum circumference (r = 0.72) and 100 seed weight (r = 0.81). Cluster analysis grouped the selected 60 mutants into four clusters with clusters I, II, III and IV consisting of 19, 4, 26 and 11 mutants, respectively, implying that crossing of mutants from these different clusters could enhance more variability for desirable traits for improvement. The present study identifies mutants T8M11-1-2, T4M18-3-1, T2M6-1-1, T8M11-4-1 and T8M23-1-2 as being superior for oil content (45%), stem diameter (4 cm), capitulum circumference (38 cm), seed weight (13 g) and seed yield per plant (26 g) respectively. Their inclusion in future breeding programs could result in the development of superior sunflower cultivars and as such, should be further evaluated in subsequent generations to stabilize the traits.

TABLE OF CONTENTS

DECLARATION i

CERTIFICATION ii

ACKNOWLEDGEMENTS iii

DEDICATION iv

ABSTRACT v

TABLE OF CONTENTS vii

LIST OF TABLES x

LIST OF FIGURES xi

LIST OF PLATES xii

LIST OF APPENDICES xiii

CHAPTER ONE 1

1.0 Introduction 1

CHAPTER TWO 4

2.0 LITERATURE REVIEW 4

2.1 Sunflower 4

2.2 Growth and development of sunflower 4

2.3 Sunflower production 5

2.7 Principles of induced mutation in crops 8

2.9 Mutation breeding strategy for obtaining mutants in crops 8

2.10 Mutagenic agents 9

2.11 Ethyl methane sulfonate (EMS) induced mutation in sunflower 10

2.12 Optimal dosage for mutagenic treatment in crops 10

2.14 Genetic variability in sunflower 11

2.16 Correlation study in sunflower 12

CHAPTER THREE 13

3.0 MATERIALS AND METHODS 13

3.1 Experimental Site 13

3.2 Genetic Materials 13

3.3 Laboratory experiment 13

3.3.1 EMS Mutagenesis 13

3.3.2 Determination of optimum mutagen dose 16

3.4 Field experiment 16

3.4.1 EMS treatment 16

3.4.3 M_2 plant population and evaluation 17

3.5 Data collected 17

3.6 Data Analysis 19

3.6.1 Analysis of variance 19

3.6.2 Genotypic and phenotypic coefficients of variation 19

3.6.3 Correlation Coefficients 21

3.6.4 Cluster analysis 21

CHAPTER FOUR.....22

4.0 RESULTS.....22

4.1 Response of sunflower varieties to different EMS doses and optimal mutagen dose determination.....22

4.2 Variability in agronomic traits of M_2 progenies derived from two sunflower varieties 24

4.4. Estimates of Phenotypic and Genotypic Coefficients of Variation 28

4.5. Variations in oil content and other agronomic traits amongst 60 selected M_2 progenies...28

4.6 Association among oil content, seed yield per plant and other agronomic traits.....33

4.7 Divergence of the 60 selected M_2 mutants.....33

CHAPTER FIVE 36

5.0 DISCUSSION.....36

CHAPTER SIX.....40

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS.....40

6.1 SUMMARY 40

6.2 CONCLUSIONS 41

6.3 RECOMMENDATIONS 42

REFERENCES 43

LIST OF TABLES

Table 3.1: Description of the two sunflower varieties 15

Table 3.2: Form of analysis of variance and Expected Mean Squares for Alpha lattice design ...20

Table 4.1: Germination percentages (GP) and Lethality (%) of two sunflower varieties treated with five EMS concentrations and four exposure durations at Zaria in 2016..... 23

Table 4.2: Analysis of variance for agronomic traits of M_2 progenies and two check varieties of sunflower evaluated at Zaria in 2016. 26

Table 4.3: Mean performance of agronomic traits of M_2 mutants progenies of sunflower varieties evaluated at Zaria in 2016 50

Table 4.4: Genetic parameters of nine important traits of M_2 mutants progenies of sunflower varieties evaluated at Zaria in 2016. 28

Table 4.5: Agronomic traits of 60 promising M_2 mutants progenies of sunflower varieties evaluated at Zaria in 2016. 30

Table 4.6: Correlation coefficients of agronomic traits among M_2 progenies of two sunflower varieties evaluated at Zaria in 2016 34

LIST OF FIGURES

Figure 1.1: Dendrogram of 60 M_2 mutants of sunflower varieties as derived by UPGMA cluster from the Euclidian distance matrix. 35

LIST OF PLATES

Plate 4.1: Morphological variations of some sunflower mutants 32

LIST OF APPENDICES

Appendix 1: Mean performance of agronomic traits of M_2 progenies of sunflower evaluated at Zaria in 2016.....50

CHAPTER ONE

1.0 Introduction

Cultivated sunflower (*Helianthus annuus* L.) is an annual, herbaceous (*2n=34*) and a cross pollinated crop, native to North America. It is a member of the Asteraceae family and it is regarded as the fourth most important oilseed crop grown worldwide after soya beans, palm and edible rapeseed (canola) (Moghaddasi, 2011; Sujatha *et al*., 2012). Sunflower is cultivated on about 18 million hectares worldwide with an annual seed production of 40 million tons (FAOSTAT, 2015). Its seeds are known for their high oil (25 - 48%) and protein (23 - 35%) content and thus used in confectionary and animal feed (Imran *et al.*, 2015). It is used as an ornamental plant due to the attractiveness of its flower (Mayor *et al.*, 2010). Sunflower seeds have abundant health benefits which can be attributed to the high levels of polyunsaturated and monounsaturated fats, phytosterols, tocopherols, protein, copper, folates, iron, zinc, and vitamin B (Nandha *et al*., 2014; Roche *et al.*, 2010). Its oil is used as raw materials in many industries due to the presence of four commercially important fatty acids namely, palmitic, stearic, oleic, and linoleic acids (Lee *et al.*, 2010).

Gene variability within a species enables the development of new improved varieties with improved characteristics. Therefore, the success of genetic improvement of sunflower depends on the magnitude of genetic variability which enables the selection of desirable genotypes for breeding purpose (Cvejic *et al*., 2011). In addition, realizing the need for the imperativeness of low-input agriculture being proffered for the 21st century, farmers require a suite of improved crop varieties that are genetically diverse in terms of climate change resilience, input use-efficiency, high yielding potential, resistance to biotic and abiotic stresses, enhanced nutritional

and other important quality attributes (Tester and Langridge, 2010; Waines and Ehdaie, 2007). However, the envisaged genetically diverse portfolios of sunflower crop varieties are often unavailable to farmers due to its extreme narrow genetic base (Seiler and Frederick, 2011). This makes its improvement through conventional breeding method alone difficult. In view of this, the induction of genetic variability for sunflower crop, particularly in the era of increasing global food crisis and changing climatic regimes is, therefore, highly desirable.

Mutagenesis is an important tool in plant breeding for increasing genetic variability and consequently, broadening the genetic base of germplasms (Ndou *et al*., 2013). Spontaneous mutations occur naturally in crops but, its rate is low and cannot always be exploited for breeding, thus the need for induced mutagenesis (Jain and Suprasanna, 2011). Mutations in plants can be induced using chemical or physical mutagens and the effectiveness and efficiency of any induced mutagenesis experiment are direct results of the choice of appropriate mutagen treatments (Rupinder and Kole, 2005).

Almost all mutagens have the property of reacting with DNA and thereby bringing about changes in nucleotide sequences. However, the mode of action of each mutagen is distinct. Besides, a mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality or sterility may decrease its efficiency (Shagufta *et al*, 2013). Thus, in order to exploit induced mutagenesis for crop improvement, a preliminary determination of the treatment that would yield the greatest amount of desirable mutation while at the same time, producing the least density of undesirable effects is necessary (Xin *et al*., 2008; Mba *et al*., 2010). Chemical mutagens as compared with physical mutagens offer high mutation rate and predominantly, point mutations (Cvejic *et al*., 2011). Among chemical mutagens, ethyl methane

sulfonate (EMS) is the most powerful and effective mutagen for creating mutations in plants (Cvejic *et al*., 2011).

Mutagenesis has become an important crop improvement tool available to breeders with no regulatory restrictions imposed as with genetically modified crops (Parry *et al.*, 2009) with mutant varieties readily accepted by consumers. Sunflower mutants with altered agronomic traits have been created through the use of induced mutations amongst which includes; early maturing, short stature, larger head diameter, thinner husk, high oil content, cytoplasmic male sterile (CMS) lines and many more (Sabetta *et al.*, 2011; Cvejic *et al*., 2015; Mostafa, 2011). Mutagenesis, in conjunction with conventional breeding methods could result in mutant varieties with desirable traits that can be used to broaden the genetic base of sunflower and to improve its various agro-morphologically important traits. There is paucity of information on the response of sunflower varieties developed by IAR, Samaru to different EMS doses, as well as, effective mutagen dose for induction of variability. The present study was focused on increasing the genetic variability within selected sunflower varieties using EMS and selecting mutants with important agro-morphological traits for further improvement. In view of these, the objectives of this study were to:

1. assess the response of the sunflower varieties to different EMS doses and determine optimal EMS doses for desirable mutation
2. estimate genetic variability for agronomic traits among M_2 mutants and identify some desirable mutants for oil content and other agronomic traits
3. assess the degree of association among oil content, grain yield and other agronomic traits
4. assess the degree of similarity among the mutants for the studied agronomic traits.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sunflower

Sunflower (*Helianthus annuus* L.) is an annual plant and its generic name Helianthus comes from the Greek words *helios* (the sun) and *anthos* (a flower). It is a diploid species (*2n= 34*) that belongs to the sub - tribe - *Helianthineae*, tribe - *Helianthea*, sub - family - *Asteroideae* and family - *Compositae* (*Asteraceae*) (Andrew *et al*., 2013). Helianthus is native to temperate North America and contains 14 annual and 37 perennial species (Shilling, 2006). They are large plants and are grown throughout the world because they are relatively short growing season species. Sunflower is an annual herb with a rough, hairy stem; broad, coarsely toothed, rough leaves, 3 to 12 inches long and circular capitulum of 3 to 6 inches or more (Khaleghizadeh, 2011). The capitula are composed of many small tubular flowers arranged compactly on a flattish disk. The face of the capitulum comprises of hundreds of disk flowers, which form into a seed (achene) (Seghatoleslami *et al*., 2012).

2.2 Growth and development of sunflower

Sunflower is a broadleaf plant that emerges from the soil with two large cotyledons. The emergence will take four to five days when planted an inch deep, but will take a few days longer when planted deeper (Rawat *et al*., 2010). Sunflower is normally a tall plant and the most cultivated ones are about 150 - 220 cm high. Both semi dwarf (100 - 150) and dwarf (50 - 100) plants have been produced in addition to those of standard heights (150 - 180) (Kaya *et al*., 2012). After reaching their full heights and blooming, the capitulum on some cultivars turn downwards, designed to make it harder for birds to eat the seed. Early maturing varieties are

ready for harvesting 90 to 120 days after planting, and late maturing varieties 120 to 160 days after planting (Khan *et al.*, 2013). The seeds are ready for harvest when the heads turn black or brown and the seed moisture content reaches 10 - 12%. Depending on climatic and agronomic conditions, yields can vary from 600 to 3000 kg per hectare (Akhtar *et al.*, 2012).

2.3 Sunflower production

In recent years, sunflower cultivated area has been steadily increasing due to the breeding of dwarf, high yielding hybrids which facilitate mechanization as well as the emphasis given to polyunsaturated acids for human consumption. Although sunflower is generally regarded as a temperate crop, it is currently cultivated on approximately 18 million hectares in 40 countries of the world, including some countries in the humid tropical Africa because it is quite rustic and can perform well under varying climatic and soil conditions (Seiler *et al*., 2011). Global production grew steadily in the last 25 years (PSD-USDA, 2011), and Food and Agricultural Organization (FAO) expects world total output close to 60 million tons towards 2050. The four largest producers (Ukraine, Russia, European Union and Argentina) account for 70% of global volume. According to data from FAOSTAT (2015), Ukraine ranked first producing 10.0 million tons of seeds. Russia Federation and European Union ranked second and third places with 9.0 and 8.8 million tons of seeds, respectively. South Africa ranked first in Africa and twelfth worldwide, producing 0.9 million tons of sunflower seeds. Although statistical data on production level and hectarage in Nigeria are not available, seed yield above 1 ton per hectare has been reported (Okonji, 2014).

2.4 Genetics and breeding of sunflower

The main aim of plant breeding is to develop new varieties to meet the needs of its end users. The objectives in sunflower breeding programs vary with specific programs, but generally place

emphasis on high grain yield, oil yield, oil quality and approaches to management of resistance genes to both biotic and abiotic stresses (Skoric *et al*., 2012). For special markets, breeding objectives may be slightly different. For confectionery sunflower, the main direction in breeding is towards increased 1,000 seed weight, protein content and quality, while lowering the seed oil content (Hladni *et al*., 2011). Another major objective in sunflower breeding is decreasing plant height as it facilitates mechanization and hybridization. In addition, reduced plant height helps ameliorate the problems associated with lack of stand-ability as it reduces capitulum, stem and root lodging. Lodging due to adverse growing conditions can reduce yield significantly causing up to 100% crop loss (Sala *et al.,* 2012).

2.5 Uses of sunflower crop

Sunflower is produced mainly for oil. Its oil is considered premium because of its high level of unsaturated fatty acids, bland flavor and high smoke points (Saima *et al.*, 2016). It supplies more Vitamin E than any other vegetable oil and contains variable amounts of vitamins A, B, D and K. The primary fatty acids in the oil are oleic and linoleic acids, with the remainder consisting of palmitic and stearic saturated fatty acids (Saima *et al.*, 2016). Sunflower seed oil has abundant health benefits such as improving cardiovascular, immune, reproductive and central nervous systems that can be attributed to the high levels of polyunsaturated and monounsaturated fats, phytosterols, tocopherols, protein, copper, folates, iron, zinc, and vitamin B (Nandha *et al*., 2014; Roche *et al.*, 2010). The seeds and its by-products are also a rich source of proteins and carbohydrates for animal feeds (Aminifar and Galavi, 2014). In addition, the use of sunflower oil as pesticide carriers, production of agrochemicals, surfactants, adhesives, plastics, vanishes, fabric softeners, lubricants and coatings have been explored. The flowers are used as a yellow dye, and the plant itself can be used for fodder, silage and as a green-manure crop (Dedio, 2005).

Furthermore, the high energy demand in the industrialized world as well as the pollution problems caused by the use of fossil fuels makes it increasingly necessary to develop a new renewable energy source. Biodiesel, which is a vegetable oil or animal fat based diesel fuel, is an attractive alternative to fossil fuels. It is carbon neutral, biodegradable, non - toxic and has low emission profiles as compared to petroleum fuels. Interestingly, sunflower oil is an important feedstock with good properties for the production of biodiesel after rapeseed oil (Alejandro, 2011).

2.6 Mutation in crops

Primarily, simple selection of desirable offspring was the first method of breeding crops and this utilized the occurrence of spontaneous mutations (Shu *et al.,* 2010). Genetics became a fundamental science of plant breeding after Gregor Johann Mendel discovered the laws of heredity in the nineteenth century. However, further advancements in plant breeding took place when the hybridization methodology was developed. Its aim was to combine desirable genes found in two or more different varieties in order to produce pure-line progeny superior to the parental types in many respects. Later, the work on the induction of genetic alterations through X-rays by Lewis John Stadler in the late 1920s and early 1930s laid the foundation of another type of plant breeding known as mutation breeding (Shu *et al.,* 2010). Mutations are the primary sources of all genetic variations existing in any organism, including plants (Kharkwal, 2012). Spontaneous mutations are very rare and random in terms of time of occurrence, which makes them more difficult to use in plant breeding programs.

2.7 Principles of induced mutation in crops

Induced mutation is aimed at creating mutants through altering one or two major traits, while maintaining the major genetic composition of the variety (Babaei *et al.*, 2010). Mutations are artificially induced by exposing plant materials to mutagenic treatments leading to a break in the nuclear DNA and subsequently, during the process of DNA repair, mutations occur randomly and are heritable (Jain and Suprasanna, 2011). Conventional breeding techniques rely on combining the available genetic variation through hybridization. The difference between induced mutation and conventional breeding is that the latter involves the production of new genetic combinations from already existing parental genes, while mutagen treatments create new gene combinations with high mutation frequency (Majeed *et al.*, 2010). Induced mutations are useful in creating variability that is not available in the gene pool (Kumar and Pandey, 2008). Mutation has been used to generate new varieties with better characteristics in sunflower (Kumar *et al*., 2013; Cvejic *et al.*, 2015) and in other crops such as groundnut (Badigannavar and Mondial, 2010; Satpute and Suradkar, 2011), cowpea (Nair and Mehta, 2014; Devmani and Dwivedi, 2014), wheat (*Triticum aestivum*) (Ndou, *et al.*, 2013) and cucumber (Wang *et al.*, 2014).

2.9 Mutation breeding strategy for obtaining mutants in crops

Genetically, M_1 mutant plants are heterozygous and only dominant mutations will be expressed in the M_1 generation while recessives will be expressed in the M_2 generation. Therefore, screening of mutants is done in subsequent generations where segregation will occur (Roychowdhury and Tah, 2013). The key point in mutation breeding is the process of identifying individuals with a target mutation, which involves mutants screening and confirmation (Forster and Shu, 2012). Mutants screening involves selection of individuals from a large mutated population that meet specific selection criteria. For example, early flowering, high yield, disease

resistance and so forth as compared to the controls. However, these selections are often regarded as putative mutants. Mutants confirmation, on the other hand, is the process of re-evaluating the putative mutants under a replicated environment using large samples. Through this process, many putative mutants are revealed to be false mutants (Mba, 2013). Caution should be taken to prevent cross pollination among the M_1 individuals as this would lead to generation of new variation that will make it difficult to differentiate the effect of mutation from hybridization (Roychowdhury *et al.*, 2011).

2.10 Mutagenic agents

Agents of artificial mutations are called mutagens and are generally grouped into two broad categories - chemical and physical mutagenic agents. The chemical mutagens which are mostly used for mutation induction belong to the class of alkylating agents such as ethyl methane sulfonate (EMS); diethyl sulphate (DES); ethyleneimine (EI); ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH), methyl nitroso urea (MNH) and azides (Ndou *et al*., 2013). Physical mutagens include ionic radiation, X-rays, UV light, gamma rays and neutrons (Velmurugan *et al.*, 2010). The effect of chemical mutagens are generally considered mild and are predominantly point mutations with lesser lethality unlike physical mutagens which induces gross lesions, such as chromosomal abbreviation or rearrangements (Oldach, 2011). However, chemical mutagens are generally carcinogenic and, therefore, extra care must be taken for health protection during the procedure (Oladosu *et al*., 2016). As pointed out by Mba (2013), the frequency and types of mutations are direct results of the dosage and rate of exposure of the mutagen rather than its type. In the end, the choice of a mutagen will be based more often than not on the particular researcher's circumstances, such as safety of usage, ease of use, availability of the mutagens, effectiveness, cost among other factors.

2.11 Ethyl methane sulfonate (EMS) induced mutation in sunflower

Among chemical mutagens, EMS with the chemical formula $C_3 H_8 SO_3$ is the most common mutagen used to create mutations in plants (Arisha *et al.*, 2014). EMS produces random mutations in genetic materials by nucleotide substitution with base pair transition (G: C to A: T), thereby producing only point mutations (Kumar *et al.*, 2013). Mutation breeding using EMS is effective in generating much desired variation for certain traits where the genetic variation was deficient (Singh and Kole, 2005). Sunflower mutants with altered agronomic traits that have been created through EMS - induced mutations include early maturing, short stature, larger head diameter, thinner husk, high oil content, branching mutants (Cvejic *et al*., 2011; Sabetta *et al*., 2011; Cvejic *et al*., 2015), high palmitic acid mutants (Velasco *et al*., 2008), cytoplasmic male sterile (CMS) lines that are resistance to biotic and abiotic stresses and many more (Mostafa, 2011).

2.12 Optimal dosage for mutagenic treatment in crops

The effectiveness and efficiency of any induced mutagenesis experiment are affected by the choice of appropriate mutagen dosage that will yield a reasonable number of desired mutations for traits of interest while inflicting the least unintended disruption to the genotypic integrity of the crop (Mba *et al.*, 2010). A mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality may decrease its efficiency (Shagufta *et al.*, 2013). It is therefore, a standard practice to precede any bulk mutagen treatment with a preliminary determination of the dosage that would yield the greatest amount of desirable mutation per unit dose, while at the same time producing the least density of undesirable effects. Jain (2010) reported that assessment of the dosage of chemical mutagens could be determined by varying EMS concentrations and exposure durations.

A determination of the mutagen dose that results in a 50% reduction in seed germination, known as LD_{50} has been used to predict accurately the most effective and efficient mutagen dose (Arisha *et al.,* 2014). However, it has been observed that doses inducing 30 to 50% lethality (LD_{30} - LD_{50}) among the M_1 plants will result in high desirable mutation rate in the M_1 generation (Devi and Selvakumar, 2013). Therefore, in practice, these lethal doses are used in induced mutagenesis for generating mutant populations for crop improvement. Determination of these parameters is fairly straight forward and involves comparison between the untreated (control) samples with the treated samples.

2.14 Genetic variability in sunflower

The development of an effective plant breeding program is dependent upon the existence of genetic variability for effective selection. An insight into the magnitude of variability present in the gene pool of a crop species is of utmost importance to a plant breeder for starting a judicious plant breeding program (Safavi *et al*., 2015). Also, it is necessary to partition the observed phenotypic variability into heritable and non-heritable components with suitable parameters such as phenotypic and genotypic co-efficients of variation. The knowledge of these parameters is essential for a breeder in selecting best mutants for further improvement (Ajayi *et al*., 2014). An investigation carried out by Nakitar *et al*. (2013) to elicit information on genetic variability for yield and its component traits in 34 mutants of sunflower showed wide ranges of variability with moderate GCV and PCV estimates for most of the traits. The induced mutation study of Cjevic *et al*. (2015) using eight sunflower inbred lines exhibited high variability amongst the mutant progenies for studied traits allowing selection of some valuable mutants for further evaluation. Mutant lines developed by Encheva *et al*. (2008), Kumar *et al*. (2013) and Sabetta *et al*. (2011) also showed wide range of variability for studied agronomic traits. The variability studies carried

out by Jagadeesan *et al.* (2008) in M_1 and M_2 generations of sunflower showed significant variation for plant height, oil content and seed yield per plant. Genetic variability assessment of 174 accessions of sunflower showed significant variation for studied agronomic traits (Naleema *et al*., 2016).

2.16 Correlation study in sunflower

Correlation is the mutual relationship between various plant traits. Correlation studies determine how far two variables are associated with each other (Jubair, 2011). Seed yield and oil content are the two most important economic traits in sunflower. However, they are under a strong influence of environmental factors (Hladni *et al.,* 2010). That is why breeding for these complex traits demands knowledge of their relationship with other agronomic traits to determine the component traits on which indirect selection can be based for genetic improvement (Hladni *et al.,* 2010). Studies on sunflower correlation analysis showed that grain yield per plant was significantly and positively correlated with plant height, stem diameter, capitulum circumference and 100-seed weight (Tyagi, 2011; Sowmya *et al.,* 2010; Hladni *et al.,* 2010). Grain yield was negatively correlated with days to flowering and days to maturity (Memon *et al.*, 2014). Some studies have also shown positive relationships between oil content and 100-seed weight in sunflower (Anandhan *et al*., 2010; Ghaffari *et al*., 2011). However, negative and significant correlations has been reported between oil content and stem diameter, capitulum circumference, plant height and grain yield per plant (Hladni *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The first stage of this research was carried out in the Biotechnology Laboratory of the Department of Plant Science, while the second stage was carried out at the Institute for Agricultural Research (IAR) Farm Samaru, Zaria during the raining and dry seasons of 2016. Zaria (Latitude $11^0$11'N and $7^0$38'E, altitude 680 m above sea level) is located in the northern guinea savannah agro-ecological zone of Nigeria. It has a mean annual rainfall of about 1,045 mm.

3.2 Genetic Materials

Two open pollinated sunflower varieties; SAMSUN 2 and SAMSUN 4, obtained from the sunflower improvement unit of the IAR, Samaru, Zaria were used for this study. A brief description of the varieties used in this study is presented in Table 3.1.

3.3 Laboratory experiment

3.3.1 EMS Mutagenesis

One thousand seeds per variety were pre-soaked in distilled water for 6 hrs to enhance the rate of uptake and the distribution of the mutagen in the target tissue. The seeds were subsequently treated with five different concentrations of EMS (v/v) at room temperature (23^0C) with gentle orbital shaking. The treated seeds were then washed with distilled water three times for five minutes and for the fourth time for 30 min (Kumar *et al*., 2013). The resulting EMS treated seeds are referred to as M_1 seeds.

Low concentrations (0.1, 0.2, and 0.3%) and higher concentrations (1.0 and 1.5%) with four different exposure times (2, 4, 6 and 8 hrs) were used, giving a total of 40 treatments for the two varieties. One thousand seeds per variety were pre-soaked in water for 6 hrs to enhance the rate of uptake and the distribution of the mutagen in the target tissue. These seeds were subsequently divided into 40 bottles which comprised of 50 seeds each. They were then treated with the above EMS concentration and exposure times at room temperature (23^0C) with gentle orbital shaking. Afterwards, the treated seeds were washed three times for 5 min and for the fourth time for 30 min according to Kumar *et al*. (2013). The resultant EMS treated seeds are referred to as M_1 seeds.

Table 3.1: Description of the two sunflower varieties

Genotypes	**Origin**	**Description**
SAMSUN 2	Canada	Medium maturing (about 80 days), medium size seed and tolerant to moisture stress.
SAMSUN 4	Nigeria	Early to medium maturing (about 72 days), medium size seed, moderately tolerant to moisture stress.

3.3.2 Determination of optimum mutagen dose

To determine the optimum dose of the EMS mutagens for the creation of desirable mutation and variability of the sunflower varieties, the treated seeds and control (untreated seeds) from each variety were placed on wet Whatman paper in Petri-dishes and transferred to a growth chamber at 12 hrs photoperiod. A total of 40 treatments with two checks were laid out in 2 x 4 x 5 factorial experiment in a randomized complete block design with two replications. The seeds were watered daily until germination and data were collected on the number of seed germinated four days afterwards. Subsequently, germination and lethality percentages were computed according to (Wang *et al.*, 2014) as follows:

Germination percentage *= [Total no of seeds germinated/Total no of seeds planted]* 100*

Lethality (%) *= (1 − Germination percentage of treated seeds/Germination percentage of control) * 100*

3.4 Field experiment

3.4.1 EMS treatment

One hundred and fifty (150) dry sunflower seeds from each variety were presoaked in water for 6 hrs to enhance the rate of uptake and the distribution of the mutagen in the target tissue. The presoaked seeds were subsequently treated with the selected EMS concentrations of 0.2 and 0.3% (v/v) in water for 4 and 6 hrs at room temperature with gentle orbital shaking. The treated seeds were then washed three times for five minutes and for the fourth time for 30 mins (Kumar *et al*., 2013). The treated seeds known as the M_1 seeds were air dried for an hr before sowing.

3.4.2 M_1 plant population

Land preparation was done by ploughing and harrowing after which the M_1 seeds (treated seeds) were sown in the field with a spacing of 0.70 x 0.25 m. Each treatment occupied 5 rows of 5 m

long with 21 plants in a row giving a total of 105 plants in a plot. The field was sprayed with Galex 500EC herbicides at the rate of 1.0 kg ha^{-1} after sowing to suppress the growth of weeds. Manual weeding was also carried out at three and six weeks after sowing. Application of NPK 15-15-15 fertilizer at the rate of 30 kg ha^{-1} was done three weeks after sowing. Application of 30 g Cypermethrin + 250 g Dimethoate/litre pesticide at the rate of 0.2 kg ha^{-1} was carried out for pest control. To allow self-pollination, sunflower capitula were bagged just before anthesis. Of 840 (for the eight treatments) expected plants, a total of 309 surviving M_1 plants at harvest were observed out of which, 78 M_1 plants with substantial seed set were harvested individually as M_2 seeds.

3.4.3 M_2 plant population and evaluation

Seeds obtained from the 78 M_1 plants were advanced to raise the M_2 plant population as progeny rows on the field under irrigation in September, 2016. The progenies and two checks (SAMSUN2 and SAMSUN4) were evaluated using 16 x 5 lattices design with two replications. Each of the mutants was sown in a row length of 2 m at spacing of 0.75 x 0.50 m. Three seeds were sown per hill and emerged seedlings were thinned to one plant per stand 2 weeks after emergence to achieve five plant stands per row. All agronomic practices were carried out as earlier described in 3.4.2. To allow self-pollination, sunflower capitula were also bagged just before anthesis. A total of 418 surviving plants at harvest were observed out of the expected 800 plants for the two replications.

3.5 Data collected

Data were recorded on individual plant basis using procedure prescribed by the sunflower improvement unit of IAR, Samaru, Zaria. Plant height (cm) at maturity was measured as the distance between the soil surface and stem attachment to the capitulum using a meter rule.

Capitulum circumference (cm) was obtained by measuring each of the plant capitulum with a meter rule. Capitulum weight (kg) was obtained by weighing each of the capitulum using a weighing scale. Number of leaves per plant was determined by counting the number of leaves per plant at maturity. Stem diameter (cm) was determined using a vernier caliper at the middle of the plant. Number of days to flowering was recorded as the number of days from emergence to flower bloom. Days to maturity was recorded as the number of days from planting to maturity (when the back of the heads had turned yellow and the outer bracts, brown). The grain yield (grams) per plant was obtained by weighing threshed seeds from individual plants. Weight of 100 seed (grams) was derived by weighing 100 seeds counted from each capitulum. Oil content was determined at the Product and Development Research Unit of the Institute of Agricultural Research, Samaru, using Soxhlet method of oil extraction. A gram of pulverized sunflower seed from each of the 60 selected M_2 mutants were weighed into a filter paper folded and assembled into the Soxhlet apparatus. Therein, sunflower meal was extracted with N-hexane for 8 hrs. Thereafter, the sunflower meal was transferred into an oven to dry. The difference between the powder weight before and after extraction was expressed in terms of oil percentage as follows:

$$\frac{W3-W1}{W2-W1}\times 100$$ Where;

W_1 = Weight of the empty filter paper

W_2 = Weight of the filter paper with sample before extraction

W_3 = Weight of the filter paper with the sample after extraction and drying

3.6 Data Analysis

3.6.1 Analysis of variance

Data collected on the different traits were subjected to analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of the Statistical Analysis System (SAS) software, version 9.2 to ascertain the existence of variability amongst the mutants. The mutants were then compared using the Least Significant Differences values (LSD) at 5% probability according to Gomez and Gomez (1984). The linear model for the Alpha Lattice design used and the form of Analysis of variance with expected mean squares is shown below.

$Y_{ijk} = \mu + m_i + \gamma_j + \rho_{i(j)} + \ell_{ijk}$

Y_{ijk} = Observation (k = 1, 2.….r : i = j = 1, 2 ..... n)

μ = Mean

τ_i = Mutant effect i = 1, 2.....m

γ_j = Replication effect j = 1, 2.....r

$\rho_{i(j)}$ = Block within replication effect k = 1, 2.....b

$\ell_{i\,jk}$ = Residual error

3.6.2 Genotypic and phenotypic coefficients of variation

Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were estimated from the analysis of variance using the formular described by Burton and Devane (1953).

GCV = $\frac{\sqrt{\sigma_g^2}}{\overline{X}} \times 100$ PCV = $\frac{\sqrt{\sigma_{ph}^2}}{\overline{X}} \times 100$

Table 3.2: Form of analysis of variance and Expected Mean Squares for Alpha lattice design

Source of variation	Df	MS	EMS
Replication	$(r-1)$		
Block(Replication)	$b(r-1)$		
Genotypes	$(g-1)$	MS_g	$\sigma_e^2 + r\sigma_g^2$
Error	$g-(rb-1)$	MS_e	σ_e^2

Where, Df = Degree of freedom; MS = Mean square; EMS = Expected mean square; r = replication; g = genotype; b = block; σ_g^2 = genotypic variance; σ_e^2 = error variance

Where $\overline{X}$ = Grand mean, σ^2_{ph} = Phenotypic variance, σ^2_g = genotypic variance

PCV and GCV values were categorized as low (0 - 10%), moderate (10 -20%) and high (>20%) (Sivasubramanian and Menan, 1973).

3.6.3 Correlation Coefficients

Correlation coefficients of the traits were estimated using Statistical Analysis System (SAS) software, version 9.2 in order to determine the association between the studied traits.

3.6.4 Cluster analysis

Hierarchical cluster analysis based on the Unweighted Pair Group Method Using Arithmetic Average (UPGMA) was carried out to group selected mutants based on trait similarity using the PAST software, version 2.12 and Euclidean distance was used as a measure of distance for cluster formation.

CHAPTER FOUR

4.0 RESULTS

4.1 Response of sunflower varieties to different EMS doses and optimal mutagen dose determination

The responses of the two sunflower varieties to different EMS doses showed a general decrease in seed germination percentage as EMS concentrations and exposure durations were increased. It was observed that, seeds treated with 0.1 and 0.2% EMS concentrations showed minimal lethality, while those treated with 0.3, 1.0% and 1.5% EMS concentrations at all exposure durations showed drastic to complete plant lethality. Optimal doses were observed when the two sunflower varieties were treated with 0.1% EMS for 6 hrs, 0.2% EMS for 4 hrs and 0.2% EMS for 6 hrs, 0.3% EMS for 4 hrs and 0.3% EMS for 6 hrs (Table 4.1)

Table 4.1: Germination percentages (GP) and Lethality (%) of two sunflower varieties treated with five EMS concentrations for four exposure durations at the Biotechnology laboratory of the Department of Plant Science, IAR, Zaria in 2016

Concentration	Time (hr)	SAMSUN 2		SAMSUN 4	
		GP	Lethality (%)	GP	Lethality (%)
Control	0	78	0	80	0
0.1	2	82	5	60	25
	4	78	0	58	28
	6	40	49	40	50
	8	16	80	38	52
0.2	2	70	10	60	25
	4	50	36	52	35
	6	52	33	40	50
	8	22	72	20	75
0.3	2	30	62	30	60
	4	28	64	26	68
	6	26	66	24	66
	8	8	90	10	88
1.0	2	2	97	4	95
	4	2	97	0	100
	6	0	100	0	100
	8	0	100	0	100
1.5	2	0	100	0	100
	4	0	100	0	100
	6	0	100	0	100
	8	0	100	0	100

4.2 Variability in agronomic traits of M_2 progenies derived from two sunflower varieties

The analysis of variance for agronomic traits amongst 239 M_2 progenies from the two varietes and two checks are presented in Table 4.2. The results revealed significant ($P<0.05$) differences amongst the mutants for number of leaves, stem diameter, capitulum weight, 100-seed weight, and grain yield per plant while highly significant ($P<0.01$) differences were also observed for plant height, days to flowering, days to maturity and capitulum circumference (Table 4.2)

Mean performance for the studied agronomic traits is presented in Table 4.3. Stem diameter had a mean of 2 cm and ranged between 1cm and 4cm in T2M14-4 and T3M31-1 which were derived from SAMSUN 2, exposed to 0.2% EMS for 6 hrs and 0.3% EMS for 4 hrs, respectively. These mutants showed approximately 300% increase over SAMSUN 2. Plant height had a mean of 160 cm and ranged between 96 cm in T2M31-4 and 229 cm in T1M44-4. Mutant T2M31-4 and T1M44-4 derived from SAMSUN 2, exposed to 0.2% EMS for 6 hrs and 4 hrs, respectively, showed approximately 53% decrease and 7% increase in plant height over SAMSUN 2.

Days to flowering recorded a mean of 67 days and ranged between 56 days in T2M31-1, T5M8-1, and T5M8-5 and 84 days in T5M15-4. Mutants T2M31-1, T5M8-1 and T5M8-5 showed approximately 12.5% decrease over SAMSUN 2. Days to maturity had a mean of 96 days and ranged between 86 days in T2M31-1 and 113 days in T5M15-4. Mutant T2M31-1 derived from SAMSUN 2, exposed to 0.2% EMS for 6 hrs showed approximately 9% decrease over SAMSUN 2. Capitulum circumference had a mean of 20 cm and varied from 10 cm in T4M36-2 to 34 cm in T8M8-4. Mutant T8M8-4 derived from SAMSUN 4, exposed to 0.3% EMS for 6 hrs showed an increment of 62% over SAMSUN 2. Weight of 100 - seeds exhibited a mean of 8 g and ranged between 4 and 14 g was recorded in mutants T3M36-1, T3M31-1 and T8M11-4. These mutants were derived from SAMSUN 2, exposed to 0.3% EMS for 4 hrs. They showed an

increment of approximately 180% over the checks. Grain yield per plant showed a mean of 12 g and ranged from 7 g in T1M32-3, T5M40-3, and T8M21-3 to 23g in T1M1-3. Mutant T1M1-3 derived from SAMSUN 2, exposed to 0.2% EMS for 4 hrs showed approximately 109% increase over SAMSUN 2 (Table 4.3)

Table 4.2: Analysis of variance for agronomic traits of M_2 progenies and two check varieties of sunflower evaluated at Zaria in 2016

Source of variation	Df	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
Replication	1	56.0*	7.6**	10441.5**	36.1	39.6*	6.5	0.1	19.6*	14.8**
Block(rep)	8	7.0	0.9**	1029.6**	14.4	12.8	27.5*	0.3	7.4*	275.4**
Mutants	238	13.0*	0.4*	566.1**	32.4**	34.6**	21.9**	0.3*	5.1*	55.7*
Error	91	9.0	0.3	317	9.7	9.4	13.1	0.2	3.6	14.1

** $P < 0.01$; * $P < 0.05$

Table 4.3: Mean performance of agronomic traits of M_2 progenies of sunflower evaluated at Zaria in 2016

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
T1M1-3	30	3	184	69	98	25	0.7	12	23
T8M8-4	32	2	175	71	101	34	0.9	13	22
T8M29-2	28	3	159	64	94	21	0.8	9	21
T5M24-1	23	2	137	58	88	20	0.4	8	20
T7M46-1	44	3	219	70	99	28	0.7	11	20
T5M21-3	30	3	166	63	93	22	1	12	19
T1M15-2	38	3	164	67	97	26	1.2	10	19
T7M27-1	28	3	159	69	99	29	0.8	8	19
T7M5-1	30	3	166	69	99	25	0.7	12	19
T5M8-1	27	2	132	56	88	23	0.6	8	18
T5M24-3	26	2	149	58	88	22	0.6	10	18
T5M24-2	25	2	152	58	88	22	0.2	11	18
T3M36-1	32	3	161	59	89	31	2.2	14	18
T6M37-1	26	2	135	60	90	18	0.3	7	18
T3M31-1	30	4	166	64	94	31	2.5	14	18
T8M11-1	32	2	171	65	95	24	0.8	10	18
T8M11-4	30	3	179	65	94	26	1.1	14	18
T8M10-3	27	2	133	66	96	14	0.1	8	18
T5M42-1	29	1	144	68	96	16	0.3	8	18
SAMSUN2	29	1	213	64	94	21	0.1	5	11
SAMSUN4	28	1	208	63	92	20	0.1	5	10
Mean	**29**	**2**	**160**	**67**	**96**	**20**	**1.0**	**8**	**12**
LSD (0.05)	**4.4**	**0.7**	**25.2**	**4.4**	**4.3**	**5.1**	**0.7**	**2.7**	**5.3**
CV (%)	**11**	**20**	**11**	**5**	**3**	**18**	**86**	**22**	**33**

4.4. Estimates of Phenotypic and Genotypic Coefficients of Variation

The estimates of phenotypic coefficients of variation (PCV) and genotypic coefficient of variation (GCV) of the studied traits are presented in Table 4.4. PCV values were higher than those of the GCV for all the traits. GCV ranged between 3.5% for days to maturity and 31.6% for capitulum weight. On the other hand, PCV ranged between 5% for days to maturity and 54.8% for capitulum weight. The difference between PCV and GCV estimates was high for all the traits except for days to flowering, days to maturity and grain yield per plant (Table 4.4).

4.5. Variations in oil content and other agronomic traits amongst 60 selected M_2 progenies

The percentage oil content and other agronomic traits of 60 selected M_2 mutants are presented in Table 4.5. Mutant T1M15-2-1 had the highest number of leaves (39); T1M47-1-2, T2M6-1-1, T3M31-1-1 and T4M18-3-1 had the highest stem diameter (4 cm); T1M12-1-1 and T4M18-3-1 showed the highest plant height (210 cm), while T2M31-4-1 and T8M23-1-1 flowered earlier (57 days). Mutant T2M6-1-1 had the highest capitulum circumference (38 cm); T7M5-3-2, T8M12-2-1, T8M12-3-1, T8M8-4-2, T8M11-4-1 and T8M8-2-1 showed the highest 100 seed weight (13 g); T8M23-1-2 showed the highest grain yield per plant (26 g), while mutant T8M11-1-2 showed the highest oil content of 45% (Table 4.5).

Table 4.4: Genetic parameters of some important traits of M_2 mutant progenies of sunflower varieties evaluated at Zaria in 2016

Traits	Error variance	Genotypic variance	Phenotypic variance	Genotypic coefficient of variation (%)	Phenotypic coefficient of variation (%)
No of leaves	9.0	2.0	11.0	4.9	11.4
Stem diameter (cm)	0.3	0.1	0.4	11.2	29.6
Plant height (cm)	317	124.5	441.6	6.9	13.1
Days to flowering	9.7	11.4	21.1	5.0	6.9
Days to maturity	9.4	11.5	20.9	3.5	4.8
Capitulum circumference(cm)	13.1	4.4	17.5	10.5	20.9
Capitulum weight (kg)	0.2	0.1	0.3	31.6	54.8
Grain weight (g)	3.6	1.6	5.1	15.3	28.2
Grain yield per plant (g)	14.1	9.8	23.9	38.0	40.7

Table 4.5: Agronomic traits of 60 promising M_2 mutant progenies of sunflower varieties evaluated at Zaria in 2016

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)	Oil content (%)
T1M1-1-1	29	2	180	67	97	26	1.0	10	16	43
T1M1-1-2	29	3	195	65	95	28	1.2	10	15	37
T1M12-1-1	36	4	210	69	99	24	0.4	11	15	35
T1M12-2-2	29	3	195	69	99	22	0.5	12	10	37
T1M1-2-2	30	3	161	65	95	28	0.7	10	16	40
T1M1-3-1	30	3	185	67	97	22	0.5	10	13	39
T1M15-2-1	39	3	175	67	97	27	1.3	10	16	37
T1M47-1-2	32	4	170	69	99	30	1.2	13	20	39
T2M29-1-2	34	3	205	64	94	25	1.1	11	20	35
T2M31-4-1	27	2	100	57	87	20	0.1	10	12	40
T2M6-1-1	32	4	200	67	97	38	3.5	13	14	40
T2M6-1-2	26	2	175	70	100	19	0.2	13	18	36
T2M6-2-2	26	2	175	70	100	25	0.9	10	14	35
T3M14-1-1	33	3	150	71	101	25	2.5	12	16	35
T3M14-3-1	28	3	140	71	101	22	1.7	10	17	27
T3M29-2-2	26	3	120	69	99	23	0.8	11	18	42
T3M31-1-1	30	4	170	66	96	30	2.3	13	15	35
T3M34-1-2	24	2	160	62	92	26	1.0	11	15	39
T3M34-2-1	29	3	170	63	93	29	2.6	11	13	40
T3M36-1-1	32	3	165	61	91	30	2.0	12	15	42
T3M36-2-1	31	3	160	61	91	22	0.7	10	13	35
T4M11-4-1	22	2	110	64	94	23	0.3	10	12	26
T4M1-1-2	18	1	130	72	102	23	0.1	10	15	32
T4M18-3-1	33	4	210	67	97	29	2.0	10	13	39
CHECK 2	29	1	213	64	94	21	0.1	5	11	29
T5M15-3-2	27	2	130	82	112	22	0.9	10	15	35
T5M21-1-2	30	3	135	63	93	24	1.0	10	17	37
T5M21-3-1	30	3	170	65	95	21	0.8	10	16	32
T5M23-1-2	27	3	180	64	94	27	0.6	12	13	43
T5M24-2-1	22	2	140	58	88	23	0.7	10	15	36
T5M24-2-2	28	3	165	58	88	22	0.1	12	25	36

Table 4.5: Continued

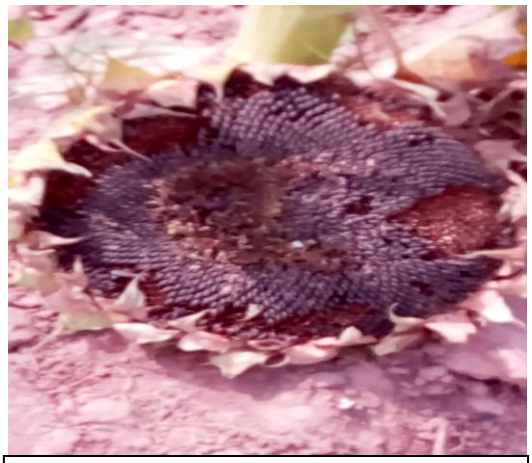
Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumferenc (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)	Oil content (%)
T5M24-3-2	25	2	140	61	91	22	0.8	10	19	41
T5M34-1-2	26	2	120	62	92	19	0.6	12	19	27
T5M42-2-1	30	2	155	67	96	18	0.3	10	14	43
T5M47-3-2	27	2	130	65	95	23	1.5	10	13	37
T6M28-2-2	27	2	140	67	97	19	0.5	10	16	44
T6M3-2-1	34	3	145	66	96	21	0.7	10	10	34
T6M37-3-1	29	2	170	61	91	21	0.5	10	11	30
T7M18-1-2	30	3	175	69	99	20	0.7	11	13	33
T7M18-2-2	33	3	160	69	99	30	1.0	10	11	35
T7M23-1-2	31	2	135	69	99	20	0.5	11	16	39
T7M31-1-1	31	3	185	65	95	21	1.0	10	8	23
T7M31-3-1	33	3	185	66	96	24	1.2	10	14	26
T7M35-1-2	30	3	170	68	98	23	0.5	10	15	30
T7M35-2-1	37	3	195	73	103	20	0.9	10	12	31
T7M46-3-1	35	3	170	69	100	21	0.9	10	12	39
T7M47-1-2	31	2	135	63	93	20	0.6	10	15	35
T7M5-1-1	30	3	170	71	101	24	0.5	10	16	35
T7M5-3-2	32	3	190	66	96	26	1.0	13	12	38
T8M11-1-2	33	2	170	68	98	26	1.1	10	21	45
T8M11-4-1	30	3	180	63	93	23	0.9	13	14	40
T8M12-2-1	30	2	165	69	99	18	0.4	13	14	40
T8M12-3-1	25	2	170	70	100	19	0.1	13	14	39
T8M21-1-2	30	3	155	68	98	28	1.0	11	20	39
T8M23-1-1	28	3	150	57	87	20	0.4	10	12	35
T8M23-1-2	28	3	150	58	88	20	0.4	10	26	36
T8M26-1-2	33	2	160	73	103	23	1.0	12	23	34
T8M26-2-2	30	2	145	73	103	21	0.5	10	11	32
T8M5-2-2	33	3	170	73	103	28	1.2	11	14	41
T8M8-2-1	36	3	190	68	98	30	0.8	13	20	38
T8M8-4-2	32	2	180	70	100	32	0.8	13	23	40
CHECK4	27	1	208	63	92	20	0.1	5	8	36



SAMSUN 2 (CONTROL)



MUTANT T4M-18-3-1



SAMSUN 4 (CONTROL)



MUTANT T2M6-1-1



SAMSUN 2 AND MUTANT T8M12-2-1



SAMSUN 4 AND MUTANT T2M6-1-1

Plate 4.1: Morphological variations of some sunflower mutants

4.6 Association among oil content, seed yield per plant and other agronomic traits

The pearson correlation matrix amongst the studied agronomic traits of sunflower is presented in Table 4.6. Positive and significant ($P<0.05$) correlation was observed between grain yield per plant and number of leaves ($r = 0.76$); stem diameter ($r = 0.61$); plant height ($r = 0.68$); days to flowering ($r = 0.77$); days to maturity ($r = 0.77$); capitulum circumference ($r = 0.72$) and 100 seed weight ($r = 0.81$), while correlation of grain yield per plant with capitulum weight ($r = 0.27$) and oil content ($r = 0.16$) was positive but non-significant. There was no significant correlation between oil content and any of the studied agronomic traits (Table 4.6)

4.7 Divergence of the 60 selected M_2 mutants

Hierarchical dendogram obtained from Euclidean distance using clustering method of UPGMA presented in Fig.1 showed that the mutants were distributed into four major clusters when the line of similarity coefficient was set at the 25% similarity level. Clusters I, II, III and IV consisted of 19, 4, 26 and 11 mutants, respectively. Cluster I majorly consisted of mutants of average height ranging between 130 and 160 cm and mutants having high means for grain yield per plant (16 g). Cluster II consisted of mutants with lower plant height ranging between 100 and 120 cm and mutants having low mean for days to flowering (64 days) and high mean for 100 seed weight (11g). Cluster III consisted of mutants with high mean for stem diameter (3 cm), 100 seed weight (11g) and oil content (37%). Cluster IV consisted of tall mutants with plant height ranging between 185 and 210cm having high mean for stem diameter (3 g) and capitulum circumference (23 cm).

Table 4.6: Correlation coefficients of agronomic traits among M_2 progenies of two sunflower varieties evaluated at Zaria in 2016

Traits	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant (g)	Oil content (%)
No of leaves	1	0.85**	0.95**	0.93**	0.95**	0.91**	0.44	0.90**	0.76**	-0.04
Stem diameter(cm)		1	0.82**	0.77**	0.78**	0.85**	0.57**	0.82**	0.61**	-0.22
Plant height(cm)			1	0.91**	0.91**	0.91**	0.40	0.85**	0.68**	-0.04
Days to flowering				1	0.99**	0.89**	0.37	0.92**	0.77**	0.13
Days to maturity					1	0.89**	0.37	0.93**	0.77**	0.13
Capitulum circumference(cm)						1	0.61**	0.89**	0.72**	0.03
Capitulum weight(kg)							1	0.43	0.27	-0.21
100 Seed weight(g)								1	0.81**	0.18
Grain yield per plant(g)									1	0.16
Oil content (%)										1

**Significant at 1% level, * Significant at 5% level of probability

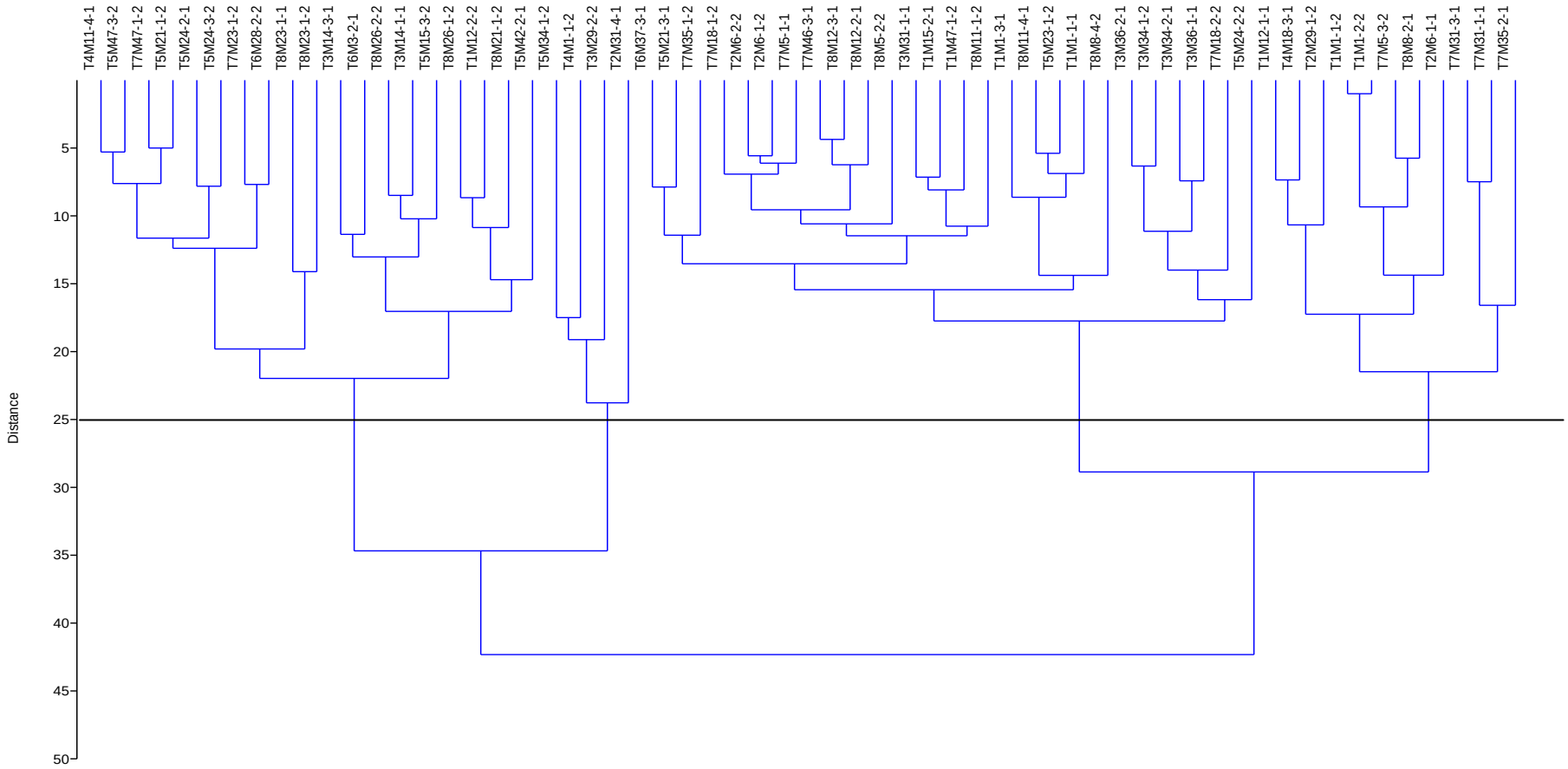


Figure 1.1: Dendrogram of 60 M_2 mutants of sunflower varieties as derived by UPGMA cluster from the Euclidian distance matrix.

CHAPTER FIVE

5.0 DISCUSSION

The availability of genetic variation is a prerequisite for genetic improvement of crops. Where sufficient variation does not exist naturally as in the case of sunflower, it can be created through targeted processes like induced mutation. The effectiveness of mutagenesis depends on appropriate mutagen dose (optimal dose) that will yield a reasonable number of desired mutations for traits of interest, while inflicting the least density of undesirable effect. In this study, the effect of EMS treatments on seed germination was assessed to determine the optimal dose of EMS that will produce a maximum mutation density without causing extensive lethality. The low germination percentages observed in the present study could be due to the toxic nature of the mutagen which could affect the physiological and biological processes that regulates seedling growth, causing the embryos to become weak or die (Devi and Mullainathan, 2012). Chen *et al*. (2012) and Kumar *et al.* (2013) also reported a decrease in seed germination with an increase in EMS concentration in wheat and sunflower, respectively.

The significant variation among the mutants for all the traits may allow significant progress from selection of promising mutant progenies for improvement. Similar results of significant variations for agronomic traits were observed in induced mutation study of Encheva *et al*. (2008) and Cvejic (2015) in sunflower, Ahmed (2016) in groundnut and Ndou (2012) in wheat. Reduced plant height observed in the mutant population as compared with the checks may be attributed to inhibition of auxin synthesis by the chemical mutagen as earlier reported by Nair and Mehta (2014). Reduced plant height and increased stem diameter can be used in sunflower breeding to ameliorate problems associated with lack of stand-ability of existing tall varieties thereby,

reducing both stem and root lodging. Encheva *et al*. (2008) and Cvejic *et al*. (2015) also reported mutants with reduced height from induced mutation in sunflower.

Development of early maturing genotypes depends on the reduction in days to flowering and it enables sunflower genotypes avoid biotic (diseases and insects) and abiotic stresses (heat and drought) at the ripening stage (Chatterjee *et al*., 2012). The delayed maturity observed in the mutant population as compared with the controls could have been attributed to a delay in cell division due to the effects of the chemical mutagen. Early and late maturing mutants were also derived from the induced mutation study of Cvejic *et al*. (2015) in sunflower.

EMS used in this study also had significant effect on 100 seed weight as approximately 15% of the mutant population weighed over twice the weight of the checks. Grain yield per plant of the mutant progenies was generally low as there was poor seed set which could be attributed to the effect of the mutagen used or the imposed self-pollination on the sunflower mutants. In addition, the increase in capitulum circumference in the mutant population implies that, more number of seeds could be obtained leading to a high grain yield. Similar outcomes of significant increase in 100-seed weight, capitulum circumference and grain yield per plant over the checks were also recorded in the induced mutation study of Nakitar *et al*. (2013) in sunflower.

The phenotypic coefficient of variation (PCV) was generally higher than the genotypic coefficient of variation (GCV). However, the high difference between the PCV and GCV estimates for number of leaves, stem diameter, plant height, capitulum circumference, capitulum weight and 100 seed weight indicates more influence of the environment in the expression of these traits than genetic. Whereas, the slight difference observed between the estimates of the

two parameters for days to flowering, days to maturity and grain yield per plant suggests low environmental influence the expression of these traits.

Oil yield is an important objective in sunflower breeding program since it is mainly cultivated for oil extraction. In this study, oil content showed considerable variation amongst the 60 selected mutants with over 50% of them having higher oil content than the checks. The increment observed could be due to the effect of the EMS. Similar outcome of increased oil content was reported in the induced mutation study of Cvejic *et al*. (2015) in sunflower, Begum and Dasgupta (2015) in sesame and Pavadai *et al*. (2010) in soya bean.

Grain yield and oil content are often affected by many other traits. Therefore, it crucial to determine their relationship with other agronomic traits so as to determine the component traits on which indirect selection can be based for genetic improvement (Jockovic *et al.*, 2012). In the present study, the positive and significant correlation observed between grain yield per plant and all the studied traits except capitulum weight and oil content indicates that as the levels of these traits increased, grain yield per plant also increased. This implies that selection for these traits could result in high grain yield per plant. This result is in conformity with the trait association analysis study of Memon *et al.* (2014) and Jockovic *et al*. (2012). The non-significant correlation observed between percentage oil content and all the studied traits implies that selection of these traits may not contribute to the improvement of oil content (%) in sunflower. This result is in disagreement with the findings of Anandhan *et al*. (2010) and Ghaffari *et al*. (2011) who reported significant relationship between oil content and 100-seed weight.

Cluster analysis was used to group mutants based on similarity for agronomic traits. In this present study, the cluster analysis grouped the 60 mutants into four clusters indicating that the

mutants were moderately divergent. Based on this result, mutants in Cluster I can be selected for reduced days to flowering, high oil content and seed yield. Mutants in Cluster III can be selected for high oil content and seed yield per plant. Also, mutants included in Cluster II can be selected for reduced plant height and days to flowering. Mutants included in Cluster IV can be selected for high stem diameter, head diameter and 100 seed weight. This implies that, crossing of mutants from these different clusters could create more variability for desirable traits for improvement.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

Sunflower is one of the most important oilseed crops because of its nutritional composition and health related value. However, its improvement is impeded by its extreme narrow genetic base. Mutation induction is a workable, efficient and unregulated technology in the breeder's toolbox for creating variability for sunflower improvement. In view of these, this research was conducted to (i) assess the response of the sunflower seed to different EMS doses and determine optimal doses for successful mutation. (ii) estimate genetic variability for agronomic traits among the mutant lines and identify some desirable mutants for oil content and some agronomic traits (iii) assess the degree of association among oil content, grain yield and some agronomic traits. (iv) assess the degree of similarity among the selected mutants for the studied agronomic traits

Two sunflower varieties (SAMSUN2 and SAMSUN4) were treated with five EMS concentrations at four different exposure durations. Thereafter, two EMS doses considered to be optimum were selected to generate 840 M_1 populations in the research field. A total of 78 surviving M_1 plants were sown in a progeny row. The mutant progenies and the checks were evaluated using 16 x 5 Alpha lattice design with two replications for agronomic traits such as number of leaves, stem diameter, plant height, days to flowering, days to maturity, head diameter, head weight, 100-seed weight, grain yield per plant. Afterwards, mutants with high seed yield per plant were selected for oil content analysis. The findings from this research are summarized as follows:

- EMS treatment of the two sunflower varieties showed decrease in seed germination as EMS concentration and exposure durations were increased suggesting that high EMS doses could be detrimental to seed germination.

- Significant ($P<0.05$) difference existed amongst the mutants progenies for all the studied traits indicating the presence of substantial variation amongst the mutant progenies which could be due to the enhancing effect of the EMS used.

- Positive and significant correlation existed between grain yield per plant and number of leave per plant, plant height, stem diameter, days to flowering, capitulum circumference and 100-seed weight indicating that as these traits increases, the grain yield per plant also increases.

- Sixty selected mutants were grouped into four clusters based on trait similarity with clusters I, II, III and IV consisting 19, 4, 26 and 11 mutants respectively.

6.2 CONCLUSIONS

EMS concentrations and exposure durations of 0.2% EMS for 4 hrs, 0.2% EMS for 6 hrs, 0.3% EMS for 4 hrs and 0.3% EMS for 6 hrs were most effective for induction of desirable mutation for the two sunflower varieties. The genetic structure of the parental materials were highly affected by the chemical mutagen as over 70% of the mutants exhibited superiority for oil content and other agronomic traits over the parent varieties. Mutants T8M11-1-2, T4M18-3-1, T2M6-1-1, T8M11-4-1 and T8M12-2-1 have been identified as superior for oil content and other agronomic traits. Plant height, stem diameter, capitulum circumference and 100 seed weight

could be selected indirectly for grain yield. Crossing of mutants from the four different clusters generated could create more variability in sunflower.

6.3 RECOMMENDATIONS

- Selection for oil content and agronomic traits in the early mutant generation may be difficult. It is therefore recommended that, mutants T8M11-1-2, T4M18-3-1, T2M6-1-1, T8M11-4-1 and T8M12-2-1 be evaluated in subsequent generations for stability.

- The use of EMS concentrations and exposure durations of 0.2% EMS for 4 hrs, 0.2% EMS for 6 hrs, 0.3% EMS for 4 hrs and 0.3% EMS for 6 hrs could be effective for inducing desirable mutants with enhanced seed yield per plant (26 g), seed weight (13 g), capitulum circumference (38 cm) and oil content (45%) of sunflower.

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APPENDICES

Appendix 1: Mean performance of agronomic traits of M_2 progenies of sunflower evaluated at Zaria in 2016

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant (g)
T1M1-1	29	3	184	67	97	29	1.2	11	17
T1M1-2	28	3	179	67	97	26	0.7	9	14
T1M1-3	30	3	184	69	98	25	0.7	12	23
T1M12-1	32	3	191	68	98	20	0.3	8	12
T1M12-2	26	2	163	67	97	20	0.3	9	13
T1M12-3	29	2	176	69	99	20	0.4	5	15
T1M15-1	33	2	179	67	97	25	0.9	9	14
T1M15-2	38	3	164	67	97	26	1.2	10	19
T1M32-1	30	3	185	69	99	21	0.3	7	12
T1M32-2	29	2	164	70	100	15	0.9	4	10
T1M32-3	29	2	159	67	97	22	1.3	4	7
T1M32-5	30	2	179	70	100	18	1.2	4	12
T1M40-1	28	2	183	60	90	20	0.8	8	13
T1M40-2	30	2	168	61	91	13	1.0	7	10
T1M40-3	31	2	163	62	92	23	1.3	8	12
T1M44-1	34	3	204	73	102	21	0.6	5	12
T1M44-2	33	3	212	73	102	23	0.7	7	11
T1M44-3	33	3	207	73	102	20	0.5	7	14
T1M44-4	35	3	229	73	102	24	0.7	7	11
T1M44-5	33	3	219	73	102	23	0.5	9	16
T1M45-1	29	2	154	68	98	16	1.1	7	15
T1M45-2	24	2	159	68	98	20	1.5	8	13
T1M45-3	26	3	149	68	98	15	1.3	7	10
T1M47-1	34	3	180	68	98	27	0.9	9	14
T1M47-2	30	3	166	68	98	23	0.7	11	18
T1M47-3	31	3	176	69	99	23	0.3	9	14
T1M8-1	30	2	191	65	95	19	0.6	8	11
T1M8-2	33	2	176	63	93	20	0.7	9	13
T1M8-3	34	2	171	62	90	19	0.6	9	16
T2M14-1	31	3	179	66	96	21	1.3	4	9
T2M14-2	29	2	144	69	99	24	1.6	6	12
T2M14-3	26	1	109	63	93	17	1.0	5	13
T2M18-2	30	3	210	61	91	17	0.5	4	9
T2M2-1	30	2	189	69	98	23	0.9	10	12
T2M2-2	26	2	169	70	99	19	0.3	6	9
T2M2-3	28	3	129	68	97	25	0.8	6	10
T2M2-4	28	3	129	69	98	31	1.1	10	13

T1= SAMSUN2 exposed to 0.2% EMS for 4hrs, T2= SAMSUN2 exposed to 0.2% EMS for 6hrs.

Appendix 1: Continued

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
T2M31-1	34	3	136	56	86	23	0.7	8	15
T2M31-2	24	1	141	57	87	15	0.3	7	11
T2M31-3	21	2	136	57	87	16	0.3	8	11
T2M31-4	27	2	96	58	88	16	0.3	12	13
T2M47-1	26	2	168	68	98	21	1.8	7	11
T2M47-2	26	3	198	68	98	32	2.0	5	12
T2M47-3	24	3	173	68	98	29	2.3	5	9
T2M51-1	30	2	146	83	113	19	1.0	9	15
T2M6-1	29	3	179	69	99	29	1.8	13	17
T2M6-2	26	2	170	71	101	27	1.0	10	13
T3M12-1	35	3	182	68	98	24	0.9	5	10
T3M12-2	32	2	162	69	99	20	0.3	6	12
T3M14-1	32	3	139	72	102	24	2.3	12	13
T3M14-2	31	2	139	75	105	17	1.3	6	9
T3M14-3	27	2	129	72	102	21	1.5	10	14
T3M18-1	28	2	144	61	91	21	0.6	8	9
T3M18-2	30	2	149	61	91	19	0.3	6	11
T3M18-1	21	2	104	62	92	23	0.9	8	11
T3M18-2	29	3	144	62	92	27	1.4	9	14
T3M19-1	28	2	158	66	96	23	0.8	4	9
T3M28-1	33	3	164	66	95	27	1.2	11	10
T3M28-2	28	2	189	66	95	19	0.7	6	11
T3M28-3	29	2	164	66	95	22	0.7	11	12
T3M29-1	31	2	149	71	101	23	0.8	7	15
T3M29-2	29	2	159	70	100	24	1.0	9	14
T3M31-1	30	4	166	64	94	31	2.5	14	18
T3M34-1	25	2	154	62	92	27	1.7	9	13
T3M34-2	24	2	149	62	92	26	1.4	9	11
T3M36-1	32	3	161	59	89	31	2.2	14	18
T3M36-2	31	3	156	59	89	23	0.9	12	16
T4M 28-1	27	2	144	66	96	25	0.2	8	16
T4M 28-2	25	2	124	66	96	23	0.8	6	11
T4M1-1	24	3	154	71	100	23	0.9	10	17
T4M1-2	25	3	155	72	102	22	0.8	7	14
T4M11-1	29	2	159	65	95	23	0.5	8	13
T4M11-2	30	2	167	65	95	23	0.3	8	10
T4M11-3	30	2	179	66	95	22	0.7	9	12
T4M11-4	22	2	129	66	95	26	0.5	12	16
T4M12-1	34	2	160	79	109	23	1.1	7	12
T4M12-2	25	2	129	73	103	18	0.0	9	12
T4M12-3	31	3	159	73	103	24	0.7	6	10

T3= SAMSUN2 exposed to 0.3% EMS for 4hrs, T4= SAMSUN2 exposed to 0.3% EMS for 6hrs

Appendix 1: Continued

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
T4M15-1	26	3	139	63	93	22	0.9	9	16
T4M18-2	28	2	149	72	102	19	0.8	5	11
T4M18-3	31	3	171	71	100	24	1.2	8	12
T4M36-1	23	2	137	71	101	13	0.1	6	13
T4M36-2	19	1	137	71	101	10	0.2	5	15
T4M39-1	30	3	156	69	99	24	1.4	10	13
T4M39-2	26	3	126	69	99	23	1.2	7	12
T4M4-1	31	2	166	61	92	22	0.9	8	13
T5M15-1	32	3	162	83	112	21	0.7	8	14
T5M15-2	29	2	143	83	112	16	0.8	6	10
T5M15-3	30	3	164	83	112	23	1.3	9	13
T5M15-4	26	1	139	84	113	16	0.3	8	14
T5M21-1	31	3	166	63	93	24	1.0	9	13
T5M21-2	31	3	208	62	92	24	1.1	8	11
T5M21-3	30	3	166	63	93	22	1.0	12	19
T5M23-1	30	2	169	67	97	23	0.8	10	14
T5M23-2	25	1	139	69	99	15	2.5	7	11
T5M23-3	26	1	161	68	98	20	0.8	9	15
T5M23-4	30	2	150	64	94	29	0.8	10	15
T5M24-1	23	2	137	58	88	20	0.4	8	20
T5M24-2	25	2	152	58	88	22	0.2	11	18
T5M24-3	26	2	149	58	88	22	0.6	10	18
T5M34-1	26	2	138	67	92	17	0.3	9	15
T5M34-2	32	2	149	70	93	18	0.4	8	11
T5M34-3	24	1	99	71	93	12	0.0	6	10
T5M34-4	29	2	144	70	91	18	0.4	8	12
T5M34-5	29	2	144	69	92	13	0.0	5	9
T5M38-1	27	2	144	65	94	18	0.0	7	16
T5M38-2	28	2	136	67	96	15	0.0	7	13
T5M38-3	31	2	156	65	95	15	0.0	6	13
T5M38-4	28	2	158	63	93	17	-0.1	6	11
T5M40-1	29	2	162	68	98	16	0.5	8	14
T5M40-2	29	2	155	68	98	20	0.7	10	14
T5M40-3	32	2	139	69	99	11	0.2	4	7
T5M42- 1	29	1	144	68	96	16	0.3	8	18
T5M42- 2	29	2	144	68	97	17	0.1	10	15
T5M42- 3	28	1	124	68	96	12	0.0	5	10
T5M42- 4	26	2	144	70	96	17	0.2	9	16
T5M42- 5	32	2	184	66	95	23	0.9	9	12
T5M42-1	26	2	164	64	94	17	0.7	8	10

T5= SAMSUN4 exposed to 0.2% EMS for 4hrs, CHECK 2 = SAMSUN 2

Appendix 1: Continued

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
T5M42-2	24	2	164	62	94	15	0.4	6	9
T5M42-3	30	2	154	64	94	19	0.7	9	12
T5M47-1	27	2	160	69	99	15	0.2	7	11
T5M47-2	27	2	153	68	98	13	0.4	7	11
T5M47-3	25	1	145	69	99	17	0.6	8	14
T5M47-4	33	3	169	71	101	19	0.4	10	15
T5M47-5	27	2	164	71	101	18	0.4	10	14
T5M8-1	27	2	132	56	88	23	0.6	8	18
T5M8-2	29	2	152	57	88	23	0.5	9	17
T5M8-3	24	2	139	57	88	19	0.3	8	13
T5M8-4	20	1	134	57	89	16	0.3	9	14
T5M8-5	27	2	154	56	88	18	0.3	6	12
T6M15-1	28	2	152	69	99	14	0.1	6	10
T6M15-2	27	2	139	69	99	14	0.1	5	9
T6M15-3	29	1	149	70	100	15	0.0	5	13
T6M15-4	30	2	177	69	99	19	0.3	7	16
T6M16-1	32	3	171	60	90	22	0.8	9	14
T6M16-2	31	2	153	63	93	19	0.5	9	13
T6M16-3	22	2	140	61	91	22	0.7	9	17
T6M16-4	27	2	120	63	93	22	0.3	8	16
T6M17-1	27	2	147	72	101	21	0.6	8	13
T6M17-2	30	2	157	72	101	20	0.5	6	11
T6M17-3	31	2	172	71	101	16	0.3	4	10
T6M17-4	31	2	144	73	102	15	0.3	5	10
T6M17-5	32	2	164	74	103	20	0.4	9	13
T6M2-1	25	2	139	62	92	19	0.3	8	10
T6M2-2	27	2	161	62	92	18	0.5	7	12
T6M2-3	22	1	124	63	93	16	0.2	5	11
T6M2-4	26	1	131	62	92	15	0.3	5	12
T6M2-5	28	2	159	63	92	19	0.6	7	14
T6M22-1	28	2	152	73	103	18	0.2	7	10
T6M22-2	29	2	162	72	102	15	0.1	8	12
T6M22-3	27	2	147	72	102	15	0.0	6	15
T6M22-4	23	1	137	72	102	13	0.0	7	16
T6M28-1	26	1	130	64	94	14	-0.1	6	10
T6M28-2	28	1	145	64	94	18	0.1	8	12
T6M28-3	27	1	157	64	94	16	0.1	4	8
T6M29-1	30	3	145	65	95	21	0.8	4	13
T6M3-1	33	2	149	68	98	19	0.6	8	12
T6M3-2	32	2	136	66	97	17	0.4	8	12
T6M37-1	26	2	135	60	90	18	0.3	7	18

T6= SAMSUN4 exposed to 0.2% EMS for 6hrs

Appendix 1: Continued

Mutants	No of leaves	Stem diameter (cm)	Plant Height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
T6M7-1	31	1	141	58	88	19	0.4	11	16
T6M7-2	26	1	131	60	90	14	0.3	7	12
T6M7-3	28	1	131	59	89	17	0.3	9	11
T6M7-5	27	1	161	59	89	17	0.3	9	14
T7M1-1	31	2	151	62	92	27	0.8	10	15
T7M1-2	29	2	156	62	92	22	0.7	11	16
T7M18-1	29	2	171	70	100	21	0.7	9	11
T7M18-2	33	3	155	70	100	32	1.1	10	12
T7M23-1	32	2	144	66	96	20	0.3	10	15
T7M27-1	28	3	159	69	99	29	0.8	8	19
T7M31-1	28	2	159	68	98	19	0.7	8	15
T7M31-2	27	2	161	67	97	18	0.5	5	12
T7M31-3	32	2	174	67	97	23	1.0	10	16
T7M35-1	30	2	167	72	102	22	0.6	9	11
T7M35-2	35	2	183	75	105	18	0.7	9	16
T7M46-1	44	3	219	70	99	28	0.7	11	20
T7M46-2	32	2	149	72	100	20	0.6	7	13
T7M46-3	35	3	169	71	101	24	1.1	12	16
T7M47-1	32	2	165	61	91	20	0.4	9	13
T7M47-2	37	2	155	64	94	22	0.6	9	16
T7M47-3	31	2	155	63	93	21	0.6	9	15
T7M47-4	32	3	180	63	93	20	0.6	8	13
T7M47-5	33	3	190	63	93	22	0.7	7	14
T7M48-1	28	1	146	64	94	17	0.4	8	11
T7M48-2	28	2	156	64	94	24	0.8	8	10
T7M5-1	30	3	166	69	99	25	0.7	12	19
T7M5-2	30	2	161	69	99	22	0.6	10	15
T7M5-3	30	3	179	68	98	23	0.7	10	12
T8M10-1	30	3	169	63	92	20	0.6	11	16
T8M10-2	29	2	158	66	96	15	0.1	6	10
T8M10-3	27	2	133	66	96	14	0.1	8	18
T8M10-4	29	2	157	69	99	13	-0.1	6	10
T8M11-1	32	2	171	65	95	24	0.8	10	18
T8M11-2	29	2	169	66	96	19	0.6	9	16
T8M11-3	32	3	184	65	94	23	0.7	10	12
T8M11-4	30	3	179	65	94	26	1.1	14	18
T8M12-1	27	2	157	68	98	13	-0.1	5	10
T8M12-2	27	1	155	69	99	15	0.1	8	15
T8M12-3	24	2	162	71	101	16	-0.1	9	16
T8M12-4	31	2	157	69	99	17	0.0	7	12

T7= SAMSUN4 exposed to 0.3% EMS for 4hrs, T8= SAMSUN2 exposed to 0.3% EMS for 6hrs

Appendix 1: Continued

Mutants	No of leaves	Stem diameter (cm)	Plant height (cm)	Days to flowering	Days to maturity	Capitulum circumference (cm)	Capitulum weight (kg)	100 Seed weight (g)	Grain yield per plant(g)
T8M16-1	25	2	176	70	100	19	0.6	11	15
T8M16-2	30	3	156	68	98	17	0.7	8	12
T8M16-3	27	2	156	71	101	14	0.3	10	16
T8M21-1	28	3	154	64	94	24	0.7	10	15
T8M21-2	25	2	139	64	94	15	0.1	8	10
T8M21-3	27	2	154	61	91	16	0.0	4	7
T8M21-4	29	3	164	61	91	15	0.4	4	9
T8M23-1	27	2	151	59	90	20	0.4	9	16
T8M23-2	31	2	161	59	90	24	0.7	8	10
T8M26-1	32	3	182	70	100	25	1.1	9	17
T8M26-2	32	3	175	72	102	24	0.8	10	13
T8M26-3	27	2	155	73	103	18	0.7	6	10
T8M26-4	30	3	145	71	101	17	0.5	4	8
T8M26-5	31	2	170	72	102	21	0.8	5	11
T8M29-1	28	2	158	60	90	18	0.4	8	14
T8M29-2	28	3	159	64	94	21	0.8	9	21
T8M5-1	28	2	158	64	94	29	0.8	8	14
T8M5-2	34	3	179	70	100	28	1.0	11	14
T8M8-1	29	2	164	70	100	22	0.5	9	12
T8M8-2	31	2	177	71	101	22	0.4	9	14
T8M8-3	30	2	185	73	103	24	1.1	9	15
T8M8-4	32	2	175	71	101	34	0.9	13	22
TIM8-4	34	2	191	60	90	23	0.7	10	13
TIM8-5	35	3	191	60	90	29	0.7	10	16
SAMSUN2	29	1	213	64	94	21	0.1	5	11
SAMSUN4	28	1	208	63	92	20	0.1	5	10
Mean	**29**	**2**	**160**	**67**	**96**	**20**	**1.0**	**8**	**12**
LSD (0.05)	**4.4**	**0.7**	**25.2**	**4.4**	**4.3**	**5.1**	**0.7**	**2.7**	**5.3**
CV (%)	**11**	**20**	**11**	**5**	**3**	**18**	**86**	**22**	**33**