ETHYL METHANE SULPHONATE (EMS) INDUCED MUTATION IN SELECTED SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES FOR AGROMORPHOLOGICAL TRAITS AND SEED RETENTION

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MTech/SLS/2018/7952

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FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGER STATE

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF TECHNOLOGY IN BOTANY (APPLIED PLANT GENETICS AND BREEDING)

SEPTEMBER, 2023

DECLARATION

I, hereby declare that this thesis titled **"Ethyl Methane Sulphonate (EMS) Induced Mutation in Selected Soybean [*Glycine max* (L.) Merrill] Genotypes for Agromorphological Traits and Seed Retention"** is a collection of my original research work and it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

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SIGNATURE/DATE

CERTIFICATION

The thesis titled **"Ethyl Methane Sulphonate (EMS) Induced Mutation in Selected Soybean [*Glycine max* (L.) Merrill] Genotypes for Agromorphological Traits and Seed Retention"** by ISAH, Bashira (MTech/SLS/2018/7952) meets the regulations governing the award of the degree of Master of Technology of the Federal University of Technology Minna, Nigeria and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This research project is dedicated to the memory of my late father, Alhaji Isah Abdullahi Zakari, who though gone today, would have been ecstatic his last girl has reached this far. May Almighty Allah Subhaanahu Wa Ta'ala forgive your short-comings and grant you eternal rest, Ameen.

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ABSTRACT

Soybean (*Glycine max)* is one of the most important legume crops worldwide grown mainly for its protein and oil rich seeds. Despite its importance, pod shattering causes about 34-99 % yield losses, with limited success in breeding cultivars for resistance to pod shattering. Hence, the need for alternative means of achieving this in a way that will be sustainable and increase its production is paramount. Four selected genotypes prone to shattering were collected from National Cereal Research Institute (NCRI), Badeggi, Niger State and treated using 0.00 % (control), 0.20 %, 0.40 %, 0.60 % and 0.80 % EMS concentration. The treated seeds were evaluated for agromorphological characters and pod shattering ability. The height of the plant at maturity ranged between 25.00-42.25 cm with an average plant height of 34.05 cm. Results of the Qualitative observation showed that all accessions had green leaves with the exception of TGX1904-6F at 0.60 % EMS and TGX1835-10E at 0.80 % EMS which had chlorosis. The leaflets were mostly 3 and ovate except the 0.60 % and 0.80 % EMS which had 4-5 leaflets and mostly lanceolate and intermediate. The flower colours were pinkish to purplish except TGX1987-10F at 0.40 % EMS which had whitish flowers. The yield result showed TGX1835-10E control had significantly ($P < 0.05$) highest number of pods per plant (188.25) while TGX1987-10F at 0.80 % EMS had the lowest (22.00). Also, for number of seeds per pod, TGX1904-6F at 0.40 % EMS had the highest number of seeds per pod (3.15), while TGX1448-2E at 0.40 % EMS recorded the least value (1.55). TGX1987-10F control had the highest shattering percentage (44.18 %) while TGX1448-2E with EMS 0.20 %, 0.40 % and 0.80 %, TGX1904-6F at EMS 0.40 %, 0.60 % and 0.80 %, TGX1835-10E at EMS 0.40 %, 0.60 % and 0.80 %, TGX1987-10F at EMS 0.40 % and 0.80 % all had the lowest (0.00 %). After the oil extraction, it was observed that the percentage oil yield of seeds ranged between 15.44-21.27 %, increase in the oil yield as well as glycerin content were observed with increase in the EMS concentration. The superiority of 0.40 % and 0.60 % EMS concentration for high yielding and resistance to shattering indicate the potential for the concentration and effectiveness of EMS for inducing useful mutation in soybean. The potential mutants generated should be selected for further evaluation in soybean breeding programme and its improvement.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Soybean [*Glycine max* (L.) Merrill] is an oil-producing legume crop in the Fabaceae family (Espina *et al.,* 2018*)*. It has become the miracle crop of the 21st century. Soybean is significant among known leguminous crops globally as it is cultivated primarily for its high protein content, and oil producing seeds (Diers and Scaboo, 2019). Soybean production has for a long time been a common production in Africa with history from Egypt in the year 1858 and the first reported cultivation in South Africa in 1903 (Shurtleff and Aoyagi, 2009). In spite of this and the extensive lands that are suitable for the production of this crop, the fertile land area has largely been under-utilized for the crop's large-scale production over the past 55 years when compared to large producing countries like Brazil and the United States (US). Low production in the Sub-Saharan Africa (SSA) have given rise to sub-optimal productivity which have disincentivized cultivation of soybean for farmers. Factors such as infertile soils, poor agronomic practices and lack of adapted/improved varieties have been found to be associated with low productivity of this crop. However, Foyer *et al*. (2019) recommends that Africa holds incredible potential for sustainable soybean production, despite the changing global climate and the already existing limitations to crop production which is mainly the shattering of its pods.

Pod dehiscence (commonly referred to as pod shattering) which results in splitting of grown pods and scattering of the seeds at maturity, an important phenomenon in several wild plants which provides progeny with sufficient space for growth as well as improves the potential

for survival under a wide variation of ecological conditions (Zhang *et al.,* 2018). Crops that have been domesticated like soybean [*Glycine max* (L.) Merr.], common vetch (*Vicia sativa* L.), sesame (*Sesamum indicum* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and rapeseed oil (*Brassica napus* L.) are most commonly predisposed to pod shattering; which is the known and significant factor responsible for significant yield losses (Bennett *et al*., 2011; Funatsuki *et al.,* 2014; Zhang *et al.,* 2018). Mature pods of soybean burst open along the sutures (dorsal and ventral) and scatter the seeds under less humid environments. The dehiscence of soybean pods is one of the major limitations to harvesting, because the loss during harvest increases the seed shattering. Therefore, pod shattering has been central part of agricultural research giving its importance in the improvement of the crop as resistance to pod shattering is essential for high yield of developed soybean (Dong *et al*., 2014; Ballester and Ferrándiz, 2017). Soybean has a narrow variability as hybridization is a tedious operation due to its small floral parts, so a lot of other methods have been used in an attempt to increase its variability which includes the use of mutagens.

Ethyl methane sulfonate (EMS) is an effective chemical mutagen used for high frequency point mutation in seeds (Talebi *et al*., 2012; Chen *et al*., 2013). Although very efficient, EMS is quite simple to use in comparison with other forms of chemical mutagens. Mutagenesis of soybean is a genetic process that has been used in agriculture to assess important agronomic traits, develop improved varieties, distinguish loci in charge of chief functions and discover novel alleles (Cooper *et al*., 2008; Khan and Tyagi, 2013).

In addition, this chemical mutagen has also been examined in soybean mutants for distinctions in phenotypes such as the colour of seeds, phenotypic presentation of roots and altered plant architectures (Bolon *et al.,* 2011; 2014). Bolon *et al*. (2011) discovered

dissimilar structure in the phenotypes of mutated soybean plants which includes curly leaves, yellow coloration, premature pod production, non-nodulation and hyper nodulation, chimeric and short trichome and petiole mutant with wrinkled leaf. Recent cultigens with more yields and additional traits should be used in developing mutant species. Therefore, mutant species are critical to the production of improved varieties (Arelli *et al*., 2015).

1.2 Statement of the Research Problem

Pod shattering is a major limitation to crop production that results in 34-99 % soybean seed losses (Kataliko *et al.*, 2019). With the risk of plants losing 100 % of their seeds, pod shattering is known as the most important limitation to soybean cultivation in sub-tropical and tropical regions (IITA, 1992; Adeyeye *et al*., 2014; Kataliko *et al.*, 2019). Most of the soybean cultivars in the tropics are introduced (in its natural form) from areas where soybean has been cultivated for decades. However, resistant cultivars that have been introduced from other regions across the globe are a risk of pod shattering when introduced into the tropics (Tukamuhabwa *et al*., 2000; Kataliko *et al.*, 2019) possibly due to variations in ecological interactions and genotypic conditions. Efforts to cultivate soybean varieties against pod shattering has been sluggish with inadequate success, there is a necessity to produce genetic variability in the crop by means of mutagenesis particularly as hybridization is nearly impossible as a result of the cleistogamous flowers of the crop.

1.3 Aim and Objectives of this Study

The aim of this study is to evaluate the effects of Ethyl Methane Sulphonate (EMS) on the agromorphological traits and seed retention capacity in selected soybean [*Glycine max* (L.) Merrill] genotypes.

The objectives of this study are to determine the;

1 effects of EMS on selected agromorphological traits of the first mutant generation (M1) lines of the soybean genotypes.
2 effects of EMS on the seed retention capacity on the first mutant generation (M1) lines of the soybean genotypes.
3 pollen diameters of the M1 lines of the soybean genotypes.
4 percentage oil yield and oil characterisation of the M1 line of the soybean genotypes

1.4 Justification for the Study

The need for selecting soybean among other crops is pertinent as this crop is very significant because of its oil-rich seeds as well as its protein content for both human and animal consumption. Such an important crop with a serious production limitation as seed shattering before harvesting have raised queries on how to retain seeds in the pods on the field before harvesting. Several mutagens have been used for mutagenesis in soybean and the effects have been observed (Khan and Tyagi, 2013). There is a need to produce mutant varieties of soybean in order to generate improved cultivars (Espina *et al.*, 2018). Mutagenesis of this crop increase soybean variability; with resultant increase in the chances of identifying new mutants which are valuable to breeding programs aimed at improved agronomical performance of the seeds (Nobre *et al.,* 2019). Ethyl methane sulphonate (EMS) have been used to generate mutations resulting in a mutant variety with improved variations in the phenotypes where mutants with high agromorphological features have been spotted (Espina *et al.*, 2018). Identifying, developing and utilizing varieties with pod shattering resistance can decrease losses in yields (Kataliko *et al.,* 2019). The extensive use of soybeans and its

co-products have raised the demand of developing new cultivars with the most advantageous characteristics (Nobre *et al.,* 2019).

Based on existing knowledge that pod shattering may negatively affect the yield and productivity of most legume plant products, findings from this study have a potential of proffering solutions to this problem by improving plant products and resulting economic stability in a long run. Thus, this research will adopt chemical mutagenesis (EMS) to significantly eliminate this limitation as this approach is proposed to prevent pod shattering in soybeans and will help farmers improve plant yield and productivity of healthy crops while encouraging and improving marketing of quality indigenous products within the country and across the globe.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Origin of Soybean Domestication

Soybean (*Glycine max* (L.) Merrill) is a common leguminous crop that produces oil and protein in animal food and is an essential food for human (Hartman *et al*., 2011). It is widely established that recent soybean that have been cultivated are domesticated from its wild cultivar (*Glycine soja* Sieb. and Zucc.) in Eastern Asia between 6000-9000 years ago (Kim *et al*., 2012). Additionally, the soybean origin has been vaguely understood due to lack of archeological and molecular evidences from studies. However, progress in genome sequencing of both wild and domesticated soybeans with recent archeological findings have expanded the history of this significant crop (Sedivy *et al*., 2017).

Although historical evidence suggests the origin of soybean origin from North-eastern China, (Eastern Zhou Dynasty agricultural revolution), the origin of soybean landraces with the main genetic diversity has been linked with the Huanghe region (Li *et al*., 2010). In this region, numerous archeological charred specimens (Lee *et al*., 2011) placed the Yellow River basin as a major origin of soybean domestication; while there are suggestions of Yangtze basin (Southern China) being the supposed birthplace of soybean based on clustering analyses and phylogenetics using nucleotide diversity and microsatellites (Guo *et al*., 2010).

Current findings are consistent with a previously established hypothesis that the transition to domesticated soybean occurred as a slow process. Before establishment of the estimated

domestication time of soybean, divergence studies of both *G. max* and *G. soja* species genomes proposed that the progenitors of domesticated soybean deviated from *G. soja*, creating a complex between *G. soja* and *G. max* (Li *et al*., 2014). Hence, the possibility of evolutionary transitional species *G. gracilis* signifies a *G. soja* – *G. max* complex that humans interacted with previously before the event of soybean domestication.

Resequencing of 302 improved, landrace or wild soybeans suggests that all domesticated soybeans are derivatives from a single cluster of *G. soja* wild soybeans, supporting the single origin hypothesis that all presently cultivated domesticated soybeans originated from a single domestication event (Zhou *et al*., 2015). This early domesticates may have either vanished among wild soybeans or have been unified into the domesticated soybean from China, which may have had more beneficial traits for cultivation, during its spread throughout the Japan and the Korean peninsula, resulting in the constant yet diverse subpopulations in these regions. Future genomic studies of more set of semi-wild soybeans from various geographic regions, as well as efforts to get genome sequence evidence from archeological constituents, will further explain the early history of soybeans in the pre-domestication era (Sedivy *et al*., 2017).

2.2 History of Soybean Introduction and Cultivation in Sub-Saharan Africa (SSA)

Introduction and commercial cultivation of soybean have quite a brief history in SSA countries (Mpepereki *et al*., 2000). In the 19th century, it was introduced to SSA along the East coast of Africa by Chinese traders (Giller and Dashiell, 2006). South Africa recorded its first soybean cultivation in 1903 (Shurtleff and Aoyagi, 2009). Soybean was first cultivated in Malawi in 1909 and in Tanzania in 1907 (Giller and Dashiell, 2006). Introduction of

soybean to Nigeria was in 1908 (Shurtleff and Aoyagi, 2009) where 'Malayan' an introduced variety was adopted and cultivated as an export crop in a small area in Benue state. The crop is usually grown as an intercrop in citrus orchards and in farms in mixed farming with sorghum or maize. Like Nigeria, Zaire has an extensive history of soybean cultivation by indigenous farmers. Before the nation gained independence, introduction and promotion of soybean was initially by missionaries and were considered as medicinal food to prevent and treat the degenerative effects of undernourishment in Zaire. Soybean first introduction to Sudan was in 1910 (Shurtleff and Aoyagi, 2009). Further introduction of soybean in the country were made in 1912 and in 1949, soybean was cultivated in Southwest Sudan to avoid severe undernourishment among babies and expectant and breastfeeding women (Ibrahim, 2012). Further introduction of soybean continued (Ibrahim *et al*., 2017) with distinct efforts to boost cultivation of soybean in SSA.

2.3 Classification of Soybean

According to Shurtleff and Aoyagi (2009), the Merrill classification of soybean is as follows:

Kingdom Plantae

Order Fabales

Family Fabaceae

Sub-Family Faboideae

Genus *Glycine*

Species *G. max*

2.4 The Process of Soybean Cultivation

According to Kalau (2017), soyabean production in Nigeria typically starts in May or June. Soyabeans grow well on nearly all types of soil apart from deep soil that have bad water retaining capacity. So, authorities say that the optimal pH is 6.5 maximum, and it may be essential to be calcified. Generally, this plant sprouts healthier in moderate areas. Soyabean is a short-day herbaceous plant and the blossoming occurs when the day shortens and nights begin to lengthen. Also, soyabean breeding and how it undergoes numerous mellowing phases has led production of crops that have big variety of sizes. Another significant condition for soyabean production is rainfall. Abundant harvest is impossible when deprived of decent watering, and regular water source is the most vital thing throughout the process of the pod and beans development. Currently, watering is a vital aspect soybean cultivation for higher revenue and security for all planters**.**

2.5 The Status of Soybean in Africa

According to Cornelius and Goldsmith (2019), African soybean breeders supply less than 1 % of the world's soybeans. Universal cultivation of soybean has reached a compound annual growth rate (CAGR) of 4.68 % since 1961, while African production rates are rising at about 48 % faster at a rate of 6.84 % annually. Both the world and Africa's growth in soybean cultivation regularly result from a growth in soybean acres cultivated and not from produce. The top three soybean producers are South Africa, Nigeria and Zambia on the African continent.

2.6 Morphological Characteristics and Biology of Soybean

Soybean is a leguminous plant that can grow in an extensive variety of soils with optimal growth in saturated alluvial soils with a decent organic content. The plant can reach a height of about 1 m (3ft) with 80 - 120 days from sowing to harvesting (Ulafić *et al.*, 2020). The

leaves, stems and pods are covered with fine hair, the primary leaves are opposite, unifoliate and ovate; the secondary leaves are usually compound, trifoliate and alternate. The rooting system is tap root, from which lateral rooting system emerges. The flowers are discreet with either purple, pink or white coloration and self-pollinated borne on the axil of the leaf; the flowers comprise of a single posterior stamen, nine fused stamens, a tabular calyx of five petals and sepals, with a pistil. The fruit is a hairy pod developing in clusters of three to five and generally contain about two to four seeds. The visible scar on the seed is referred to as the hilum (Acquaah, 2007).

Shilpashree *et al.* (2021) observed that the variability of the leaf shape of soybean is round ovate, pointed ovate and lanceolate with the coloration of the leaf showing obvious variation between various shades of green between normal to dark green, the varieties having white or purple flowers. Young pod coloration varying from light to dark green, with flat, slightly curved and curved pod forms. According to a study conducted by Ningsih *et al.* (2019), pod length ranges between 43.358 mm and 35.596 mm (4.34 - 4.56 cm); and these developing pods would keep growing until they have reached 15 - 20 days of age (Liu, 2004). According to Gopinath and Pavadai (2015), the maximum height of the plant grew to about 82.91 cm, the number of pods per plant is between 62.31 - 40.19 pods and percentage oil content between 21.85 - 18.95 %.

2.7 Cultural Practices of Soybean

(a) Crop rotation

The crop planted before the soybeans has an impact on possible yield. If soybeans are planted after soybeans, diseases and other pest problems may increase after years of successive soybean cultivation. In addition, study has shown that growth inhibiting allelopathic elements

are released from soybean deposit as it decomposes in the soil, these negates cultivation and production of soybeans. Cultivating soybeans after soybeans will not provide a sufficient yield. More yields result from soybeans grown in rotation, compared to yields gotten from cultivating soybeans after soybeans (Shea *et al*., 2020)

(b) Land preparation

Land is ploughed early to allow moisture conservation and weed control. The seedbed should be properly prepared and all grassy weeds should be eliminated (Vivian *et al.* 2013).

(c) Planting

Planting time of soybean differs and it include early to mid-April, early to late May, and mid to late June (Taghavi *et al*., 2012; Xiaoming and Qiong, 2018) with soil temperature above 68^{o}F (Shea *et al*., 2020)

(d) Harvesting and drying

Cultivated soybean are usually harvested by uprooting the whole plant or using a sickle to cut the stalk except some when planted over a very large area of land where it is harvested by bush cutter that is attached to a gasoline engine and binder. Recently, at large scale farm household a combined harvester is used. Harvested soybeans are normally dried in the sun after tying them up in bundles (Shea *et al*., 2020).

(e) Weed control

To successfully control weed, preparing the seed bed early is a pre-requisite. The chemical and cultural are the two main methods of weed control. In chemical method, before

germination or pre-emergent (planting), herbicides can be used either before or after post-emergent, while cultural method deals with weeding by hand to free the field of weeds (Vivian *et al.* 2013).

2.8 Ecological Requirements of Soybean

1 Rainfall

Soybean requires a minimum of 400 mm of well dispersed rainfall, which makes it a moderately drought tolerant plant. The period of its vegetative growth lasts between 3-4 months. The crop requires high moisture as at the time of its germination, flowering and pod-formation stage. Varieties that have short duration are recommended in parts where soybean is cultivated under rain-fed conditions, though dry weather is essential for its ripening (Ulafić *et al*., 2020).

2 Temperatures

Soybeans develop well in humid and warm conditions. Soil temperatures have to be above 15 °C for decent germination and about 20-25 °C for growth (Shea *et al*., 2020).

3 Soils

Soybeans will grow on wide variety of soils but prosper best on alluvial and clay loam soil of good fertility. The soils have to be fertile, well drained and rich in calcium with 5.6-7.0 pH range (Hoeft *et al.*, 2000).

4 Altitude

Soybeans develop well within 0-2000 m above sea level. At higher altitudes of more than 2000 m, it takes as long as 180 days (6 months) for the late maturing varieties, though its yield is more than the varieties that mature early (Ulafić *et al*., 2020).

2.9 Management of Pests and Diseases of Soya Beans

Soya beans has some pests and diseases resistance due to its anti-nutritional components. However, some pests and diseases may reach economic threshold levels (Lahiri and Reisig, 2016).

2.9.1 Pests of soybean

Soybean major pests mainly include insects like aphids, cucumber beetles and army worm; others include, African bollworms, beanflies, cutworms, nematodes, semi-looper caterpillars and storage weevils. These pests will bore holes on soybean leaves, stems and pods causing reduced yield (Xiaoming and Qiong, 2018; Shea *et al*., 2020). Management of pests could be achieved by biological methods using natural enemies, crop rotation, application of appropriate insecticides, handpicking of larvae, timely dusting of eggs (Shea *et al*., 2020) and planting of resistant varieties (Leskey *et al.,* 2012).

2.9.2 Major diseases of soybean

Diseases of soybean are caused by virus, bacteria, fungi and nematodes; these can affect leaves, stems, roots and even pods and seeds of the soybean. These diseases include: bacterial blight, bacterial pustule, rhizoctonia stem rot, rust, soybean mosaic, sudden death syndrome (Malvick, 2018; Xiaoming and Qiong, 2018; Shea *et al.,* 2020). Diseases are usually managed using pathogen free seed, planting early in the season may be helpful, cultivars with some level of resistance to some diseases should be planted (Niblack, 2005; Leskey *et al.,* 2012), crops should be rotated to manage diseases. Other strategies include reducing excessive soil moisture with drainage, minimizing compaction, and staggering planting dates (Athow, 1981; Shea *et al.,* 2020).

2.10 Economic Importance of Soybean

Soybean is one of the cheapest and richest sources of protein, it is a staple food in the diets of animals and people in several parts of the world. Soybean is one of the major sources of vegetable oil and animal protein feed in the world (Sugiyama *et al.,* 2015). Its protein content is the highest (40–42 %) of all other food crops and it is second only to groundnut in terms of oil content (18–22 %) among leguminous crops (Robert, 1986; Pagano and Miransari, 2016). Food gotten from soybean include baby food, baked goods, candy, cereal, cooking oil, imitation meats, processed meats, salad dressings, soy sauce, tofu, margarine, miso, (Ogedengbe and Bello, 2018). Additionally, muscle fatigue and obesity can be prevented by soy protein (Agyei *et al*., 2015) as soybeans has no starch content, they are good source of protein especially for diabetics.

Industrial uses for soybeans consist of candles, fertilizers and pesticides, medicines, plywood and wallboard, linoleum, varnish, soaps and disinfectants, fire extinguisher fluid and paint. There is triglyceride in the oil content which is considered as a possible source of oil for biodiesel production. Soybean is useful for aquaculture (Masuda and Goldsmith, 2009). Impact in the influence of agriculture on soil structure or changing soil species inhabitants has improved (Pagano *et al*., 2011; Wall and Nielsen, 2012). Soybean is one of the main crops cultivated globally that can affect different aspects of the ecosystem. Among the most significant components of the ecosystem are the microbes in the soil.

Among the main cultivated crops (maize, rice, wheat), the only leguminous crop associated with arbuscular mycorrhizal fungi and rhizobia is soybean, with the possibility for further exploitation. Pagano and Covacevich (2011) stated the previous information on the advantage of arbuscular mycorrhizal fungi in agro-ecosystems, and how there increase

recognition of the effects of intensify agriculture and agrochemicals usage adversely have effect on the diversity and activity of the soil microbiota as well as the soil quality; modify the number and the populations of symbiotic fungi. Mutualistic associations such as arbuscular mycorrhiza fungi are essential for soybean production (Simard and Austin, 2010; Pagano, 2012). There is an increasing use of beneficial rhizospheric microorganisms as biofertilizers in agriculture with a necessity to better recognise the impacts of various inocula on soybean physiology and growth. According to Dwivedi *et al*. (2015), soybean production improves fixation of symbiotic nitrogen. The important function of nodules on soybean roots is fixing atmospheric nitrogen through symbiotic nitrogen fixation, nitrogen supply for seed production and plant growth.

2.11 Pollen Studies

Pollen grains are the carrier of male gamete in plants and differ in various morphologies. The main function of the pollen is to transfer the male genetic material into embryo sac through a process called "double fertilization" (Mendieta and Granados, 2015). Pollen represents the substantial stage in plants and fertile pollen are important for proficient plant reproduction (Razzaq *et al*, 2019). Pollen viability can be indicated through various methods (Riano and Dafni, 2000). However, pollen viability and in-vitro germination have been extensively exploited to estimate pollen viability (Satish and Ravilumar, 2010). High crop yield also depends upon pollen viability and it has paramount significance in the hybridization programme (Patel and Mankad, 2014). Pollen quality can be estimated on the basis of vigour and fertility. If pollen viability of genotypes is high, therefore; genotypes can be considered good pollinator and assessment of pollen fertility and germination potential are important criterions for pollen evaluation (Gaaliche *et al*., 2013). Different stains such as pollen

viability tests have been used in the past for the assessment of pollen viability and the relative estimate of fertilization potential (Huang *et al.*, 2004; Ilgin *et al.*, 2007; Frescura *et al*., 2012). The use of reliable methods for functional quality of pollen is important in the evaluation of pollen during storage, crop improvement, genetics and fertility studies, in the breeding of crops (Radicevic *et al.*, 2013). Studies have indicated that, fresh pollen must be analysed in minimum time after collection. The pollen viability tests used in the past had certain drawbacks including staining of viable and nonviable pollen grains. There are numerous methods of estimating pollen quality: tests based on pollen cell membranes, staining of pollen, in vitro pollen germination, pollen germination on stigma (Razzaq *et al.*, 2019).

2.12 Shattering Resistance Associated with Domestication of Soybean

Seed dispersal loss is one crucial part of agronomical trait adopted by ancient human selection (Dong *et al*., 2014). As a result, this technique has been identified as a milestone to crop domestication (Lenser and TheiBen, 2013). Soybean [*Glycine max* (L.) Merr.] is one of the legume crops from which vegetable oils is extracted and has proteins necessary for humans' nutritional needs (Khan and Tyagi, 2013). The leguminous crop possesses excessive lignified Fibre Cap Cells (FCC) that defines its key cellular feature of the shattering-resistant trait leaving the abscission layer unmodified in the pod ventral suture (Doebley *et al*., 2016).

One key advancement in agriculture is the advent of the Neolithic (Neolithic Revolution) approximately 10,000 years ago. This innovation has led to the possibility of domesticating plant and animal species from their respective wild predecessors (Diamond, 2002; Purugganan and Fuller, 2009). Loss of seed dispersal or fruit dehiscence is an agronomic trait which was targeted to ensure a more efficient means of cultivation with crop domestication (Diamond, 2002; Purugganan and Fuller, 2009). Loss of seed shattering in cereal crops has

been found to be responsible for abscission layer (AL) elimination or alterations between the pedicel and the lemma (Simons, 2005; Li *et al.,* 2006; Taketa *et al*., 2008; Lin *et al*., 2012; Olsen and Wendel, 2013; Lenser and TheiBen, 2013; Doebley *et al*., 2016). However, there are differences in anatomy between the structures of the fruits of monocotyledons (cereals) and eudicotyledon crops (legumes). This is due to the varying mechanism that underlies shattering of pods and its seed dispersal (Tiwari and Bhatia, 1995; Christiansen *et al*., 2002; Lenser and TheiBen, 2013). It has long been established that domesticated soybeans were derived from its annual wild varieties, *Glycine soja* Sieb. and Zucc., about 5,000 years ago in East Asia. Also, researchers in the field of biology and agriculture have continued to explore the tissue contributing to resistance to pod shattering and its basic genetic mechanism in soybean that were cultivated (Tiwari and Bhatia, 1995; Christiansen *et al*., 2002).

One of the effective approaches in dissecting the genetic materials that are responsible for domestication in several species of crops is Quantitative trait locus (QTL) mapping. In rice (*Oryza sativa*), maize (*Zea mays*) and tomato (*Lycopersicon esculentum*), QTL mapping has been successful in isolating and characterizing genes that determine plant architecture, fruit size and seed shattering. Research conducted over the past 15 years have shown that a series of genomic regions associated with pod shattering in several linkage groups using QTL mapping in soybean (Suzuki *et al.,* 2010). A newly identified and fine-mapped pod shattering QTL on chromosome 16 is qPDH1 which produces 10 putative candidate genes (Suzuki *et al*., 2009, Suzuki *et al*., 2010). However, none of the 10 candidate genes has been found to be associated as the pod shattering resistance regulator. Additionally, there is no difference was observed between shattering-susceptible and shattering-resistant phenotypes of lines that are near-isogenic and influence resistance to pod shattering (Suzuki *et* al., 2009).

Consequently, it was proposed that pod shattering resistance had been attained through subtle yet undetectable changes in the morphology of fruits, or other unknown factors than morphology (Suzuki *et al*., 2010). It is important to understudy the exact structure and the genes responsible for shattering targeted by artificial selection as this would decipher the genetic mechanisms responsible for the fixation of such trait in soybeans that were domesticated. This will also better offer insights about the evolution of complex morphological traits that exist in nature (Olsen and Wendel, 2013).

2.13 Developing an Ethyl Methane Sulfonate (EMS) Mutant Population in Soybean

Soybean mutagenesis is widely used in characterizing loci in developing new varieties, regulating important functions, screening for important agronomic traits and discovering alleles (Cooper *et al*., 2008; Khan and Tyagi, 2013). EMS is a chemical mutagen that is often utilized for the mutation of seed. This is because of its efficacy and ability to induce high frequency point mutations. This chemical mutation has also been found to result in the novel stop codon for different genes (Talebi *et al*., 2012; Chen *et al*., 2013). Apart from its effectiveness, the ease in handling EMS compared to other chemical mutagens such as nitroso compounds makes it a more preferred mutagen. EMS utilized can also be detoxified via hydrolysis for disposal (Pathirana, 2011). Analysis of EMS mutagenized population can be done using two different approaches. The first include forward genetics which requires that apparent phenotypes be characterized before identifying the underlying gene; and the second approach is reverse genetics which involves the detection of mutations in the genes of interest before they are later linked to a specific function or phenotype (Peters *et al*., 2003).

EMS has been used to effect DNA mutations which has resulted in generating mutant population with increased variation in the phenotype. Suitability of soybean mutants with high oil, sucrose, oleic acid, protein and low linolenic acid contents have been established for use in breeding program (Espina *et al*., 2018). The general goal is to adopt EMS mutagenesis in the development and improvement of soybean germplasm. This population will then be used for characterization of about 50,000 predicted genes in soybean as a reverse genetic tool. The adverse effect of climate change on dryer growing seasons and emerging pest and diseases infestation remains a major concern. Therefore, it is important that soybean improvement and its increasing genetic diversity is explored and adopted (Espina *et al*., 2018).

2.14 Oil Characterisation of Soybean

On a normal dry matter basis, soybean has about 20 % oil content. Soybean is very nourishing and its oil content are not just in high amount but also in excellent quality. There's high proportion of unsaturated fatty acids in soy oil, which makes it a healthy oil. Mutant soybean population using EMS treatment have high oil content when compared to other type of mutagenesis like colchicine, gamma rays and diethyl sulphate leading to a higher oil content level (Pavadai *et al.*, 2010). The iodine value is expressed in grams of iodine for the number of halogens linked with 100 g test sample, and is used as degree of unsaturated bonds of fats and oils. The higher the iodine value is linked to the greater degree of unsaturation (Lofty *et al.*, 2015). According to FAO 2011, the standard for iodine content is 124-139 mg. Saponification value (SV) is expressed by potassium hydroxide in mg required to saponify one (1) gram of fat. Saponification is the process of breaking down a neutral fat into fatty acids and glycerol by treating with alkali. The FAO standard is 189 -195 mgKOH/g. Ester value (EV) is defined as the milligrams of KOH required to react with glycerin after

saponification of 1 g of lipid. It is calculated from the saponification value (SV) and acid value (AV) (Analytical methods to measure the constants of fats and oils, 2011). Therefore, EV = SV – AV %. While glycerin = EV × 0.05466. Glycerin is generally not harmful to the body no matter the quality. FDA (Food and Drugs Administration) declared glycerin safe for consumption, though excess can cause a potential laxative effect (Kriss, 2020).

Acid value in terms of acidity is a direct measure of the quality of the oil and reflects the care taken right from blossoming and fruit setting to the eventual sale and consumption of the oil (Lofty *et al.*, 2015). This is defined as the mg of KOH necessary to neutralize the fatty acids present in 1 g of lipid. The acid value of oil must not be too high, as this denotes an excessively high content of free fatty acids, which causes the oil to turn sour. In general, it gives an indication about edibility of the lipid (Analytical methods to measure the constants of fats and oils, 2011). The acceptable standard according to FAO is 0.6 -4.0 %, while free fatty acid is not harmful to the body, the WHO standard stands at 0.75 %.

Peroxides are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation. According to Lofty *et al.* (2015), a lower number of peroxide value indicates a good quality of cooking oil and at such should be around 10 mEq/kg. The WHO recommended value is between 10-15 mEq/kg (FAO, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was carried out at the experimental field, Department of Plant Biology, Federal University of Technology (FUT) Minna, Niger State, Nigeria. Minna is the state capital of Niger state and lies between latitude $9^{O}31^{I}$ and $9^{O}40^{I}$ North of the equator and longitudes $6^{O}29^{I}$ and $6^{O}35^{I}$ East of Greenwich with a landmass of 884 hectares. Minna is located in the North-Central geopolitical zone of Nigeria. Minna has annual temperature of 20 ^{o}C to 30 ^{o}C and relative humidity of 61 %. The area has two seasons; raining season between May to October and dry season between November to April each year. It has a low humid soil type with favorable climatic condition for planting.

3.2 Sources of Research Materials

Four (4) selected soybean genotypes seed prone to shattering were obtained from National Cereal Research Institute NCRI), Badeggi, Niger State, Nigeria. The selected genotypes are TGx1448-2E, TGx1835-10E, TGx1904-6F and TGx1987-10F.

3.3 Preparation of Mutagenic Concentration

To prepare the different concentrations, a stock solution containing 100 % of EMS was first prepared. A stock solution is a concentrated solution that will be diluted to some lower concentration for actual use. Preparation of the required concentration (0.2 %, 0.4 %, 0.6 %, and 0.8 %) was done from the stock solution using standard volumetric flask. Concentration of 0.2 % was prepared by measuring 2 ml of the stock solution and make to the mark with

98 ml of distilled water. Other concentrations (0.4 %, 0.6 % and 0.8 %) were prepared by measuring appropriate amount of stock solution (4 ml, 6 ml and 8 ml) into the volumetric flask and diluted with appropriate quantity of distilled water (96 ml, 94 ml and 92 ml) respectively to make up to the mark. The solutions were then homogenized for proper mixing of the solution.

3.4 Mutagenic Treatment of Seeds

The mutagenic treatment was conducted at the laboratory of the Department of Plant Biology, Federal University of Technology, Minna. About 150 soybean seeds from each of the four (4) genotypes were presoaked in distilled water for 4 hours under room temperature. This allowed the mutagen to diffuse more rapidly to the tissues of interest (Espina *et al.*, 2018). The method for the mutagenic treatment of seed were the steps stated by Jankowicz-Cieslak and Till (2016). The water in the seeds was decanted and the seeds were soaked again for 4 hours in different concentrations of Ethyl Methane Sulphonate (0.20 %, 0.40 %, 0.60 %, 0.80 % and 0.00 %). The solutions were decanted after the soaked hours and the seeds were rinsed under running tap for five (5) minutes. The control seeds were soaked in distilled water for the same period of four (4) hours. These seeds were bloat dry with Whatman filter paper and packaged in properly labelled envelopes.

3.5 Experimental Design and Seed Planting

The experiment was laid in a randomized complete block design (RCBD) with four (4) replicates. Each block comprised of twenty (20) plants with four (4) replicates. Two seeds were sown per hole. Inter and intra row spacing of 40 cm × 25 cm was observed respectively. The experiment was conducted between the months of May to September and the

recommended agronomical and plant protection practices by International Board for Plant Genetic Resources (IBPGR, 1984) was observed for successful growing of the plants.

3.6 Pollen Diameter

Freshly opened flowers buds were randomly collected from selected plants at the early hours of the morning. Matured anthers of the flowers were collected and squashed on a microscopic slide. A drop of 2 % acetocarmine stain was added and covered with a cover slip. A total of twenty (20) pollens were randomly selected from the microscopic slides for pollen diameter estimate. Diameters of selected pollens were measured using the eye piece graticule and recorded in micrometer (µm) with the microphotographs of the pollens taken at x 400 (Kim *et al.,* 2020).

3.7 Measurement of Morphological and Yield Parameters

The measurement of both the vegetative as well as yield parameters was recorded during the planting period following the methods stated by International Board for Plant Genetic Resources (IBPGR, 1984)

3.7.1 Plant height

The height of the plant was measured from the stem at soil level to the last node at four different stages of the plant growth (week 2, week 4, week 8 and at maturity respectively) using a tape rule in centimeters (cm).

3.7.2 Leaf length

Length of leaf was measured in centimeters (cm) using a tape rule, from the tip of the leave to the base where the leaf joins the stalk.

3.7.3 Leaf width

The width of the leaf was measured in centimeters (cm) across the leaf from side to side using a tape rule.

3.7.4 Leaf shape

This was determined from the ratio of length/width of fully developed terminal leaflet on the middle part of the stems and scored 3 for narrow "lanceolate" (L/W 2.2 or more), 5 for Intermediate (L/W 1.9-2.1) or 7 for broad "ovate" (L/W 1.8 or less).

3.7.5 Leaf size

This was estimated as the product of length (cm) and width (cm) of a fully developed terminal leaflet on the middle part of the stems and scored 3 for "small" (LW 70 cm^2 or less), 5 for "medium" (LW 70-149 cm^2) or 7 for "large" (LW 150 cm^2 or more).

3.7.6 Number of leaflets

The leaves on the leaflets are counted and scored 3 for 3 leaves, 5 for 4-6 leaves and 7 for 7 leaves or more.

3.7.7 Number of pods per plant

The number of pods on each plant were counted manually.

3.7.8 Weight of 100 pods

The weight of 100 randomly selected pods on each plant were weighed in grams (g) using an electronic weighing machine.

3.7.9 Weight of 100 seeds

The weight of 100 randomly selected seeds from each plant were weighed in grams (g) using an electronic weighing machine.

3.7.10 Pod length

The length of approximately 10 pods were taken from each plant in centimeters (cm) using a tape rule and the value was recorded.

3.7.11 Shattering percentage

This was determined by taking the percentage of the ratio of the number of shattered pods to the total number of pods plant for each plant.

3.7.10 Shattering score

Estimated percentage of pod splitting and seed shattering at a comparable time after maturity were recorded and scored; 1 (no shattering), 2 (slight shattering), 5 (medium shattering),7 (shattering) and 9 (highly shattering) as detailed below:

Table 3.1: Shattering descriptor

Scattering Score	Scattering Percentage (%)	Description
1	0	No shattering
2	1-20	Slight shattering
5	21-40	Medium shattering
7	41-60	Shattering
9	61-100	Highly shattering

3.8 Oil Extraction Process for Oil Characterization

Oil extraction using solvent methods by soxhlet apparatus was done following the methods stated by Association of Official Agricultural Chemists (AOAC, 2000) using food grade hexane as solvent. The soybean seeds were cleaned, cracked, dehulled, oven dried and grinded to powdery form. Grinded powder of the milled seeds at 50 g, with particle sizes of 0.18 - 0.9 mm was covered in filter papers and the oil was extracted using a Soxhlet extractor with n-hexane as the solvent. The solvent to solid ratio was mixed from 9:1-11:1 and the extraction temperature was carried out from 65 - 75 ^{o}C. The hexane in the solvent-oil combination was dissolved using a rotary vacuum evaporator and the oil was then collected and weighed.

3.8.1 Determination of the soybean oil yield

The percentage oil yield at the end of the extraction was determined and calculated using:

$$\text{Percentage oil yield (\%)}: \frac{\text{Weight of oil}}{\text{Total weight of sample}} \times 100 \quad (3.1)$$

3.8.2 Free fatty acid determination

The free fatty acid is the percentage by weight of a specified fatty acid. The percentage of free fatty acid in the oil was calculated as follows:

$$\text{\% Free fatty acid} = \frac{\text{V X N X 28.2}}{\text{Weight of sample}} \times 100 \quad (3.2)$$

Where,

V = average volume of NaOH (ml)

N = normality of NaOH (0.1)33

3.8.3 Iodine value determination

$$\text{\% Iodine} = \frac{(\text{V2} - \text{V 1}) \text{ X N X } 12.69}{\text{Weight of sample}} \times 100 \quad (3.3)$$

Where,

V2 = Titration of $Na_2S_2O_3$ blank (ml)

V1 = Titration of $Na_2S_2O_3$ sample (ml)

N = Normality of $Na_2S_2O_3$ solution (ml)

3.8.4 Acid value determination

$$\text{Acid value} = \frac{\text{A X B X (N)} X\ 56.1}{\text{Weight of sample}} \quad (3.4)$$

Where:

A = KOH used in titration (ml)

B = KOH used in the blank (ml)

N = normality of KOH

3.8.5 Saponification value determination

The saponification value was determined according to (Akpan *et al*., 2006)

$$\text{Saponification value} = \frac{(\text{B}-\text{S})\ X\ N\ X\ 65.1}{\text{Weight of sample}} \qquad (3.5)$$

Where;

B = 0.5N HCl required to titrate blank (ml)

S = 0.5N HCl required to titrate sample (ml)

N = normality of HCl solution (ml)

3.8.6 Ester value and glycerin determination

Ester value (EV) is calculated from the saponification value (SV) and acid value (AV) (Analytical methods to measure the constants of fats and oils, 2011).

Therefore, EV = SV – AV

While glycerin = EV × 0.05466.

Where,

EV = ester value

SV = saponification value

AV = acid value.

3.9 Data Analysis

Quantitative morphological values obtained were pooled for analyses. Analysis of variance (ANOVA) was used to determine the level of significance among the mutant lines and their respective control. Duncan's Multiple Range test (DMRT) was used to separate the mean among the pairs where there were significant differences among the means. Simple percentages and charts were used to show the seed retention capacity of each treatment. All data analysis was carried out using statistical package for social science (SPSS) version 24.0 at 5% level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Plant height

Presented in table 4.1 is the results of the plant height of the soybean genotypes. The analysis of variance (ANOVA) showed significant differences in the plant height of all the soybean genotypes. At week two (2) after planting, TGX1987-10F with EMS concentration 0.80 % was the lowest in plant height measuring 4.88 cm, while the highest plant height was recorded in TGX1987-10F, control with 8.38 cm. These values were significantly different from each other; however, TGX1987-10F with EMS concentration 0.40 % and TGX1987-10F, EMS concentration 0.20 % having the values 7.95 cm and 8.13 cm, respectively are not significantly different ($P>0.05$) from the highest plant height at week two.

At week four (4), TGX1987-10F with EMS concentration 0.20 % significantly had the highest plant height at 21.00 cm with the least being TGX1987-10F, EMS concentration 0.80 % with 8.50 cm. These values were significantly different from each other; however, TGX1835-10E control, TGX1987-10F control, TGX1448-2E control, TGX1904-6F (0.40 %) and TGX1987-10F (0.40 %) with values 19.25 cm, 19.50 cm, 19.75 cm, 20.25 cm and 20.25 cm respectively are not significantly different ($P>0.05$) from the highest plant height at week four.

Similarly, at week eight (8), statistical analysis showed that there was significant difference among the various genotypes with TGX1987-10F (0.20 %) having the highest plant height of 31.00 cm while TGX1904-6F (0.60 %) has the least with 15.50 cm. These values were significantly different from one another. However, TGX1987-10F (0.80 %) with 15.65 cm is not significantly different ($P<0.05$) from the least plant height (15.50 cm) at week eight; while TGX1987-10F (EMS concentration 0.40 %), TGX1904-6F (EMS concentration 0.40 %) and TGX1835-10E control with values 29.50 cm, 29.75 cm and 30.14 cm respectively are not significantly different ($P<0.05$) from the highest plant height at week eight.

At maturity, statistical analysis revealed that there was significant difference among the treated genotypes and their control, TGX1987-10F (0.60 %) and TGX1904-6F (0.60 %) both had the least plant height with 25.00 cm while TGX1835-10E (control) recorded the highest height with 42.25cm. This value recorded in the tallest plant was not significantly different from TGX1904-6F (0.40%) and TGX1987-10F (0.40%) both plants with the value of 41.00 cm and 38.75 cm obtained in TGX1448-2E (control) (Table 4.1).

Table 4.1 Plant Height of M_1 Soybean Genotype Recorded Different Weeks After Planting

	Plant Height (cm)			
Parameter	2 WAP	4 WAP	8 WAP	Maturity
TGX1448-2E (control)	7.63±0.55^{def}	19.75±1.55^{b}	28.25±1.44^{ab}	38.75±2.29^{b}
TGX1448-2E (0.20 %)	7.25±0.63^{bcdef}	17.00±2.12^{ab}	22.75±1.65^{ab}	32.75±3.15^{ab}
TGX1448-2E (0.40 %)	7.20±0.46^{bcdef}	12.75±1.11^{ab}	17.75±2.10^{ab}	29.25± 2.50^{ab}
TGX1448-2E (0.60 %)	7.00±0.41^{bcdef}	14.75±2.29^{ab}	22.88±3.73^{ab}	30.50±4.25^{ab}
TGX1448-2E (0.80 %)	5.63±0.63^{ab}	13.75±1.65^{ab}	22.75±2.60^{ab}	33.75±0.85^{ab}
TGX1835-10E (control)	7.00±0.58^{bcdef}	19.25±2.75^{b}	30.14±5.00^{b}	42.25±7.76^{b}
TGX1835-10E (0.20 %)	6.00±0.41^{abcd}	15.25±3.42^{ab}	21.88±5.10^{ab}	29.75±3.94^{ab}
TGX1835-10E (0.40 %)	5.88±0.52^{abc}	16.50±2.33^{ab}	24.00±3.19^{ab}	36.00±2.61^{ab}
TGX1835-10E (0.60 %)	6.13±0.24^{abcde}	14.25±2.66^{ab}	19.63±3.31^{ab}	36.25±1.65^{a}
TGX1835-10E (0.80 %)	6.00±0.20^{abcd}	14.25±1.93^{ab}	22.75±3.09^{ab}	33.75±4.05^{ab}
TGX1904-6F (control)	7.50±0.29^{cdef}	14.50±0.96^{ab}	25.00±3.08^{ab}	34.50±2.99^{ab}
TGX1904-6F (0.20 %)	7.08±0.33^{bcdef}	16.75±3.09^{ab}	21.75±3.53^{ab}	35.00±3.81^{ab}
TGX1904-6F (0.40 %)	7.75±0.32^{ef}	20.25±3.28^{b}	29.75±7.49^{b}	41.00±3.58^{b}
TGX1904-6F (0.60 %)	6.00±0.4^{abcd}	12.00±2.16^{ab}	15.50±2.95^{a}	25.00±3.72^{a}
TGX1904-6F (0.80 %)	5.75±0.32^{ab}	16.50±4.21^{ab}	21.38±6.06^{ab}	33.50±4.97^{ab}
TGX1987-10F (control)	8.38±1.03^{f}	19.50±3.18^{b}	24.25±3.57^{ab}	34.50±2.99^{a}
TGX1987-10F (0.20 %)	8.13±0.43^{f}	21.00±2.55^{b}	31.00±3.63^{b}	35.00±3.81^{ab}
TGX1987-10F (0.40 %)	7.95±0.71^{f}	20.25±4.11^{b}	29.50±5.24^{b}	41.00±3.58^{b}
TGX1987-10F (0.60 %)	6.13±0.31^{abcde}	15.25±3.07^{ab}	18.63±4.48^{ab}	25.00±3.72^{a}
TGX1987-10F (0.80 %)	4.88±0.43^{a}	8.50±0.65^{a}	15.63±1.84^{a}	33.50±4.97^{ab}

Values are means ± standard error, values followed by the same alphabet(s) on the column are significantly different at P<0.05 tested by Duncan Multiple Range Test.

WAP = Week after planting

4.1.2 Phenotypic characterisation

The results of the qualitative observations in the treated phenotypes and controls are shown in table 4.2. In terms of leaf shape, three of the five (5) genotypes of V1; (TGX1448-2E; control, EMS Concentration 0.20 % and 0.40%) have ovate shaped leaves (Plates V and VII), and the remaining two genotypes (TGX1448-2E; EMS Concentration 0.60 % and 0.80 %) having intermediate leaves. Three (3) of all five (5) genotypes of V2 (TGX1835-10E, control, EMS Concentration 0.20 % and 0.40 %) have ovate shaped leaves (Plates V and VII) except the EMS concentration 0.60 % and 0.80 % which had intermediate and lanceolate leaves (Plates VI and VIII) respectively. Two genotypes of V3 (TGX1904-6F) and V4 (TGX1987-10F) including their controls have ovate leaves (Plates V and VII). Two of V3 (TGX1904-6F; 0.40 % and 0.60 % EMS concentration) have intermediate leaves and 0.80% EMS concentration had lanceolate leaves (Plates VI and VIII). Similarly, two of the five genotypes of V4 (TGX1987-10F), that is, 0.60 % and 0.80 % EMS concentration have intermediate leaves while 0.40 % EMS concentration have lanceolate leaves (Plates VI and VIII). In terms of leaf size, all the genotypes had small leaflet sizes.

The leaflet number ranged from three to five leaflets. It was noted that the control, 0.20 % and 0.40 % EMS concentration of all varieties had three leaflets (as shown in plates VII and VIII) while the 0.60 % and 0.80 % EMS concentration of all varieties had five leaflet number (as shown in plates V and VI). The colour of the leaves appeared in different shades of green except in TGX1904-6F with 0.60 % EMS concentration and TGX1835-10E with 0.80 % EMS concentration which had chlorosis (as shown in plates III and IV). In the flowers, the colour of the corolla were purple (as shown in plate II) except TGX1987-10F with 0.40 % EMS concentration which had white corolla (as shown in plate I) (Table 4.2).

Table 4.2 Some Phenotypic Characterisation of the M_1 Soybean Genotype

Parameter	Leaflet Shape	Leaflet size	Leaflet number	Corolla colour	Leaf colour	Mature pod colour
TGX1448-2E (control)	Ovate	Small	Three	Purple	Green	Tan
TGX1448-2E (0.20 %)	Ovate	Small	Three	Purple	Green	Tan
TGX1448-2E (0.40 %)	Ovate	Small	Three	Purple	Green	Brown
TGX1448-2E (0.60 %)	Intermediate	Small	Five	Purple	Green	Tan
TGX1448-2E (0.80 %)	Intermediate	Small	Five	Purple	Green	Tan
TGX1835-10E (control)	Ovate	Small	Three	Purple	Green	Tan
TGX1835-10E (0.20 %)	Ovate	Small	Three	Purple	Green	Tan
TGX1835-10E (0.40 %)	Ovate	Small	Three	Purple	Green	Brown
TGX1835-10E (0.60 %)	Intermediate	Small	Five	Purple	Green	Tan
TGX1835-10E (0.80 %)	Lanceolate	Small	Five	Purple	Green/yellow	Brown
TGX1904-6F (control)	Ovate	Small	Three	Purple	Green	Tan
TGX1904-6F (0.20 %)	Ovate	Small	Three	Purple	Green	Tan
TGX1904-6F (0.40 %)	Intermediate	Small	Three	Purple	Green	Brown
TGX1904-6F (0.60 %)	Intermediate	Small	Five	Purple	Green/yellow	Brown
TGX1904-6F (0.80 %)	Lanceolate	Small	Five	Purple	Green	Brown
TGX1987-10F (control)	Ovate	Small	Three	Purple	Green	Tan
TGX1987-10F (0.20 %)	Ovate	Small	Three	Purple	Green	Tan
TGX1987-10F (0.40 %)	Lanceolate	Small	Three	White	Green	Tan
TGX1987-10F (0.60 %)	Intermediate	Small	Five	Purple	Green	Tan
TGX1987-10F (0.80 %)	Intermediate	Small	Five	Purple	Green	Brown

Plate I: TGX1987-10F, EMS Concentration 0.40 % having white corolla

Plate II: TGX1904-6F, control having purple corolla

Plate III: TGX1904-6F, EMS Concentration 0.60 % having leaves with chlorosis

Plate IV: TGX1835-10E, EMS Concentration 0.80 % having leaves with chlorosis

Plate V: TGX1448-2E, EMS Concentration 0.80 % having pentafoliate ovate leaves

Plate VI: TGX1904-6F, EMS Concentration 0.80 % having pentafoliate lanceolate leaves

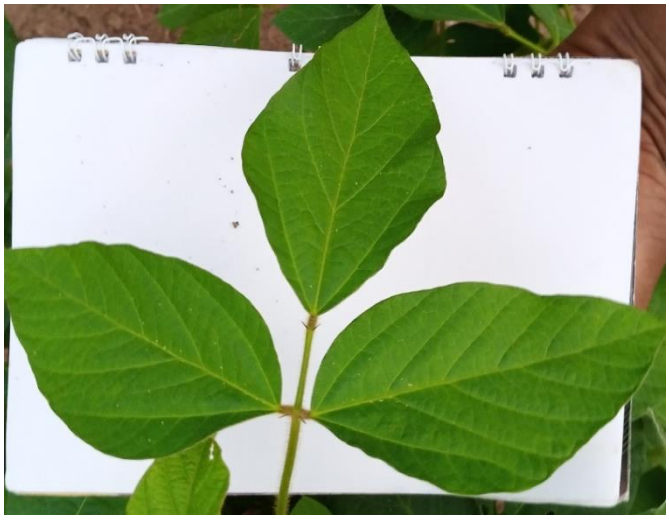

Plate VII: TGX1448-2E, EMS Concentration 0.40 % having trifoliate ovate leaves

Plate VIII: TGX1835-10E, EMS Concentration 0.20 % having trifoliate lanceolate leaves

Plate IX: Pod colour variation

(L-R: brown, tan, brown, tan, tan)

Plate X: Variation in pod sizes

(L-R: 4 seeds, 3 seeds, 2 seeds, 1 seed)

4.1.3 Number of pods per plant and number of seeds per pod

The number of pods per plant was highest in TGX1835-10E (control) with 188.25 pods while the lowest was recorded in TGX1987-10F (0.80 % EMS concentration) with 22.00 pods. Statistical analysis shows that these values were significantly different from each other and all the values from the other genotypes (Table 4.3).

Also, genotype TGX1904-6F (EMS Concentration 0.40 %) have the highest number of seeds per pod with the value of 3.15 seed per pod, while TGX1448-2E (EMS concentration 0.40 %) with 1.55 seed per pod recorded the least value. These values were significantly different from each other. However, TGX1448-2E (EMS concentration 0.40 %), TGX1835-10E (EMS concentration 0.40 %) and TGX1987-10F (EMS concentration 0.40 %) all having the value of 3.05 seeds per pod are not significantly different ($P>0.05$) from the highest number of seeds per pod (Table 4.3).

4.1.4 Length of pod, weight of 100 Pod and weight of a 100 seed

The least pod length was recorded in TGX1835-10E, EMS Concentration 80 % with 3.20 cm and the longest pod was obtained in TGX1448-2E (0.20 %) with the value of 4.40 cm, these values were significantly different from each other, however, TGX1448-2E, EMS concentration 0.40 %, with 3.25 cm was not significantly different from the least pod length, while TGX1904-6F (EMS Concentration 0.40%) with 4.38 cm was not significantly different from the longest pod length. (Table 4.4).

Statistical analysis shows that there were significant differences among genotypes in table 4.4 with regards to 100 pod weight. The least 100 pod weight was recorded in TGX1987-

10F (0.60 % EMS Concentration) with the value of 22.13 g and the highest weight was recorded in TGX1987-10F with EMS Concentration 0.40 % having 39.13 g. These values were significantly different from one another. More so, there was also significant differences between TGX1448-2E, with EMS concentration 0.20 % and TGX1904-6F with EMS Concentration 0.40 % compared to other genotypes (Table 4.3).

The highest value for 100 seed weight was recorded in TGX1987-10F (EMS Concentration 0.20 %) with 14.75 g and the lowest value was recorded as 9.20 g in TGX1904-6F, 0.60 % EMS Concentration (Table 4.3). These highest and lowest values were Statistically significant different from one another and from all the value of other genotypes.

Table 4.3 Some Yield Parameters of the M_1 Soybean Genotypes

Parameters	Number of Pods Per Plant	Number of Seeds Per Pod	100 pods Weight(g)	100 seed Weight (g)	Pod Length (cm)
TGX1448-2E (control)	116.00 ± 59.65^{bcde}	2.00±0.00^{bcd}	29.88 ± 1.97^{cdef}	12.03 ± 0.13^{bcde}	3.45 ± 0.13^{ab}
TGX1448-2E (0.20 %)	104.25 ± 31.67^{abcde}	2.15±0.05^{cd}	32.40 ± 0.75^{efg}	12.38 ± 0.43^{def}	4.40 ± 0.20^{e}
TGX1448-2E (0.40 %)	46.50 ± 17.91^{abc}	3.05±0.05^{e}	26.03 ± 2.41^{abcc}	11.38 ± 0.43^{bc}	3.25 ± 0.06^{a}
TGX1448-2E (0.60 %)	134.75 ± 38.90^{cde}	2.05±1.00^{bcd}	31.20 ± 1.91^{def}	11.33 ± 0.24^{bc}	3.63 ± 0.06^{abc}
TGX1448-2E (0.80 %)	75.00 ± 9.63^{abcd}	1.55±0.17^{a}	29.40 ± 1.83^{cde}	12.63 ± 0.33^{defg}	4.18 ± 0.12^{de}
TGX1835-10E (control)	188.25 ± 25.39^{e}	2.00±0.00^{bcd}	34.78 ± 3.23^{fgh}	11.38 ± 0.24^{bc}	3.60 ± 0.12^{abc}
TGX1835-10E (0.20 %)	101.00 ± 27.24^{abcde}	2.10±0.10^{cd}	34.48 ± 0.19^{fgh}	13.40 ± 0.08^{gh}	4.30 ± 0.11^{de}
TGX1835-10E (0.40 %)	63.75 ± 29.81^{abcd}	3.05±0.05^{e}	27.13 ± 0.92^{bcd}	13.00 ± 0.39^{fgh}	3.43 ± 0.22^{ab}
TGX1835-10E (0.60 %)	88.50 ± 25.80^{abcd}	2.40±0.22^{d}	27.03 ± 0.51^{bcd}	12.45 ± 0.26^{def}	3.45 ± 0.18^{ab}
TGX1835-10E (0.80 %)	57.75 ±21.84^{abc}	1.80±0.14^{abc}	29.90 ± 0.04^{cdef}	11.80 ± 0.16^{bcd}	3.20 ± 0.12^{a}
TGX1904-6F (control)	87.25 ± 14.56^{abcd}	2.00±0.81^{bcd}	26.30 ± 0.59^{abcd}	12.88 ± 0.17^{efgh}	3.50 ± 0.17^{abc}
TGX1904-6F (0.20 %)	134.00 ± 30.58^{cde}	2.05±0.05^{bcd}	34.58 ± 1.69^{fgh}	12.18 ± 0.41^{cdef}	3.58 ± 0.21^{abc}
TGX1904-6F (0.40 %)	150.75 ± 15.72^{de}	3.15±0.10^{e}	34.95 ± 0.18^{gh}	13.70 ± 0.33^{h}	4.38 ± 0.10^{e}
TGX1904-6F (0.60 %)	55.25 ± 27.53^{abc}	1.90±0.17^{abc}	22.75 ± 2.04 ^{ab}	9.20 ± 0.36^{a}	3.45 ± 0.26^{ab}
TGX1904-6F (0.80 %)	47.50 ± 10.68^{abc}	1.65±0.21^{ab}	27.18 ± 1.99^{bcd}	11.15 ± 0.33^{b}	3.53 ± 0.14^{abc}
TGX1987-10F (control)	128.75 ± 7.92^{cde}	2.00±0.00^{bcd}	29.50 ± 0.33^{cde}	11.78 ± 0.23^{bcd}	3.50 ± 0.04 ^{abc}
TGX1987-10F (0.20 %)	96.25 ± 22.12^{abcd}	2.00±0.14^{bcd}	24.03 ± 1.78^{ab}	14.75 ± 0.26^{i}	4.13 ± 0.14^{de}
TGX1987-10F (0.40 %)	127.25 ± 42.49^{cde}	3.05±0.05^{e}	39.13 ± 1.57^{h}	13.63 ± 0.12^{h}	4.13 ± 0.17^{de}
TGX1987-10F (0.60 %)	24.75 ± 5.14^{ab}	2.05±0.05^{bcd}	22.13 ± 0.51^{a}	11.28 ± 0.13^{bc}	3.88 ± 0.17^{bcd}
TGX1987-10F (0.80 %)	22.00 ± 5.31^{a}	1.75±0.26^{abc}	31.30 ± 0.82^{defg}	11.78 ± 0.18^{bcd}	3.98 ± 0.06^{cde}

Values are means ± standard error, values followed by the same alphabet(s) on the column are significantly different at P<0.05 tested by Duncan Multiple Range Test.

4.1.5 Pod shattering

The result of pod shattering of the various soybean genotype is presented in Table 4.4. There was a statistically significant difference between the highest and the lowest shattering percentage. TGX1987-10F (control) had the highest shattering percentage of 44.18 % followed by TGX1904-6F (control) with 37.00 %. The lowest shattering percentage was noted in TGX1448-2E (0.20%), TGX1448-2E (0.40 %), TGX1448-2E (0.60 %), TGX1835-10E (0.60 %), TGX1835-10E (0.60 %), TGX1835-10E (0.80 %), TGX1904-6F (0.40 %), TGX1904-6F (0.80 %), TGX1987-10F (0.40 %), TGX1987-10F (0.60 %) and TGX1987-10F (0.80 %) which all recorded 0.00 %. More so, TGX1448-2E (0.60 %), TGX1904-6F (0.60 %) and TGX1904-6F (0.20 %) recorded slight shattering with percentage of 1.18 %, 1.50 % and 2.50 % respectively. These values were statistically insignificant from one another and from the lowest shattering percentage (Table 4.4). It was also observed that all the treated genotypes with 0.40 % and 0.80 % EMS concentration produced non-shattering pods in all the genotypes.

Table 4.4 Shattering Percentage, Score and Description of the M_1 Soybean Genotype

Parameters	Shattering percentage (%)	Scattering score	Description
TGX1448-2E (control)	12.50 ± 7.50 [ab]	2	Slight shattering
TGX1448-2E (0.20 %)	0.00 ± 0.00[a]	1	No shattering
TGX1448-2E (0.40 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1448-2E (0.60 %)	1.18 ± 0.87 [a]	2	Slight shattering
TGX1448-2E (0.80 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1835-10E (control)	26.35 ± 10.42[bc]	5	Medium shattering
TGX1835-10E (0.20 %)	15.00 ± 6.12 [ab]	2	Slight shattering
TGX1835-10E (0.40 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1835-10E (0.60 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1835-10E (0.80 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1904-6F (control)	37.00 ± 5.07[cd]	5	Medium shattering
TGX1904-6F (0.20 %)	2.50 ± 2.50 [a]	2	Slight shattering
TGX1904-6F (0.40 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1904-6F (0.60 %)	1.50 ± 1.50 [a]	2	Slight shattering
TGX1904-6F (0.80 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1987-10F (control)	44.18 ± 9.26[d]	7	Shattering
TGX1987-10F (0.20 %)	17.50 ± 11.09[b]	2	Slight shattering
TGX1987-10F (0.40 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1987-10F (0.60 %)	0.00 ± 0.00 [a]	1	No shattering
TGX1987-10F (0.80 %)	0.00± 0.00 [a]	1	No shattering

Values are means ± standard error, values followed by the same alphabet(s) on the column are significantly different at P=0.05 tested by Duncan Multiple Range Test.

Plate XI: TGX1987-10F, control:
A highly shattering plant

Plate XII: TGX1904-6F, with EMS
Concentration 0.40 %: A no-shattering plant

4.1.6 Pollen diameters

With the exception of TGX1904-6F which had the highest pollen diameter at the control (25.00 µm), the highest pollen diameter for both TGX1835-10E and TGX1448-2E (27.50 µm) as well as the highest for TGX1987-10F (25.00 µm) were obtained at 0.40 % EMS concentration. This highest value (25.00 µm) obtained in TGX1987-10F 0.40 % EMS concentration was statistically insignificant when compared to the control in the same genotype. Significantly lowest pollen diameter was recorded at 0.80 % concentration for all the genotypes with the value of 22.00 µm, 22.50 µm, 22.00 µm and 22.50 µm for TGX1448-2E, TGX1835-10E, TGX1904-6F and TGX1987-10F respectively (Table 4.5).

Table 4.5 Pollen Diameter of the M_1 Soybean Genotype

Treatment	TGX1448-2E (µm)	TGX1835-10E (µm)	TGX1904-6F (µm)	TGX1987-10F (µm)
Control	25.00 ± 0.00^{bc}	22.50 ± 0.00^{a}	25.00 ± 0.00^{bc}	25.00 ± 0.00^{bc}
0.20	23.75 ± 1.25^{ab}	27.00 ± 0.00^{cd}	23.75 ± 1.25^{ab}	23.75 ± 1.25^{ab}
0.40	27.50 ± 0.00^{cd}	27.50 ± 0.00^{cd}	23.75 ± 1.25^{ab}	25.00 ± 0.00^{bc}
0.60	22.50± 0.00^{a}	25.50 ± 0.00^{bc}	22.50 ± 0.00^{a}	23.75 ± 1.25^{ab}
0.80	22.00 ± 0.00^{a}	22.50 ± 0.00^{a}	22.00 ± 0.00^{a}	22.50 ± 0.00^{a}

Values are means ± standard error, values followed by the same alphabet(s) on the column are significantly different at P=0.05 tested by Duncan Multiple Range Test.

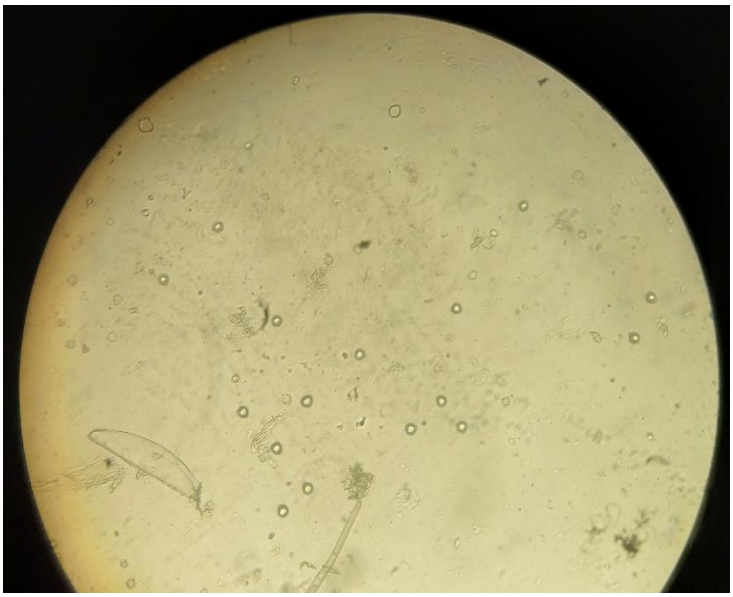

Plate XIII: Microscopic presentation of Pollen grains (magnification X10)

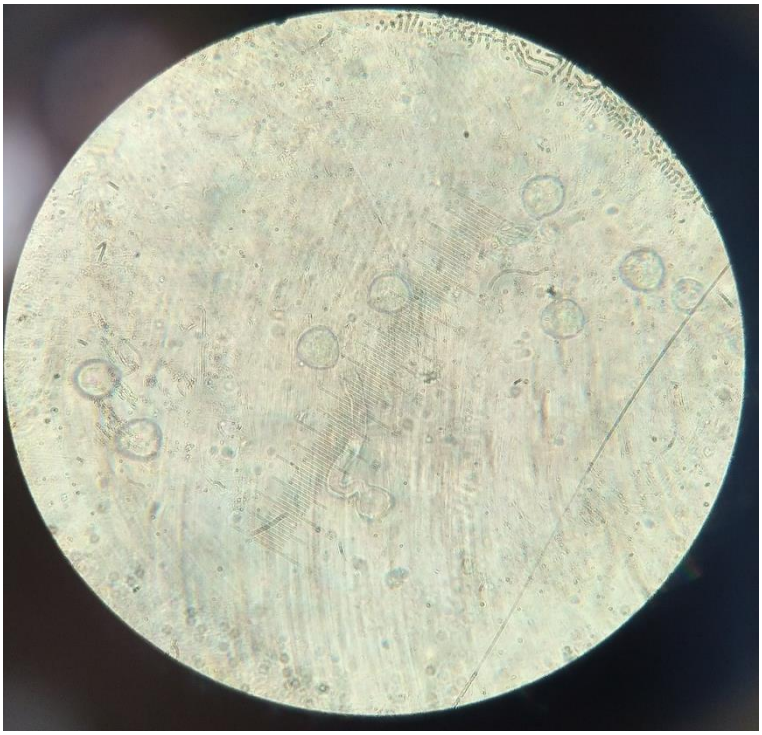

Plate XIV: Microscopic presentation of pollen grains using the eyepiece graticule for measurement (magnification X40)

4.1.7 Percentage oil yield and characterization of the soybean genotype

Only twelve (12) genotypes were selected for percentage oil yield and characterization out of the total of twenty (20) soybean genotypes (including control) used for this study. This was to ensure that the EMS treatment did not have potentially negative effect on the yield and characterization the genotypes with the maximum yield potential.

The percentage oil that was extracted from soybean seeds accounted for a certain percentage of the weight of the soybean seeds. Statistics revealed significant differences among the various genotypes as represented on Table 4.6. TGX1904-6F with 20 % EMS Concentration have the highest percentage oil yield of 21.27 %, followed by TGX1835-10E (0.60 %), with 21.20%, while TGX1448-2E, with EMS Concentration 0.20 % have the lowest percentage oil yield of 15.44 %. The free fatty acid in percentage of the soybean genotypes ranged between 0.44-2.88 %. The least free fatty composition is 0.44 % recorded in TGX1835-10E, control, while TGX1448-2E, with EMS Concentration 0.60 % have the highest free fatty acid percentage of 2.88 %. These values were statistically different from one another and from the other genotypes ($P<.05$). The percentage glycerin value ranged between 7.12-10.25 % with TGX1904-6F (EMS Concentration 0.40 %) recording the highest value and TGX1448-2E (control) the least value. These values differed from each other significantly as well as from all other genotypes.

The statistical analysis of the acid value of the soybean genotypes revealed significant differences among the different genotypes; with TGX1835-10E (control) having the least value of 0.87 % and TGX1448-2E (0.60 %) having the highest value of 5.87 %. These values were different from each other significantly as well as from the value of all other genotypes.

TGX1904-6F, with EMS Concentration 0.20 % recorded the highest iodine value of 11.83 (I_2/100g). The lowest iodine value is recorded in TGX1448-2E, with EMS Concentration 0.60 % with the value of 3.11 (I_2/100g). These values were statistically significant in the difference between each value as well as from the value of other genotypes. The peroxide value of the soybean genotype ranged between 1.95-16.55 mEq/kg with TGX1987-10F (control) recording the highest value and TGX1448-2E (control) having the lowest (Table 4.6).

Analysis in the study reveals a significant difference in the saponification value and ester value among the genotypes, with TGX1904-6F (EMS Concentration 0.40 %) being the highest with the values of 188.50 mgKOH/g and 187.41 mgKOH/g, respectively; The lowest value in both was recorded in TGX1448-2E (control) with the value of 132.40 mgKOH/g and 129.81 mgKOH/g, respectively. These were significantly different from each other and from the values of the different genotypes ($P<.05$). It was noted that the value of the refractive index of all twenty (12) genotypes is 1.47.

Table 4.6 Oil Yield and Other Characterization of the M_1 Soybean Genotypes

Parameter	Oil yield (%)	Free Fatty acid (%)	Glycerin (%)	Acid value (%)	Iodine value (I_2/100g)	Peroxide value (mEq/kg)	Saponification value (mgKOH/g)	Ester value (mgKOH/g)	Refractive index
TGX1448-2E (control)	20.00±0.69^{de}	1.06±0.05^{e}	7.12±0.07^{a}	2.11±0.09^{h}	6.73±0.01^{d}	1.95±0.05^{a}	132.40±1.41^{a}	129.81±1.81^{a}	1.47±0.00
0.20%	15.44±0.81^{a}	0.81±0.02^{d}	8.07±0.08^{c}	1.60±0.03^{e}	5.42±0.01^{b}	6.25±0.05^{e}	149.23±1.41^{c}	147.63±1.43^{a}	1.47±0.00
0.60%	19.60±0.28^{def}	2.88±0.08^{g}	8.46±0.24^{d}	5.87±0.04^{i}	3.11±0.01^{a}	5.10±0.10^{d}	157.64±1.40^{d}	154.69±4.35^{c}	1.47±0.00
TGX1835-10E (control)	19.32±0.01^{de}	0.44±0.02^{a}	7.77±0.12^{b}	0.87±0.03^{a}	8.62±0.01^{f}	2.16±0.06^{a}	142.92±2.11^{b}	142.05±2.14^{b}	1.47±0.00
0.20%	20.67±0.02^{def}	0.64±0.02^{c}	7.94±0.01^{bc}	1.27±0.03	5.58±0.04^{b}	3.40±0.10^{c}	146.56±0.14^{bc}	145.30±0.17^{b}	1.47±0.00
0.60%	21.20±0.02^{ef}	0.58±0.02^{bc}	7.95±0.08^{bc}	1.15±0.03^{cd}	7.52±0.00^{e}	2.70±0.10^{b}	146.42±1.40^{bc}	145.27±1.37^{b}	1.47±0.00
TGX1904-6F (control)	19.35±0.20^{def}	0.90±0.03^{d}	7.83±0.00^{bc}	1.80±0.06^{f}	6.50±0.01^{c}	9.35±0.05^{g}	145.02±0.00^{b}	143.22±0.06^{b}	1.47±0.00
0.20%	21.27±0.41^{f}	0.52±0.01^{ab}	7.92±0.02^{bc}	1.04±0.03^{bc}	11.83±0.01^{i}	7.15±0.05^{f}	145.86±0.28^{bc}	144.82±0.25^{b}	1.47±0.00
0.40%	19.01±0.92 ^{cd}	0.55±0.02^{bc}	10.25±0.08^{f}	1.10±0.03^{bc}	11.21±0.01^{j}	12.68±0.13^{i}	188.50±1.40^{f}	187.41±1.38^{e}	1.47±0.00
TGX1987-10F (control)	16.54±0.12^{ab}	0.99±0.03^{c}	8.67±0.02^{d}	2.00±0.06^{d}	9.36±0.10^{g}	10.60±0.10^{h}	160.45±0.28^{d}	158.48±0.22^{c}	1.47±0.00
0.20%	17.50±0.36^{bc}	0.64±0.02^{de}	9.79±0.02^{e}	1.280.04^{g}	10.59±0.13^{h}	16.55±0.05^{k}	180.36±0.28^{e}	179.09±0.33^{d}	1.47±0.00
0.40%	20.57±1.08^{def}	0.50±0.03^{ab}	8.70±0.02^{d}	0.99±0.06^{ab}	10.57±0.11^{h}	13.85±0.15^{j}	160.10±0.35^{d}	159.12±0.41^{c}	1.47±0.00
FAO/WHO standard	~ 19.00	~ 0.75	~ 10.00	0.60 -4.00	12.40-13.90	10.00-15.00	189.00-195.00	188.40-191.00	1.466-1.470

Values are means ± standard error, values followed by the same alphabet(s) on the column are significantly different at P=0.05 tested by Duncan Multiple Range Test.

4.2 Discussion of Results

The success of breeding using mutagenesis depends firstly on the efficiency and effectiveness of the mutagen of interest (Arisha *et al*., 2015). However, mutagen variations have varying effects either as a result of the concentration used or the materials used on. Optimizing the concentration of the mutagen to be used is necessary before treatment of the materials in order to guarantee high frequency of mutation as well as obtain enough viable seeds. When mutagens are used in high concentration, it effects to plants detrimental; however, higher mutation frequency could also be an effect of higher concentrations of the mutant used (Shah *et al*., 2015).

4.2.1 Plant height

Soybean is a leguminous plant that can survive in a varying range of soils, although soyabeans grow optimally in humid alluvial soils that have good organic content. This study found that at maturity, the range of the plant height between 25.00cm and 42.00 cm, with an average of 34.05 cm. This is within the range of 19.93-67.00 cm with an average of 45.12 cm reported by Rajkumar *et al.* (2010). Pushpa and Ketoswara (2013) also reported a similar finding with the mean plant height value of 38.35 cm and range between of 30-58 cm. These similar findings could be associated with similar genotype of seed. Ulafić *et al.* (2020) opined that soybean could attain a height of around 1 m (3ft), while Acquaah (2007) also reported that soybean height at maturity varies from less than 2.0 m, these varying results could be caused by variation in agro-ecological zones where experiments were carried out. Sagel *et al.* (2017) also reported similar result to that recorded of this study in the range of plant height at maturity as 35.00 – 56.30 cm and a mean value of 46.18 cm, this similarity could be

attributed to the use of the same chemical mutagen. Gopinath and Pavadai (2015), in their study noted the highest height of the plant grew to reach 82.91cm. These differences in the highest/tallest plants can be due to the different mutagen used as well as its concentration.

4.2.2 Morphological observation

Changes in several morphological characters were observed in this study especially changes in leaf morphology which were very visible in the narrow/lanceolate leaves, tetrafoliates, pentafoliates, leaves in varying shades of green with some having chlorosis. The flowers were purple color except a few plants with white corollas. These observations concede with the findings of Espina *et al.* (2018) where several phenotypic variations were observed in leaf morphology/leaf phenotypes with narrow leaf, tetra-foliate and penta-foliate. With the leaf known as the main site of photosynthesis, increase in the number of leaves results in larger leaf surface area, which in turn increases the rate of photosynthesis, as the leaf receives maximized sunlight for photosynthesis. Variations in the leaf shapes in some of the treatments from ovate to intermediate and lanceolate could be attributed to variations in the physiological process of leaf formation due to the mutagenic effects. These observations are also in agreement with the findings of Shilpashree *et al.* (2021), who noted the leaf shape variability as lanceolate and ovate; with the leaf color showing noticeable variation from normal to dark green, the genotypes having white or purple flowers. Zhou *et al.* (2019) also reported that in many mutants, EMS mutagenesis resulted in a wide range of morphological phenotypes, such as chlorotic leaves and numerous leaflets at a single node, while most wild-type soybean leaves were trifoliolate.

4.2.3 Number of pods per plant and number of seed per pod

The mean value of pod in every plant as recorded in this study is 92.48 pods with a range of 22.00 – 188.25 pods per plant. Contrary to the highest pod per plant, Ningsih *et al.* (2019) observed that the highest number of pods per plant was between 62.31 pods. Also, Olena *et al*. (2020) reported 138.73 per plant to be the highest number of pods per plant. The differences identified in these results could be associated with the differences in the genetic make-up of the variety used as well as the soil composition.

The number of seeds per pod observed in this study was between 1.55 – 3.15 with an average of 2.19 seed per pod and is consistent with findings from Pushpa and Ketoswara (2013) which reported 1.00- 3.00 number of seeds per pod; and Kataliko *et al.* (2019) who recorded number of seeds per pod ranging between 2.03 – 2.54 seeds. This reveals that the number of seeds per pod is significantly affected by soybean genotypes. In agreement with this finding, Nwofia *et al*. (2016) opined that the number of seeds per pod are influenced by the genetic make-up.

4.2.4 Length of pod and weight of a 100 seed

The length of pod recorded in this research ranged from 3.20 – 4.40 cm with an average value of 3.75cm. This finding is similar to a study which reported that length of the pod to be between 3.56-4.34 cm (Ningsih *et al.*, 2019). Olena *et al*. (2020) reported an average pod length of 3.90 cm.

The 100 seed weight recorded in this study ranged between 9.20 and 14.75 g which is similar to the findings of Saha and Matiul (2022) that recorded 100 seed weight to vary from 8.39 g to 13.15 g. These findings have close conformity with the work of Pankaj (2013) which revealed that the

weight of100 seed among varieties ranged between 10.55 and 16.34 g. These findings are in line with the findings of Pusha and Ketosawara (2013) with the range of 7.26 to 15.38 g. The small variations in the value range reported by the various authors and this study could be due to the differences in the number of genotypes used and the environment of the study.

4.2.5 Pod shattering

In this study, the highest shattering percentage was reported at 44.18 %, which genotypes with shattering resistance ranged between 0.00 - 15.00 %. According to a study by Krisnawati *et al*. (2020), Krisnawati and Adie (2016) based on the shattering incidence, resistant genotypes were observed to have pod shattering percentage ranging from 7–10 %. While susceptible genotypes had 100% shattering severity. In another study, susceptible genotypes records 34 to 99 % seed losses in soybean (Kataliko *et al.,* 2019). These various finding could be attributed with genetic make-up as well as induced breeding of the soybean genotypes. The non-shattering between 0.40 - 0.80 % treated plants could also be associated with the presence of the induced resistance gene(s) in the genotypes by the mutagen. This indicates the effectiveness of concentrations in inducing the seed retention trait in the plants.

4.2.6 Pollen diameter

A study conducted by Kim *et al.* (2020) revealed that the diameter of pollen grain of non-transgenic soybean was between the ranges of 24.8 and 27.3 μm while that of transgenic soybean ranged between 25.1 and 26.6 μm. Similarly, Kaltchuk-Santos *et al*. (1993) reported that the diameter of pollen grain ranged from 25.23 to 26.85 μm. The ranged pollen diameter (22.00 – 27.50 μm) obtained in this study confirmed the earlier report of Kim *et al.* (2020) and Kaltchuk-Santos *et al*. (1993). Horak *et al*. (2015) reported that the pollen grain diameter

of soybean observed were between the ranges of 21.6 to 23.7 μm, which was slightly smaller than the value estimated in this study. Though, the mean diameter of pollen grain of soybean was found to be larger, ranging between 27.3 μm and 30.4 μm (Yoshimura, 2011).

4.2.7 Percentage oil yield of the soybean genotype

The percentage oil yield recorded in this study has an average of 19.20 % and ranged between 15.44 - 21.27 % which is in line with the study conducted by Ningsih *et al.* (2019), who recorded 18.95 - 21.85 %. Olena *et al.* (2020) also reported oil yield to range between 18.8 - 19.4 % and 15–22 % was recorded by Singh (2010). Zhou *et al.* (2019) recorded 20.7 % as the highest percentage oil yield. Contrary to these results, Espina *et al.* (2018) reported higher oil yield of 25 % and attributed the spike to mutagenesis.

Study has shown that optimum yield potential of soybeans is significantly influenced by the number of pod in a plant, number of seed in a pod, and the weight of seed (Taghavi *et al.*, 2012) as well as its seed retention capacity. The maximum yield parameter in this study was recorded in plants treated with 0.40 and 0.60% EMS concentration. These genotypes had a combination of increased number of pod per plants, seed per pod, seed weight and most importantly, a very high seed retention capacity. This finding is agreeable to a study by Gopinath and Pavadai (2015) who recorded maximum yield parameter at 0.50 % of EMS treatments, Archana *et al.* (2004) also recorded optimum yield at 0.50 % of EMS treatments. Findings from the above studies are also consistent with the study conducted by Espina *et al.* (2018) which recorded optimum yield at 50 % of EMS treatment.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The present study provides evidence about the enormous differences that exist between the different soybean genotypes and the possibility of an immense variability that exists in EMS as a chemical mutagen for soybean mutagenesis.

TGX1448-2E (EMS Concentration 0.20 %), TGX1448-2E (EMS Concentration 0.60 %), TGX1904-6F (EMS Concentration 0.20 %), TGX1904-6F (EMS Concentration 0.40 %) and TGX1987-10F (EMS Concentration 0.40 %) showed excellent agromorphological parameters having recorded high yield, bigger seeds, more seeds per pod and recording little to no shattering.

Based on the findings noted in this study, application of EMS for soybean chemical mutagenesis is effective for seed retention, greatly reducing pod shattering.

The largest pollen diameters were obtained at 0.40 % EMS concentration for mostly all genotypes and notably smallest at 0.80 % concentration for all the genotypes.

There was increase in percentage oil with increase in EMS concentration especially in TGX1835-10E and TGX1987-10F. Glycerin percentage also increased in all genotypes with increase in EMS concentration.

5.2 Recommendations

I. To promote high yield for large scale production, TGX1448-2E (EMS Concentration 0.20 %), TGX1448-2E, (EMS Concentration 0.60 %), TGX1904-6F (EMS Concentration 0.20 %), TGX1904-6F (EMS Concentration 0.40 %) and TGX1987-10F (EMS Concentration 0.40 %) is recommended for its high yield with little or no shattering qualities.

II. Further research should be carried out on the use of mutagenesis to improve seed retention capacity of soybean in subsequent mutant lines and favorable characteristics noted these mutants can be exploited for future soybean breeding programs.

III. It is proposed that this EMS mutant population should be explored in further studies that include the screening for amino acid pathways, phytic acids, allergens, and other agronomic traits in soyabeans.

Contributions to Knowledge

The thesis established that Ethyl Methane Sulphonate (EMS) induced mutation has provided beneficial effects on the agromorphological and seed retention capacity of the soybean genotype.

The phenotypic assessment revealed that TGX1987-10F (0.40%) had whitish flowers as opposed to the pinkish to purplish flowers observed in all the other plants; and TGX1904-6F (0.60%) and TGX1835-10E (0.80%) had chlorotic leaves.

The quantitative parameters reveled that TGX1904-6F (0.40%) had the highest number of seed per pod at 3.15 seeds per pod. TGX1987-10F (0.40%) at 39.13 g recorded the largest weight for 100 pods and at 0.20 %, same plant recorded largest weight of 100 seed at 14.75 %.

TGX1448-2E (0.20 %, 0.40 %, 0.60%), TGX1835-10E (0.40 %, 0.60 %, 0.80 %) TGX1904-6F (0.40%, 0.60%, 0.80%), TGX1987-10F (0.40%, 0.80 %) all had perfect seed retention capacity having 0. 00 % seed shattering.

The thesis further showed that TGX1448-2E, TGX1987-10F and TGX1835-10E at 0.40 % recorded the largest pollen diameter at 27.50 µm. TGX1904-6F (0.20 %) recorded the highest oil yield percentage at 21.27 %.

Summarily, all these findings imply that the superiority of 0.20 %, 0.40 %, 0.60% EMS concentration makes them the most suitable mutants for producing beneficial soybean mutants.

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APPENDICES

PLANT HEIGHT

		Sum of Squares	Df	Mean Square	F	Sig.
Plant Height @Wk2	Between Groups	71.982	19	3.789	3.844	.000
	Within Groups	59.128	60	.985		
	Total	131.110	79			
Plant Height @Wk4	Between Groups	795.200	19	41.853	1.511	.114
	Within Groups	1661.500	60	27.692		
	Total	2456.700	79			
Plant Height @Wk8	Between Groups	1639.184	19	86.273	1.407	.158
	Within Groups	3678.813	60	61.314		
	Total	5317.997	79			
Plant Height @Maturity	Between Groups	1668.800	19	87.832	1.506	.116
	Within Groups	3499.000	60	58.317		
	Total	5167.800	79			

LENGTH AND WIDTH OF LEAVES

		Sum of Squares	Df	Mean Square	F	Sig.
Leave length	Between Groups	68.157	19	3.587	1.477	.128
	Within Groups	145.773	60	2.430		
	Total	213.930	79			
Leave width	Between Groups	33.521	19	1.764	1.515	.113
	Within Groups	69.878	60	1.165		
	Total	103.399	79			

YIELD PARAMETERS

		Sum of Squares	Df	Mean Square	F	Sig.
Pod/plant	Between Groups	147357.950	19	7755.682	2.659	.002
	Within Groups	175004.000	60	2916.733		
	Total	322361.950	79			
Seed/pod	Between Groups	18.258	19	.961	15.625	.000
	Within Groups	3.690	60	.062		
	Total	21.948	79			
100 pod weight	Between Groups	1536.527	19	80.870	8.635	.000
	Within Groups	561.898	60	9.365		
	Total	2098.425	79			
100 seed weight	Between Groups	108.920	19	5.733	18.065	.000
	Within Groups	19.040	60	.317		
	Total	127.960	79			
Pod Length	Between Groups	11.263	19	.593	6.473	.000
	Within Groups	5.495	60	.092		
	Total	16.758	79			
Shattering percentage	Between Groups	13874.677	19	730.246	8.189	.000
	Within Groups	5350.205	60	89.170		
	Total	19224.882	79			

POLLEN DIAMETER

		Sum of Squares	df	Mean Square	F	Sig.
Pollen diameter	Between Groups	117.869	19	6.204	7.941	.000
	Within Groups	15.625	20	.781		
	Total	133.494	39			

OIL YIELD AND OTHER SEED CHARACTERIZATION

		Sum of Squares	Df	Mean Square	F	Sig.
Oil yield	Between Groups	74.623	11	6.784	11.438	.000
	Within Groups	7.117	12	.593		
	Total	81.740	23			
Free fatty acid	Between Groups	9.651	11	.877	460.776	.000
	Within Groups	.023	12	.002		
	Total	9.674	23			
Glycerin	Between Groups	17.236	11	1.567	101.801	.000
	Within Groups	.185	12	.015		
	Total	17.420	23			
Acid value	Between Groups	40.479	11	3.680	899.359	.000
	Within Groups	.049	12	.004		
	Total	40.528	23			
Iodine value	Between Groups	161.839	11	14.713	2361.898	.000
	Within Groups	.075	12	.006		
	Total	161.914	23			
Peroxide value	Between Groups	536.910	11	48.810	3127.178	.000
	Within Groups	.187	12	.016		
	Total	537.098	23			
Sap. value	Between Groups	5688.736	11	517.158	211.799	.000
	Within Groups	29.301	12	2.442		
	Total	5718.036	23			
Ester value	Between Groups	5805.881	11	527.807	96.046	.000
	Within Groups	65.944	12	5.495		
	Total	5871.825	23			
Refractive index	Between Groups	.000	11	.000	.	
	Within Groups	.000	12	.000		
	Total	.000	23			