EVALUATION OF GAMMA RADIATION-INDUCED MUTANT LINES OF SESAME (*SESAMUM INDICUM* L.) FOR GENETIC IMPROVEMENT OF SOME DESIRABLE TRAITS

BY

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ABSTRACT

The creation, evaluation and selection of mutants with desirable traits have served as source of genetic variability for breeding programmes of many crops. Thus, eleven mutant lines of sesame developed through induced gamma irradiation were evaluated in the M_4 generation alongside the three parental varieties [NCRIBEN 04E (check-1), NCRIBEN 01M (check-2) and NCRIBEN 03L (check-3)]. The mutant lines and their respective checks were laid in a randomized complete block design (RCBD) with three replicates each. All the parameters were studied following standard procedures. The results of vegetative parameters revealed that mutant lines ML-6 (123.83 cm) and ML-8 (121.59 cm) had highest plant height at maturity with 5 % level of significance while ML-7 had the highest number of branches per plant. There was no significant difference ($P > 0.05$) in number of days to 50 % flowering. ML-2 (77.30), ML-3 (105.33), ML-7 (151.00) and ML-8 (163.00) produced higher number of capsules per plant than their respective checks. Mutant lines with improved capsule characteristics comprised of ML-2 (2-3 capsules per leaf axil), ML-6 (1-2 capsules per leaf axil), ML-9 (1-2 capsules per leaf axil), ML-10 (2-3 capsules per leaf axil) and ML-11 (1-3 capsules per leaf axil). All the M_4 mutants had adequate pollen viabilities (over 80 %). The highest pollen germinability was recorded at 20 % sucrose concentration for all the mutant lines. Suboblate shaped pollens with 10-13 colpi were observed in all the mutant lines and the checks. Significant increase was observed in oil contents of ML-2 (40.32 %) and ML-10 (40.05 %) over their check groups. All mutants derived from Check-1 [ML-1 (22.83 %), ML-2 (28.63 %), ML-3 (27.41 %)] and Check-2 [ML-4 (23.72 %), ML-5 (22.85 %), ML-6 (23.41 %) and ML-7 (32.83 %)] showed significant enhancements in protein content of the seeds with ML-7 (32.83 %) having the highest value. Similarly, all mutants showed significantly higher ($P < 0.05$) moisture contents than their checks except ML-8 (3.38 %). ML-10 (0.303 %) had the highest tannin value while the least oxalate and phytate contents were recorded in ML-1 (2.545 %) and Check-2 (0.532 %), respectively. All the mutants obtained from check-1 and check-3 showed significantly higher free fatty acid except ML-8 (1.13 %). Result on oil composition revealed that the physical properties of oil obtained from all the mutant lines and checks are within the acceptable limits by Codex. The variability observed in morphological, yield, seed nutritional composition and oil properties of the M_4 lines reflects the existence of genetic diversity as a result of gamma irradiation and suggests potential genetic improvements for sesame. Mutant lines with higher pollen viability could be used as male parents in controlled pollinations for increasing the productivity of sesame and further improvement of the crop.

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ABBREVIATIONS, GLOSSARIES AND SYMBOLS

AOAC: Association of Official Analytical Chemists

DES: Diethyl Sulphate

EMS: Ethyl Methane Sulphonate

FAO: Food and Agriculture Organisation

FNI: Fast Neutron irradiation

Gy: Gray

IAEA: International Atomic Energy Agency

IPGRI: International Plant Genetic Resources Institute

ML: Mutant Lines

MMS: Methyl Methane Sulphonate

NBPGR: National Bureau of Plant Genetic Resources

NAERLS: National Agricultural Extension and Research Liaison Services

WAP: Weeks After Planting

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Sesame (*Sesamum indicum* L.), also referred to as the queen of oil seed crops is said to be the most traditional and the oldest oil seed crop that is valued for its high-quality oil seeds (Sruba and Amitava, 2017). According to Rizki *et al.* (2015), sesame plants are considered to have originated from Africa and have been utilized extensively for thousands of years as a seed of worldwide significance for edible oil, cake, paste and for confectionary purposes. Sesame oil can be used for cooking, salad oils and margarine, manufacture of soaps, pharmaceuticals, insecticides, perfumes and paints (Tadese and Misgana, 2017). Sesame oil is preferred as cooking oil among many Nigerian families (Falusi *et al.*, 2001) and is increasingly becoming a popular seed oil (Biabani and Pakniyat, 2008). In Nigeria, sesame is locally called by different names; 'Ridi' in Hausa, 'Esso' in Nupe, 'Eeku' in Yoruba and 'Ekuku' in Igbo (Muhammad, 2018).

Sesamum indicum L. (2n = 26) is a self-pollinated crop that belongs to the family Pedaliaceae (Falusi *et al.*, 2001). The genus *Sesamum* consists of 36 species, most of which are wild with *S. indicum* being the most commonly cultivated species (Purselglove, 1974; Falusi, 2006; Abejide *et al*., 2013). Sesame is primarily grown for its oil rich seeds which are used for oil extraction (El Khier *et al*., 2008; Olaleye *et al*., 2018). Approximately, half of the seeds weight is its oil [International Plant Genetic Resource Institute and National Bureau of Plant Genetic Resources (IPGRI and NBPGR, 2004)]. Sesame oil contains two major constituents; sesamolin (0.3 - 0.5 %) which yields a powerful antioxidant (sesamol) on hydrolysis that gives excellent stability to oil and sesamin (0.5 - 1.0 %), both of which

are absent in other fixed oils (Pathak *et al.*, 2018; Saha, 2018). Anilakumar *et al.* (2010) reported that sesame seeds contain 43.3 - 44.3 % oil and around 39.0 % of oil present in sesame consists of monounsaturated fatty acid, 46.0 % polyunsaturated fatty acid and 14.0 % saturated fatty acid. According to Savant and Kothekar (2011), the oil content of sesame seeds and its fatty acid compositions are greatly influenced by both the genetic makeup as well as environmental conditions during oil accumulation. Oil characterization is a vital parameter used in determining the quality of oil seed crops (Mohammed, 2019).

According to Food and Agricultural Organization (FAO), (2019), Nigeria is the world's fourth largest producer of sesame seed with an annual production of about 550,000 tons placed after Tanzania (805,691 tons), Myanmar (764,320 tons), and India (751,000 tons). The productivity is however relatively low (global production of 4.04 million tons annually) compared to other oil seed crops (Hota *et al.*, 2016). The major yield constraints are lack of novel hybrids, narrow genetic base, low harvest index, lack of shattering resistance, and longer days to maturity and prevalence to abiotic and biotic stress conditions (Kumari *et al.*, 2016).

Genetic variability as a result of induced mutations by various mutagens has contributed to modern plant breeding and has played a major role in the development of superior plant varieties (Kharkwal and Shu, 2009; Audu *et al*., 2018). Mutation breeding has played a key role in the improvement of self-pollinated crops with limited genetic variability (Girija and Dhanavel, 2013). It has been used for the improvement of cowpea by Dhanavel *et al.* (2008), black gram by Thilgavathi and Mullianathan (2009), wheat by Sirvastava *et al.* (2011), rye by Jong-jin *et al.* (2012), Sorghum by Murali *et al.* (2013), rice by Omorigei *et*

al. (2014), sesame by Aliyu *et al.* (2017), in pepper by Yafizhan and Herwibawa (2018) and in so many other crops.

In sesame plant, Muhammad (2018) studied the M_2 lines of gamma-irradiated sesame seeds and revealed desirable traits like multicapsule per leaf axil and multicarpellate capsules. Similar studies of Mohammed (2019) on M_3 lines of the same mutants of sesame revealed lower oxalate and peroxide values and a rise in free fatty acid. Further research on these lines might lead to the discovery of more desirable traits. Similarly, there is a need to further test for the stability of the observed desirable traits.

1.2 Statement of the Research Problem

In the recently released lists of mutant varieties throughout the world by International Atomic Energy Agency (IAEA) (2019), no single mutant variety of sesame has been released in Nigeria despite the fact that Nigeria remains one of the major producers of sesame. This might be due to lack of continuity in mutation breeding programmes.

Although studies of Muhammad (2018) on M_2 generation of these mutants has revealed promising desirable traits like multicapsule per leaf axil and multicarpellate capsules, the stability of these traits is yet to be ascertained. Similarly, reductions in oxalate and peroxide values as well as a rise in free fatty acid in the M_3 lines (Mohammed, 2019) are not yet ascertained.

Information on irradiation induced changes in pollen viability and germinability that will eventually affect the yield attributes of sesame is scanty (Falusi *et al*., 2013). In addition, there is dearth of information concerning the nutritional composition and oil properties of sesame cultivars grown in Nigeria in general as well as mutant lines and breeder's lines.

1.3 Aim and Objectives of the Study

The aim of this research was to evaluate the fourth mutant (M_4) generation of sesame for genetic improvement of some identified desirable traits.

The objectives of this study were to determine the:

i. vegetative and yield parameters of M_4 lines
ii. pollen parameters of M_4 lines
iii. proximate composition and anti-nutritional factors of M_4 sesame seed
iv. quantitative and qualitative attributes of M_4 sesame seed oil

1.4 Justification for the Study

For many decades, mutagenesis has been successfully utilized as a tool for inducement of genetic variability in many crops, giving room for the isolation of mutants with desirable characters of economic importance and as a result, many new cultivars have been directly or indirectly released in the world (Diouf *et al*., 2010). The success of this work will thus ensure continuity in the evaluation of the mutant lines which will eventually lead to varietal release.

In mutagenesis, many phenotypic traits displayed by mutant lines in their early generations are not true and might be due to certain environmental factors. Thus, further evaluation of the mutants will determine whether they are true mutants or not.

Evaluation of pollen parameters of the mutant lines will add more information to the few existing literature. The extent of pollen viability and germinability will indicate how effective the mutant lines would be as a male parent or pollinator and can be used in future breeding programmes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of Sesame

Sesame is an erect annual shrub of varying sizes ranging from about 50 - 100 cm in height (Iwo *et al*., 2005; Abejide *et al*., 2013). The plant has a characteristic determinate and or indeterminate growth (Miraj and Kaini, 2016) with obtusely square stems that are yellow green. Many a times, they are highly branched with as many as twenty-six (26) stems depending on the variety. However, some varieties are un-branched. Leaves are generally bluish-green and the basal leaves are enormously divided into three or five foliate (Bedigian, 2004).

Sesame flowers are white and bell-shaped with a hint of yellow, red or blue (Martin and Leonard, 1967; Anilakumar *et al*., 2010). Flowers are hermaphroditic and are found either in cluster of two or three, or singly in the leaf axil (Andrade *et al*., 2014; Muhammad, 2018). Sesame plant bears between 15 - 20 fruits. Fruits are tiny, flat ovals and contain numerous seeds ranging from 70 - 100 seeds (McCormick, 2001). Seeds are small in size measuring to about 4mm in length, 2 mm in width and about 1 mm in thickness (Pusadkar *et al*., 2015) and come in different colours ranging from charcoal black to creamy-white when husked (Anilakumar *et al*., 2010), other colours found are yellow, red, black and brown (Naturland, 2002; Olaleye *et al*., 2018).

2.2 Origin and Domestication of Sesame

The origin of sesame has been argued for over a century (Candolle, 1886; Vavilov, 1926; Hilterbrandt, 1932; Darlington, 1963; Nayar and Mehra, 1970; Nayar, 1995; IPGRI and NBPGR, 2004). Vavilov (1926) opined that sesame plant has a polygenic origin and he

suggested India and Abyssinia as the primary centers of origin of sesame, and later thought of China as the secondary center of origin for a specific group of dwarf cultivars. Other numerous locations including India, Africa and Middle East have also been suggested as centers of origin of sesame (Nayar and Mehra, 1970).

According to Bedigian (2004), Africa and India are the two major suggestions with regards to place of origin of sesame. Hiltterbrandt (1932) however, considered Africa as the center of origin due to the multiplicity of wild species. Falusi *et al.* (2001) and Rizki *et al.* (2015) also felt likewise. It has been well established that sesame was first cultivated and domesticated in India during Harappa and Anatolian eras around 4000 years ago (Iqbal *et al*., 2018; Bedigian and Vander-Maesen, 2003) and was taken to Mesopotamia during the early bronze age (Bedigian and Harlan, 1986). This has been supported with morphological and cytogenetic evidences revealing close relationship between the native south Indian sesame and domesticated sesame (IPGRI and NBPGR, 2004).

2.3 Systematic Position of Sesame

S. indicum (Sesame) belongs to:

Kingdom Plantae

Sub-kingdom Tracheobionta

Super-division Spermatophyta

Division Magniolophyta

Class Magnoliopsida

Sub-class Asteridae

Order Tubiflorae

Family Pedaliaceae

Genus *Sesamum*

Species S. *indicum*

Source: (https://plants.usda.gov)

2.4 Agronomy of Sesame

According to Langham *et al.* (2010), sesame is well adapted to environments with well-drained soils and long growing seasons. Sesame plant prefers moderately acidic to alkaline soils with pH ranging from 5 to 8. Outstanding performance of sesame plant is achieved on fertile and well drained soils that are not even slightly crusted (Zerihan, 2012). Soil temperature of 70 ^{o}F is needed for sesame seed to germinate and the plant produces excellent yields in very hot temperatures of about 120 ^{o}F.

Sesame plants that are planted early produces the best yield with fewer problems. However, planting too early can result in reduction in yields due to the slow growth rate of seedlings in cold weather (Langham *et al*., 2008). Sesame plants require a rainfall of between 500 - 650 mm per annum (Olowe, 2009). Once the plants attain physiological maturity (usually between 113 to 146 days after planting), they are uprooted from the field and hung upside down with a mat spread beneath to collect seeds. Seeds are then cleaned and dried to a moisture content of about 80 % and then stored (Anilakumar *et al*., 2010).

2.5 Production of Sesame

In 2015, the global production of sesame seed was estimated at 5,702,809 tonnes having risen from 3.15 million tonnes in 2005 to 3.66 million tonnes in 2008 to 4.32 million tonnes in 2010. However, the global production has dropped from 5,702,809 tonnes in 2015 to

about 5,531,948 tonnes in 2017, of which about 57 % was produced in Africa and about 40 % was produced in Asia (FAO, 2019).

Nigeria's sesame production has on the contrary saw a great increase over the past few years from 158,000 tonnes in 2012 to 171,900 tonnes in 2015 which was tripled to 550,000 tonnes in the year 2019 placing Nigeria as the fourth largest producer of sesame seed in the world (FAO, 2019).

Currently, Republic of Tanzania, Myanmar and India occupy the top three positions of world sesame producers.

Table 2.1: Top 10 Countries with Highest Production of Sesame Seed in Tonnes

Country	Value
Tanzania	805961
Myanmar	764320
India	751000
Nigeria	550000
Sudan	550000
China	366000
Ethiopia	231187
South Sudan	204068
Burkina Faso	163787

Source: FAO, 2019.

2.6 Economic Importance of Sesame

According to Iqbal *et al.* (2018), oil seed crops account for the major agricultural crop after food grains and sesame is among the most important oil yielding plants and has been nicknamed "Queen of oil seeds" due to its high-quality oil (Bedigian and Harlan, 1986; Saha, 2018).

Sesame seeds are used as bread and cracker toppings and also serve as a primary source of vegetable oil in the orient (Langham *et al.*, 2008). In Ethiopia, sesame is ranked as the second most important crop (next only to coffee) in earning foreign currency (Fiseha and Zenawi, 2019). In Africa, sesame seeds are made into soups and porridges (Gooding *et al*., 2000) and are enjoyed as an appetizing delicacy when combined with roasted groundnut (Falusi *et al*., 2001). Sesame seeds are also an important cuisine in Asian meals. It is a nutritious oil seed crop and serves as a source of carbohydrate (13.5 %), protein (18 - 25 %), minerals (such as phosphorus, calcium, potassium) and healthy fatty acid (omega 6 fatty acid) but lacks omega 3 fatty acid (Pusadkar *et al*., 2015). After oil extraction, the cake is used as protein supplement in animal feed industry [National Agricultural Extension Research Liaison Services (NAERLS, 2010)].

Sesame seed oil is not only used for culinary purposes but has a wide application in industry, engineering and pharmaceuticals (Anilakumar *et al*., 2010). Medicinally, sesame leaves are helpful in treating infant cholera, dysentery, urinary infections and diarrhea. Sesame flowers are handy in treatment of constipation, alopecia and cancer while the roots possess antifungal activity (Pusadkar *et al*., 2015). Sesame seeds are used in treatment of piles, ulcer and cough (Chopra *et al*., 1958).

Sesame seed oil also has antioxidant activity, serum lipid and blood pressure lowering potential (Sirato-Yasumoto *et al*., 2001), protects liver from oxidative damages, serves as natural antibacterial for pathogens such as *Streptococcus* spp. and *staphylococcus* spp. and also inhibits growth of malignant melanoma and the rapid increase of human colon cancer cells (Smith and Salerno, 1992) and protects against air borne bacteria and viruses when swabbed in nose (Johnson *et al*., 2001; Morris, 2002).

2.7 History and Nature of Mutation Breeding

Kharkwal (2012) opined that mutation is a natural process that results in the creation of new variants of a gene and these variations provides the new material for natural selection and the driving force for evolution.

According to Oladosu *et al.* (2016), the term mutation breeding (mutationszuchtang) was first coined by Freisleben and Lein in 1942 to refer to the purposive induction and development of mutant lines for crop improvement. It has also been used in a broad sense to encompass the exploitation of natural and spontaneous mutants as well as the development of any variety possessing known mutations from whatever source. Mutations were first identified as a mechanism for creating variability by Hugo De Vries in the late 19^{th} century. He considered this variability as hereditary changes by mechanism very distinctive from segregation and recombination (Kharkwal, 2012).

The construction and utilization of mutants have a long history in plant breeding. The history could be traced back to 300 BC with reports of mutant crops in China. After the discovery of the mutagenic actions of X-ray in maize, barley and wheat by Stadler (1930), radiation-induced mutagenesis gained wide acceptance as a tool for generating novel

genetic variability in plants. The first commercial mutant variety was produced in tobacco in 1994 and before 1995, about 77 cultivars were reported to have been developed via mutagenesis (Acquaah, 2006). By 1995, the commercially released varieties increased to 484. Mutagenesis as a breeding tool became very popular in 1995 when a large range of plant species were treated with irradiation.

Even though mutants can be developed through different kinds of experimental mutagenesis, mutation breeding is frequently restricted to the use of physically and chemically induced mutagenesis (Forster and Shu, 2012).

2.7.1 Physical mutagenesis

Physical mutagens include electromagnetic radiations such as cosmic, gamma and x-rays and accounts for over 70 % of the methods used in developing new mutant plants (Oladosu *et al*., 2016). Physical mutagens mostly ionizing radiations have been widely and routinely used to induce heritable variations in many crop species including sesame (Tomlekova, 2010).

Radiation types vary in their physical properties and hence in their mutagenic activity. Fast neutron irradiations have a higher relative biological effectiveness (RBE) than gamma rays which implies that in order to obtain the same biological effect, a higher dose of gamma irradiation must be given (Kodym and Afza, 2003).

Among all the physical mutagens, gamma irradiation has been reported to be the most economical and the most effective for crop improvement (Muhammad *et al*., 2017). Successful doses of 250 Gy has been reported in *Sorghum* sp., and *Zea mays*, 100 - 200 Gy

in *Glycine max*, 100 - 300 Gy in *Triticum aestivium*, 200 - 400 Gy in *Oryza sativa* (Kodym and Afza, 2003) and 250 - 550 Gy in *Sesamum indicum* (Muhammad *et al*., 2017).

2.7.2 Chemical mutagenesis

According to Shiekh *et al.* (2012), chemical mutagenesis is a simple approach and is regarded as an effective and central tool for the improvement of yield and quality characters of crops. They generally produce an induced mutation leading to base pair substitutions that result in a change of amino acid and thus, a change in protein function (Khan *et al*., 2009). Chemical mutagens include hydroxylamine, nitrous acid, ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diethyl sulphate (DES) and acridine orange.

Among the chemical mutagens, EMS has been reported to be the most effective and efficient mutagen and has probably become the most popular mutagen (Van-Harten, 1998).

Currently, more than three hundred and seventy mutant varieties have been developed through chemical mutagenesis (IAEA, 2017).

2.8 Global Impact of Mutagenesis

Kharkwal and Shu (2009) opined that mutation breeding has played a significant role in the development of superior plant varieties during the last fifty years and new varieties have been derived either as direct mutants or through crosses with mutant cultivars (IAEA, 2017). Mutation breeding has resulted in two major outcomes: new varieties that are utilized for commercial cultivation and new genetic stock with improved characters such as high yield, early maturity, pest and disease tolerance, drought and salt tolerance and lodging resistance among others (Roychowdhury and Tah, 2013).

Mutant varieties that are resistant to biotic stresses such as tomato (resistance to bacterial wilt), rapeseed (resistance to stem rot), apple (resistance to powdery mildew and apple scab), chick pea (resistance to *Ascoctyta* blight and *Fusarium* wilt), mungbean (resistance to yellow mosaic virus) and various abiotic stress including acidity and drought tolerance in Lentil, salinity tolerance in rice, tolerance to cold and high altitude in rice have been developed through mutation breeding hence increasing productivity. In spite of the fact that development of new cultivars has been the main objective of mutation breeding, the new genetic stocks developed have many applications in plant breeding, from being used as a parent in hybrid breeding programmes to being used as a donor parent in conventional plant breeding (Oladosu *et al.*, 2016).

Induced mutations have contributed greatly in increasing crop production and will continue to play a significant role in the development of crop varieties with traits such as higher protein and starch quality, enhanced uptake of nutrients, modified oil and better tolerance and resistance to a wide range of biotic and abiotic stress conditions. The report of IAEA (2019) depicts that more than 3222 new varieties developed through mutagenesis have been released and it is expected that its activities would make notable contributions that would be otherwise impossible.

2.9 Mutation Breeding of Sesame

Non-shattering, bushy phenotype, branching from base, increment in capsule number on main axis, early maturity and resistance to pest and diseases are considered to be the most significant plant type mutants in Sesame as they all bring about enhancement in yield (Saha, 2018).

The earliest irradiation work on sesame was by Chaudhary and Das (1954) where they reported a significant increase in yield of west Bengal types number 12 and 6 at 14.4 KR and 20 KR doses of X-rays (Muhammad, 2018). Nayar (1961) reported increased oil content (from 47.82 % to 55.48 %) in small seeded types (T_{10} variety) using X-rays.

Sengupta and Datta (2004a) reported that nine (9) desirable macro mutants; namely, broad leaf (high number of capsules per plant), early flowering (synchronous maturity and enhanced fatty acid content), diffused branching and thick leaf (increased seed protein and fatty oil contents and higher number of capsules), viridis (higher seed protein, seedling colour as marker), white flower (marker trait), non-shattering capsules (intense pigmentation on flowers), globular fruit (increased oil content in seed and higher number of seeds per capsule), dark reddish-brown seed coat (high oil content) and bold seeded (high protein content) have been reported in sesame, all of which were outcome of induced mutagenesis.

Boureima *et al.* (2010) evaluated the radio sensitivity of African Sesame cultivars to gamma rays using two Sesame cultivars from Senegal. Sesame seeds were irradiated with 0 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy and 800 Gy to assess their effect on germination, seedling height and survival rate. A significant decrease with increase in irradiation doses was reported. The study revealed a more depressive effect of gamma irradiation on germination and drastic morphological abnormalities at higher doses of irradiation.

Begum and Dasgupta (2010) investigated the effects of physical and chemical mutagens in Sesame using three Sesame phenotypes viz Rama, SI 1666 and IC 21706. Seeds were

treated with physical (gamma rays: 200 Gy, 400 Gy and 600 Gy) or chemical (ethylmethane sulphonate EMS: 0.5 %, 1.0 %, 1.5 % and 2.0 %) mutagens and mutagenic effectiveness and efficiency was estimated in M_2 generation. It was reported that the average effectiveness of EMS was much higher than that of gamma rays. It was concluded that 0.5 % concentration of EMS was the most effective treatment for inducing mutations.

Diouf *et al.* (2010) studied the spectrum and frequency of different mutants induced by gamma rays in three genotypes of Sesame extensively grown in Senegal. Results showed that at least, one closed capsule mutant could be induced from each of the three genetic backgrounds using gamma irradiation. They recommended the use of medium dose range for induction of viable and useful mutations in sesame.

Savant and Kothekar (2011) examined the induction of variability in fatty acid profile of five macro mutants of Sesame variety JLT-7 using different concentrations of two chemical mutagens; ethyl methane sulphonate and sodium azide. Results revealed that the mutagenic treatments used induced significant variations in oil content and fatty acid composition of the different mutants and majority of the mutant revealed enhancement in seed oil content. They reported high oleic acid and decreased palmitic and stearic content in the high yielding branched mutant.

Falusi *et al.* (2013) investigated irradiation induced polygenic mutations in two common Nigerian Sesame cultivars (Ex-Sudan and Kenana-4) using Fast Neutron Irradiation. Results showed that 12 and 16 μSv were the most potent dose of fast neutron irradiation for the induction of viable mutants in sesame especially Ex-Sudan accessions, which produced better yield.

Falusi *et al*. (2015) evaluated three Nigerian sesame varieties after treatment with fast neutron irradiation. Seeds of Kenana-4, E-8 and Ex-Sudan were exposed to varying doses of fast neutron irradiation and results showed a corresponding increase in selected vegetative traits with an increasing dose of the radiation.

Kumari *et al.* (2016) investigated the effect of mutagenesis on germination, growth and fertility in Sesame (*Sesamum indicum* L.) Seeds of a local variety 'LTK-4' was irradiated with six gamma irradiation doses *viz.*, 150 Gy, 300 Gy, 450 Gy, 600 Gy, 750 Gy and 900 Gy. The seeds were also treated with 0.5 %, 1.0 % and 1.5 % EMS. Results revealed that higher doses of gamma irradiations and EMS both caused considerable reduction in all biological parameters. Based upon the sensitivity of mutagens, it was concluded that EMS treatments were highly effective for modifying majority of the traits in the crop.

Saravanaraman *et al.* (2016) studied the reaction of Sesame mutant generations against webworms. They treated three accessions of Sesame namely IVTS 2001-7, KMR-102 and TMV-3 with gamma rays, EMS and DES and reported that among M_1 and M_2 plants, IVTS 2001-7 and TMV-3 were resistant to webworm.

Aliyu *et al.* (2017) investigated the induction of phenotypic variants in Sesame using fast neutron irradiation. Sesame seeds were irradiated for 2 hours, 4 hours, 6 hours and 8 hours at 0.16 Sv, 0.32 Sv, 0.48 Sv and 0.64 Sv doses of fast neutron irradiations. Results showed that Fast Neutron Irradiation (FNI) significantly induced beneficial variability on the agronomic traits evaluated and mutation frequency, mutagenic efficiency and lethality induced by fast neutron irradiation were not dose dependent.

Muhammad *et al.* (2017) irradiated three varieties of Sesame viz NCRIBEN-04E, NCRIBEN-01M and NCRIBEN-03Lwith five different doses of gamma irradiation to investigate its effect on seed retention indices on the three varieties. The study revealed that gamma irradiation at 550 Gy had the highest score for NCRIBEN-04E and NCRIBEN-01M and were categorized as non-shattering type (NSH). It was concluded that dose 550 Gy was promising in generating mutants with high resistance to capsule shattering in sesame.

Saha and Paul (2017a) investigated the effects of gamma irradiation on yield and yield attributing traits of Sesame in M_1 generation using two varieties of sesame (Rama and Tillotoma). Seeds of the two varieties were exposed to varying doses of gamma irradiation (250 Gy, 300 Gy, 350 Gy, 400 Gy and 450 Gy) to study their effect on plant height, number of capsule per plant, number of branches per plant, days to first flowering, days to 50 % flowering, flower duration, seeds per capsule, capsule length, days to maturity, yield per plant and thousand seed weight. The result revealed that all the quantitative traits proportionately decreased or increased with increase in dose of irradiation and was attributed to the physiological disturbances and chromosomal damage caused by irradiation. They concluded that gamma irradiation can be successfully utilized for the inducement of variability in sesame.

Muhammad *et al.* (2018) evaluated the spectrum and frequency of mutations induced by gamma irradiations in three Nigerian Sesame varieties namely NCRIBEN-04E, NCRIBEN-01M and NCRIBEN-03L. Results obtained revealed four mutant fruit traits: multicarpellate capsule, multiple capsule per leaf axil, indehiscent capsule and terminal capsules. It was concluded that the dose range of 250 - 550 Gy was effective in inducing viable mutations in sesame.

Maibam *et al.* (2018) studied induced mutagenesis in three Sesame entries viz Gujarut Til-4 (GT-4), Gujarat Til-10 (GT-10) and Patan-64. The seeds of the three Sesame entries were treated with 0.5 %, 1.0 %, 1.5 %, 2.0 % and 2.5 % doses of ethyl methane sulphonate (EMS). Results from M1 generation revealed a gradual reduction in germination percentage with the increase in dose indicating the inhibitory effect on seed germination. In M_2 generation, plant height, number of capsules per plant and yield per plant displayed substantial variability in the three types but did not give improved yield.

Ariharasutharasan *et al.* (2019) evaluated the radio sensitivity of white seeded Sesame. Seeds of two white-seeded Sesame varieties (SVPR1 and VRI3) were irradiated with gamma rays at 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy. The results revealed a reduction in all morphological parameters with increase in irradiation dose in both varieties. In biochemical traits, photosynthetic pigment increased at lower doses of irradiation in both varieties. It was concluded that doses above 300 Gy are not suitable to enhance trait values in white seeded Sesame.

Kalaiyarasi *et al.* (2019) investigated the genetic variability, heritability and genetic advance in sesame genotypes available in Tamil Nadu using fifteen Sesame genotypes. Results revealed a high PCV and GCV and a high heritability and genetic advance.

Pradhan and Paul (2019) studied the induction of genetic variability for different quantitative characters in Sesame using gamma irradiation. Dry and homogeneous seeds of two varieties of Sesame (Rama and Tillotoma) were irradiated with different doses of gamma rays viz. 250 Gy, 300 Gy, 350 Gy and 400 Gy. Results revealed pronounced effect of doses in inducing genetic variability for all the characters. In addition, a significant

increase in number of capsules per plant was observed at 350 Gy and 400 Gy in both the varieties.

Over the years, numerous researches have been carried out on mutation breeding in Sesame and some of the noticeable achievements are presented in the table below.

Table 2.2: Achievements in Sesame through Mutation Breeding

S/n	Treatment	Character improvement	Author
1.	X-ray irradiation	High oil content	Rai and Jacob (1956)
2.	X-ray irradiation	higher oil content 55.48 % than the parent var. 47.82 %	Nayar (1961)
3.	X-ray and fast neutron (35S and 32P)	Increased number of fruits with heavier seeds with increase oil content	Kobayashi (1965)
4.	X-ray irradiation	Indehiscent capsule and early maturing traits	Kobayashi (1973)
5.	Maleic hydrazide and dalapon	Male sterility	Chauhan and Singh (1971)
6.	gamma-rays and colchicine	higher seed yield (3 - 30 %) and oil (5 - 13 %) content than parent varieties	Kamala and Sasikala (1985)
7.	sodium azide treatment	Lodging resistance with good yield potential when planted in high density	Kang *et al.* (1996)
8.	Gamma irradiation	Multicapsule per leaf axil, semishattering capsule, early maturity	Govindarasu and Ramamoorthi (1998)
9.	EMS and gamma irradiation	Male sterility	Ganesan (1995)
10.	EMS, DES, NH_2OH and HNO2	Viridis, thick leaf, broad leaf, diffused branching early flowering, white flower white flower non-shattering capsule and bold seeded	Sengupta and Datta (2004b)

Table 2.2: Achievements in Sesame through Mutation Breeding (Cont'd)

11.	EMS	late flowering plant type was protein rich	Sengupta and Datta (2004a)
12.	X-ray and gamma rays	Oil rich mutant plants	Chowdhury *et al.* (2009)
13.	Gamma irradiations and EMS	Determinate plant type Altered phyllotaxy multicapsules per axil multilocules	Boranayaka *et al.* (2010)
14.	EMS, gamma irradiations and nanoparticles (Cu-, CdS-, CuO- and ZnO-NPs)	Bushy Unbranched Globular fruit Dwarf Early flowering Narrow leaf Broad leaf I and II, Triaxillary fruit, Quadraxillary fruit, Tripetiolar node Multilocular fruit Branching from base	Das *et al.* (2017)
15.	Gamma irradiation	Early maturing Determinate growth Monostem	Saha and Paul (2017b)

Source: Saha, 2018.

2.10 M_3 Generation of Sesame Seeds

Sesame seeds studied at M_3 generation showed various kinds of capsules in the three sesame lines ranging from bicarpellate single capsule, bicarpellate multicapsulate, multicarpellate single capsules and multicarpellate multicapsulate. The capsules were arranged either oppositely, alternate to the other or in whorls. Seed colours ranged from red, brown to white. Seed textures were mostly smooth. Seed shapes were elongated with each having different weight (Mohammed, 2019).

A significant decrease was reported in the number of capsules per plant of some irradiated mutant lines and was attributed to the formation of sterile capsule manifestation transmitted through organogenesis. Increased petiole length was reported with an increasing dose of irradiation in the sesame lines. A significant decrease was observed in plant height of the three sesame lines at early stage (3 weeks). However, plant height of the irradiated sesame lines was significantly increased at six (6) weeks (Mohammed, 2019).

Most acid values observed were higher than the maximum (4 KOH/g) for vegetable oil. Low acid values were observed in some of the mutants with the least being 1.670 mg/g indicating high oil quality and stability. Lower oxalate and peroxide values were also observed in the mutants and were significantly different from one another. These variations were attributed to increased rates of reaction due to gamma irradiation leading to a rise in acid values and free fatty acids. However, some were attributed to varietal differences and environmental factors (Mohammed, 2019).

2.11 Stability of Traits: Mutant lines have been reported by various authors to usually show a wide variation in various traits, mostly showing superiority over the control varieties. However, the increased characters may sometimes decline after successive generations. Thus, the evaluation for stability performance has become a vital part of plant breeding programmes.

In sesame breeding, Patil *et al*. (2007) investigated M_3 and M_4 generations of desirable variants for stability of beneficial mutated traits. The results obtained showed that only variants with high oleic percentage were stable in M_3 and M_4 generations with ratio of essential fatty acid being within the World Health Organization (WHO) recommended value (5 - 10 %). However, Rahman and Das (2001) has reported that in true breeding lines of sesame developed through combined mutagenesis from gamma rays and EMS and isolated in M_3 generation, seed yield was observed to be significantly higher in preliminary yield trial in M_4 generation.

In lax branching and small flower mutants of sesame, Chowdhury *et al*. (2009) observed that seed yield and fatty acid contents were significantly higher in the mutants than parental cultivar and yield related traits were also enhanced, although protein contents were lower at M_4 generations.

In a study carried out by Begum and Dasgupta (2015), thirty mutant lines were selected from three widely adapted genotypes to evaluate yield and yield related attributes in M_4 generation of sesame, the analysis of variance for the characters investigated revealed mean squares that were highly significant for all traits excluding capsule length. This revealed the existence of high genetic variability in mutant lines, even at M_4 generations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was conducted at the experimental field of Upper Niger River Basin Development Authority, Minna, Niger State, Nigeria. Minna is the capital city of Niger state and lies between latitude $9^{o}31^{1}$ and $9^{o}40^{1}$ north of the equator and longitudes $6^{o}29^{1}$ and $6^{o}35^{1}$ east of Greenwich meridian with a landmass of 884 hectares.

3.2 Source of Research Materials

The experimental materials comprising of fourteen (14) mutant lines including three (3) controls were obtained from breeder's line from the Department of Plant Biology, Federal University of Technology, Minna, Nigeria.

Originally, three varieties of sesame (NCRIBEN 04E, NCRIBEN 01M and NCRIBEN 03L) (Table 3.1) were exposed to four different doses of gamma radiation (250 Gy, 350 Gy, 450 Gy and 550 Gy) to generate twelve treatment combinations. The twelve treatments were raised alongside the three parental stocks as checks making it a total of fifteen treatment combinations to generate M_1 population.

Table 3.1: Description of Parental Stock

Agronomic traits		Variety	
	NCRIBEN 04E	NCRIBEN 01M	NCRIBEN 03L
Number of capsules/axils	Multicapsular	Unicapsular	Unicapsular
Number of carpels/capsules	Bicarpellate	Bicarpellate	Bicarpellate
Maturity	Early	Mid	Late
Nature of capsule	Dehiscent	Dehiscent	Dehiscent

In M_2 generation, eight mutant lines were selected and evaluated to generate M_3 lines. Some of the M_3 lines segregated and a total of eleven M_4 lines were obtained and used as the experimental materials for this work (Table 3.2).

Table 3.2: Description of Planting Material

Mutant lines	Mutant Name	Major Features
ML-1	$04E450G_{1-3}$	3 carpels per capsule, single capsule per leaf axil
ML-2	$04E450G_{2-3}$	2 carpels per capsule, single capsule per leaf axil
ML-3	$04E450G_{3-3}$	2 carpels per capsule, 2-3 capsules per leaf axil
ML-4	$01M350G2-_{22}$	2 carpels per capsule, 2-3 capsules per leaf axil
ML-5	$01M350G_{1-21}$	3-4 carpels per capsule, 2-3 capsules per leaf axil
ML-6	$01M550G_{2-2}$	2 carpels per capsule, single capsule per leaf axil
ML-7	$01M350G_{1-2}$	3 carpels per capsule, single capsule per leaf axil
ML-8	$03L550G_{1-2}$	2 carpels per capsule, single capsule per leaf axil
ML-9	$03L450G_{2-2}$	3 carpels per capsule, single capsule per leaf axil
ML-10	$03L250G_{1-1}$	2 carpels per capsule, single capsule per leaf axil
ML-11	$03L250G_{1-11}$	3 carpels per capsule, single capsule per leaf axil

Source: Muhammad, 2018; Mohammed, 2019.

3.3 Experimental Design

Seeds of eleven (11) mutant lines were grown alongside their respective checks in a Randomized Complete Block Design (RCBD). Each block comprised of two hundred and eighty (280) plants with each of the eleven (11) mutant lines and checks being equally represented. This gave rise to eight hundred and forty (840) plants for the whole experiment. Four seeds were sown per hole and were later thinned down to two plants per stand at two weeks after planting (WAP). Inter and intra row spacing of 40 cm and 25 cm were observed respectively and each plot had a dimension of 5.6 m × 2.5 m. The experiment was conducted between the months of August, 2020 and December, 2020 and all the recommended agronomic and plant protection practices IPGRI and NBPGR (2004) were followed for successful raising of the plants.

3.4 Data Collection

Data on vegetative parameters as well as yield parameters were recorded during the planting period following the methods stated in the standard descriptors of sesame by IPGRI and NBPGR (2004).

3.4.1 Vegetative parameters

i. Plant height: The plant heights were recorded during the seedling stage at two weeks after planting (WAP), juvenile stage at five weeks after planting (WAP) and at maturity. The length of the shoots (base of the stem to the terminal bud) of ten randomly selected plants from each mutant line as well as the control was measured using a meter rule.

ii. Number of branches per plant: The number of branches per plant was recorded by direct counting.

iii. Height of first capsule on main stem: The height of first capsule on main stem was measured from 10 randomly selected plants from each mutant line as well as the control groups by using a meter rule from the base of the plant to the first capsule on the main stem.

iv. Leaf morphology: Leaf colour, leaf shape and lobe incisions were recorded at the onset of flowering using fully formed functional leaves. Leaf colour was classified into green (1), green with yellow cast (2), green with blue-green cast (3) and green with purple cast (4). Leaf shape were classified as linear (1), lanceolate (2), elliptic (3), ovate (4) and narrowly cordate (5) while lobe incisions were classified as entire (0), weak (3), medium (5) and strong (7).

3.4.2 Yield parameters

i. Days to first flowering, 50 % flowering and days to physiological maturity: The number of days from planting until the number of days to first flowering, 50 % flowering and the number of days taken for 75 % of each mutant line to attain physiological maturity was counted by direct counting of days.

ii. Number of flowers per plant: The number of flowers per plant was recorded as mean of ten randomly selected plants from each mutant line and control groups by direct counting of the flowers produced.

iii. Exterior corolla colour: This was evaluated by using five (5) randomly selected flowers and was scored using a colour chart. The flower colour were classified as white (1), white with pink shading (2), white with deep pink shading (3), pink (4), light violet (5), dark violet (6), purple (7), red (8) and maroon (9).

iv. Number of capsules per leaf axil and number of capsules per plant: both were determined as mean of ten randomly selected plants from each mutant line as well as control by direct counting.

v. Bi-carpellate capsule shape, capsule hairiness, capsule arrangement and capsule angle on stem: This was evaluated by using randomly selected capsules from the middle of the main stem. Capsule shape was classified into those with tapering apex (1), narrow oblong (2), broad oblong (3) or square (4). Capsule hairiness was scored on a scale of 0-7 described by 0 (glabrous), 3 (weak), 5 (medium) and 7 (strong). Capsule arrangement was recorded as mono capsular or multi capsular. Capsule angle on stem were classified into mixed or segregating (0), narrow or snug on stem (3), medium about 30^{o} (5) or wide about 45^{o} (7).

vi. Capsule length and width: Both were measured by a meter rule using ten randomly selected capsules from the middle of the stem, each from a different plant at physiological maturity.

vii. Fresh and dry weight of capsules: weight of ten randomly selected capsules (fresh and dry) from each of the mutant lines as well as control was measured using a weighing balance.

viii. Colour of sundried capsules: The colours of sun-dried capsules were observed using a colour chart and were recorded as green, straw/ yellow, brown/tan and purple.

ix. Number of seeds per capsule: Seeds from ten randomly selected capsules from ten different plants taken from the middle of the main stem were counted for each mutant line as well as the control groups.

x. Hundred seed weight: weight in grams of hundred (100) random seeds taken from the bulk harvest was measured using an electronic weighing balance.

xi. Seed colour: The seed colour were observed using a colour chart and were recorded as white (1), cream (2), beige (3), light brown (4), medium brown (5), dark brown (6), brick red (7), tan (8), olive (9), grey (10), dull black (11) and bright black (12).

3.5 Pollen Parameters

At the onset of reproductive phase, the flower buds of each mutant line and control were collected for pollen viability and germinability test.

3.5.1 Pollen viability test: Pollen viability test was conducted following the method of Abejide *et al.* (2013). Ten flower buds were collected from five randomly selected plants of each mutant line as well as control. A drop of 2 % aceto carmine stain was placed on a cleaned, dried glass slide. Pollens from the flowers were carefully transferred unto the stain by tapping the flowers at a short distance above the stain layer. Three flowers were used per slide in order to transfer enough pollen for microscopic observation. The preparation was left for 30 minutes to allow pollens pick up enough stain. Afterwards, the slide was mounted on a light binocular microscope for observation and pollens were examined at magnification of ×40. The viability was scored according to the staining level and. Pollens that were well stained were considered viable, those that were slightly stained were considered semi-viable and pollens that were not stained were considered as non-viable. Percentage viability was calculated as:

$$\text{Percentage pollen viability} = \frac{\text{number of well stained pollen grain}}{\text{total number of pollen grains}} \times 100 \quad \ldots\text{.equation 1}$$

3.5.2 Pollen morphology (diameter and shape): The diameter of thirty (30) different pollens selected at random were measured from each slide using the microscope eye piece graticule measuring glass (Abubakar *et al.*, 2015) and pollen shapes were determined following the procedure of Akhila and Beevy (2015).

3.5.3 Pollen germinability test: Pollen germinability test was conducted following the standard procedure of Abejide *et al*. (2013). Different concentrations (10 %, 20 % and 30 %) of sucrose solution were prepared by adding 10 g, 20 g, and 30 g of sucrose to 1 g of nutrient agar and 100 ml of distilled water respectively. The prepared mixture was properly stirred and evenly spread on a petri dish and pollens were sprinkled onto the medium gently by tapping the flowers at a short distance above the petri dish. The petri dishes were properly covered to prevent loss of water and were kept at ±28 ^{o}C for twenty-four (24) hours. After twenty-four (24) hours, the petri dishes were stored in a refrigerator until the pollen's germinability was observed. Three petri dishes were used per sucrose concentration for each mutant line and the pollen germinability was expressed in percentages. Pollens with protrusions around the edges were considered to have germinated.

$$\text{Percentage pollen germinability} = \frac{\text{number of germinated pollen grain}}{\text{total number of pollen grains}} \times 100 \quad \ldots\text{.equation 2}$$

3.6 Proximate Composition Analyses:

3.6.1 Protein content: The protein content was determined using a micro-Kjedhal method (AOAC, 2010) which involves wet digestion, distillation, and titration. An aliquot 3 g of each sample was weighed into separate boiling tubes that contained one catalyst tablet (0.15 g $CuSO_4$, 5 g K_2SO_4, and 0.1 g TiO_2) and 25 ml of concentrated sulfuric acid. The

tubes were gradually heated for digestion to occur. The digest was diluted with 10 ml of 40 % NaOH, 100 ml distilled water, and 5 ml $Na_2S_2O_3$ anti-bumping agent was added. Exactly 10 ml of boric acid was then added to the sample. The NH_4 content in the distillate was determined by titrating with 0.1 N standard HCl using a 25 ml burette. A blank was prepared without the sample. The protein value obtained was multiplied by a conversion factor, and the results were expressed as the amount of crude protein. The percentage crude protein was calculated as:

$$\text{Crude protein} = \frac{\text{actual titre value} - \text{titre value of blank} \times 0.1 \times 0.014 \times conversion\ factor \times 100}{\text{Weight of food sample}} \quad \text{.....equation 3}$$

3.6.2 Crude fibre: Crude fibre was determined using the method of AOAC (2010). A measure of 5 g of each sample was placed into separate 500 ml Erlenmeyer flask and 100 ml of TCA digestion reagent was added. It was then brought to boiling point and refluxed for exactly 40 minutes counting from the start of boiling. The flask was removed from the heater, cooled for about 10 minutes, and then filtered with a Whatman paper. The residue was rinsed with hot water and was stirred continuously using a spatula. The sample was dried overnight at 105 °C. After drying, it was transferred to a desiccator and was allowed to cool. The sample was then weighed as W_1. It was then burnt in a furnace at 500 °C for six (6) hours and allowed to cool, and reweighed as W_2. The crude fibre content was calculated as:

$$\text{Crude fibre (\%)} = \frac{W1 - W2}{Wo} \times 100 \quad \text{..... equation 4}$$

W_1= Weight of crucible + fibre + ash

W_2= Weight of crucible + ash

W_O= Dry weight of food

3.6.3 Ash content: Ash contents were determined following the procedure of AOAC (2010). Exactly 2 g of each sample were weighed into separate crucibles and were burned in a furnace at 550 °C until a light grey ash was observed. The samples were transferred to the desiccators and were allowed to cool and were weighed to obtain ash content.

$$\text{Ash content (\%)} = \frac{\text{change in weight}}{\text{Initial weight of food before drying}} \times 100 \ldots\ldots \text{ equation 5}$$

3.6.4 Moisture content: Moisture content was determined using AOAC (2010). An aliquot 2 g of the samples were weighed into separate Petri dish of known weight. They were oven-dried at 105 ± 1 °C for four (4) hours. The samples were placed in a desiccator for cooling and were later weighed. The moisture content was calculated as follows:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ equation 6}$$

W_1= Weight of sample before drying

W_2= Weight of sample after drying

3.6.5 Total carbohydrate content: Carbohydrate content was determined by difference using the method of Muller and Tobin (1980), by subtracting the total sum of the percentage of moisture, ash, crude fibre, and crude protein from hundred (100).

Total carbohydrate (%) =100 – (% Moisture + % Ash + % Fat + % Protein + % Fibre)…equation 7

3.7 Anti Nutritional Factors

3.7.1 Determination of tannin: This involves weighing a 2 g of sample into a 250 ml volumetric flask and 150 ml of distilled water was added. The mixtures were heated for one (1) hour and allowed to cool. The mixture was then filtered and an aliquot 1 ml of the

filtrate was taken and 5 ml of indigo carmine was added into a 250 ml volumetric flask and 200 ml of tap water was added. The mixture was stirred against 0.05 N $KMnO_4$ until it turned yellow.

i. Titre value was titrated as 'X ml'

ii. Blank was titrated using 5 ml of indigo carmine and 200 ml of distilled water and was recorded as 'Y ml'

$$\% \text{ tannin} = \frac{(X-Y) \times 500 \times 0.00623}{25 \times \text{Weight of sample}} \quad \ldots\ldots\ldots\ldots \text{ equation 8}$$

3.7.2 Determination of flavonoids: Aluminium chloride method was used to determine the total flavonoid contents. Exactly 1 ml of test samples were weighed in a volumetric flask and 4 ml of water was added to each sample. An aliquot 0.3 ml of 5 % sodium nitrite and 0.3 ml of 10 % aluminium chloride were added after 5 minutes. The mixtures were then incubated at room temperature for 6 minutes and then 1 ml of 1 M sodium hydroxide was added to each mixture. Spectrophotometer was used to measure the absorbance of the sample against the blank at 510 nm.

3.7.3 Determination of alkaloid: Alkaloid contents were determined following the method of Haborne (1973). An aliquot 2 g of each sample was weighed and dispersed into 50 ml of 10 % acetic acid solution in ethanol. The mixtures were stirred using a glass rod and were allowed to stand for about 4 hours before they were filtered. The filtrates were then evaporated to one quarter of their volume with the use of a hot plate and a few drops of ammonium hydroxide were added. The precipitates were filtered off using a pre-weighed filter paper and the filter papers containing the residue were dried in an oven at 60 ^{o}C for 30 minutes, transferred into the desiccator to cool off and then reweighed until constant

weights were recorded. Weight differencing was used to determine the weight of the alkaloid and was expressed as a percentage of the sample weight analyzed.

3.7.4 Determination of oxalate: The oxalate content was determined using the method of Nwosu (2011). A measure of 1g of each sample were grounded separately into powder and poured into a 100 ml container. Exactly 20 ml of 0.30 N HCL was heated to a temperature of 40 - 50 ^{o}C and was stirred for an hour using a magnetic hot plate. The mixture was extracted three times using a 20 ml flask. The mixed residue was diluted into 100 ml mark of the volumetric flask. An aliquot 5 ml of the extract was pipetted into a conical flask and made alkaline with 1.0 ml of 5 N ammonium hydroxide. The alkaline regions were determined by placing an indicator paper in the conical flask. A 1 ml of 5 % $CaCl_2$ was added to the mixture and allowed to stand for 3 hours. The mixture was then centrifuged at 300 rpm for 15 minutes. The precipitate was discarded and 2 ml of 3 N H_2SO_4 was added to the test tubes and the suspension was dissolved by heating in a water bath to a temperature of (70 - 80 ^{o}C). The content of the pipette was carefully transferred into a conical flask and titrated against 0.01 N KM_nO_4 until a pink colour was observed. The solution was kept until it turned colourless and then heated to a temperature of 70 - 80 ^{o}C. The solution was then titrated until a permanent pink colour that persisted for 30 minutes was observed.

3.7.5 Determination of phytate content: The phytate content was determined following the method of Lucas and Markakes (1975). Exactly 2 g of the sample was weighed into a conical flask. The sample was soaked with 100 ml of 2 % concentrated HCL for 3 hours and then filtered. A 50 m^3 of the filtrate and 10 cm^3 of distilled water were added to the sample to give proper acidity. A 10 ml of 0.3 % ammonium thiocyanate solution was added into the solution as indicator and treated with standard Iron II chloride solution containing

0.00195 g Iron/ml. The percentage phytic acid was determined when a yellow coloured point that persisted for 5 minutes was observed and calculated as

$$\% \text{ phytic acid} = y \times 1.19 \times 10 \quad \text{..... equation 9}$$

Where y= titre value×0.00195g

3.7.6 Determination of hydro cyanide: Different concentrations of KCN solution containing 0.1 - 1.0 mg/ml cyanide were prepared. Exactly 4 ml of alkaline picrate solution were added to 1 ml of the sample filtrates and standard cyanide solution in the test tubes and were incubated in the water bath for 15 minutes. After colour development, the absorbance was read at 490 nm against a blank containing 4 cm^3 alkaline picrate solution and 1 mL distilled water. The cyanide content was then extrapolated from the cyanide standard curve and calculated as thus

$$\text{Cyanogenic glycoside (mg/100g)} = \frac{C(mg)}{\text{Weight of sample}} \times 100 \quad \text{..... equation 10}$$

Where C (mg) = Concentration of cyanide content read off the graph

3.8 Quantitative and Qualitative Analysis of Oil

3.8.1 Determination of percentage oil: Oil extraction was carried out according to AOAC (2010). The petroleum ether extract was obtained by complete extraction using soxhlet extractor. A measure of 10 g of the powdered seeds sample was put into a porous thimble and placed in a soxhlet extractor, using 300 ml of petroleum ether (with boiling point of about 40 – 60 °C.) as extracting solvent for six (6) hours repeatedly until the required quantity was obtained. The oil was obtained after evaporation using a water bath at 70 °C to remove the excess solvent from the extracted oil. The oil was kept in the refrigerator without further treatment until needed for further analysis.

Percentage oil was determined according to the method of AOAC (2010). Exactly 5 g of dried powdered sesame seeds was weighed and enveloped with a filter paper. The sample was set into a thimble in a soxhlet extractor. A clean round conical flask was weighed and 120 ml of petroleum ether was poured into the flask. The conical flask was placed on a sample holder of the soxhlet extractor and was gradually heated for six (6) hours. The flask containing the lipid was dried in an oven at 160 ^{o}C for few minutes to eliminate the residual solvent. The flask containing the oil was placed in a desiccator to cool and was reweighed.

$$\text{Oil (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 10 \quad \text{.....equation 11}$$

3.8.2 Determination of acid value: Acid value was determined following the pattern of Olaleye *et al*. (2018). Exactly 1 g of the oil was weighed in 250 ml flasks and 5 cm^3 of isopropyl alcohol was added and the mixture was stirred thoroughly. Three (3) drops of phenolphthalein indicator were added into the prepared sample and titrated against 0.1 N KOH solution while shaking persistently until a faint pink colour appears. The titre value was recorded and the acid value was calculated as:

$$\text{Acid value} = \frac{\text{Molar concentration} + \text{Titre value} + 56.1}{\text{Weight of sample}} \quad \text{..... equation 12}$$

$$\text{Free Fatty Acid (\%)} = \frac{\text{Molar concentration of KOH} + \text{Titre value} + 28.2}{\text{Weight of sample}} \quad \text{.....equation 13}$$

3.8.3 Determination of peroxide value: Peroxide value was determined following the pattern of AOAC (2010). Exactly 3 g of melted filtered sesame was weighed to 0.001 accuracy and was inserted in 250 ml capacity Erlenmeyer flask and 15 ml of glacial acetic acid, 10 ml of chloroform, 2 ml of starch solution, 1 ml of potassium iodide saturated solution and 75 ml of distilled water were added. Standardized 0.01 N Sodium thiosulphate

solution was used to titrate the resultant mixture until the colour of the mixture turned to white. Peroxide value was calculated as:

$$\text{Peroxide value (\%)} = \frac{V \times T}{M} \times 100 \ldots\ldots\ldots\ldots \text{ equation 14}$$

Where,

V= Amount in ml of standardized sodium thiosulphate used for the test corrected to take into account the blank test

T= Exact normality of the Sodium thiosulphate solution used

M= Mass in grams of the test portion

3.8.4 Determination of saponification value: Exactly one (1) gram of oil sample was weighed into a 250 ml conical flask and 25 cm^3 of 0.1 M alcoholic potassium hydroxide solution was added. The conical flask was heated for an hour in a water bath with constant shaking. The flask was then taken out of the water bath and exactly 5 cm^3 of 1 % phenolphthalein indicator was added and was titrated with standard 0.5 M hydrochloric acid.

$$\text{Saponification value} = \frac{(V2-V1)cm3 \times 26.05}{\text{Weight of oil}} \text{ (mgKOH/g) } \ldots\ldots\ldots\ldots\ldots\ldots \text{equation 15}$$

3.8.5 Determination of iodine value: Iodine value was determined according to AOAC (2010). An aliquot 0.2 g of oil sample was weighed into a 250 cm^3 glass stoppered flask and 10 cm^3 of carbon tetrachloride was added to the oil and dissolved. Exactly 20 cm^3 Wijs' solution was equally added to the mixture and the content was corked with a stopper that initially moistened with potassium iodide solution. The mixture was titrated with 0.1 M standard sodium thiosulphate solution using starch as an indicator just before the end point.

$$\text{Iodine value} = \frac{(V2-V1)cm3\times 1.269}{\text{Weight of oil (g)}} gI2/10 \quad \text{.....equation 16}$$

3.8.6 Determination of refractive index: Measurement of the refractive index of the samples was done by means of Abbe Refractometer by the method of AOAC (2010).

3.9 Data Analysis

Data were expressed as mean ± standard error of mean. The data obtained were subjected to one-way analysis of variance (ANOVA) test to determine whether there were significant differences, and Duncan's multiple range test (DMRT) was used to separate the means where there were significant differences. Pearson's linear correlation was used to determine the relationship among various parameters. Simple percentages were used to show variations in pollen viability and germinability of the M_4 lines.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1 Vegetative parameters of M_4 lines of gamma irradiated sesame

4.1.1.1 Plant height

The M_4 lines showed significant variation in plant height at different stages (Table 4.1). The highest seedling height taken at two weeks after planting was observed in ML-6 (1.84 ± 0.18 cm) (Table 4.1). The least seedling height was observed in ML-9 (1.04 ± 0.12 cm). At juvenile stage (five weeks after planting), ML-6 also had the highest plant height (7.59 ± 1.20 cm). The least plant height at juvenile stage was observed in ML-1 (3.78 ± 0.88 cm) which was not significantly different ($p > 0.05$) from that of ML-2 (3.95 ± 0.20 cm) (Table 4.1).

The highest plant height at maturity was observed in ML-6 (123.83 ± 4. 88 cm) which was not significantly different ($p > 0.05$) from ML-8 (121.59 ± 4.39 cm) (Table 4.1). ML-1 (76.00 ± 3.04 cm) and ML-4 (74.62 ± 2.47 cm) had the least plant height at maturity. The Mutant lines revealing significant enhancement in seedling height over the checks comprise of ML-6 (1.84 ± 0.18 cm) and ML-8 (1.66 ± 0.14 cm). The mutants exhibiting significant increment in height over the checks at maturity comprise of ML-2 (117.45 ± 5.85 cm), ML-3 (109.09 ± 8.21 cm), ML-6 (123.83 ± 14.88 cm), ML-8 (121.59 ± 4.39 cm) and ML-10 (115.66 ± 6.14 cm).

4.1.1.2 Branches per plant

The ML-7 had the highest number of branches per plant (3.27 ± 0.37) (Table 4.1). The least number of branches per plant was observed in ML-1 (1.86 ± 0.24) which was not

significantly different ($p > 0.05$) from Check-1 (1.87 ± 0.13). Mutant lines ML-2 (2.20 ± 0.50), ML-3 (2.07 ± 0.35), ML-7 (3.27 ± 0.37), ML-8 (3.20 ± 0.30) and ML-9 (2.87 ± 0.07) showed significant increments over their respective check varieties (Table 4.1).

4.1.1.3 Height of first capsule on main stem

The line ML-7 was observed to have the highest height of first capsule on main stem (43.07 ± 3.22 cm) (Table 4.1). The least height of first capsule on main stem was observed in ML-5 (24.50 ± 2.78 cm) and was not significantly different ($p > 0.05$) from ML-4 (26.46 ± 6.04 cm) (Table 4.1).

4.1.1.4 Leaf morphology

Leaves were observed to be greenish in colour with no leaf hairs present and were mostly oppositely arranged in all the mutant lines except in Check-3, ML-9 and ML-2 which had mixed arrangement and ML-3 and ML-6 which had alternate leaf arrangement. Upper leaves were lanceolate in shape while the middle and lower leaves were mostly ovate and narrowly cordate in all mutant lines and check groups (Plate I). Basal leaf margin was observed to be serrated in most of the lines except in Check-1, ML-7 and ML-11 that showed an entire basal leaf margin. Lobe incisions were entire except in ML-10 which showed medium lobe incision. Petiole colour was mostly purple except in ML-8 and ML-10 where green coloured petioles were observed (Plate I).

Table 4.1: Vegetative Parameters of M_4 Generation of Irradiated Sesame Lines

Mutant lines	SH (cm)	JH (cm)	HM (cm)	BPP	HFC (cm)
ML-1	1.26 ± 0.14^{ab}	3.78 ± 0.88^{a}	76.00 ± 3.04^{a}	1.86 ± 0.24^{a}	29.98 ± 1.92^{a}
ML-2	1.22 ± 0.08^{ab}	3.95 ± 0.20^{a}	117.45 ± 5.85^{de}	2.20 ± 0.50^{abc}	41.31 ± 0.48^{c}
ML-3	1.39 ± 0.13^{abc}	4.88 ± 0.53^{ab}	109.09 ± 8.21^{bcde}	2.07 ± 0.35^{ab}	33.99 ± 1.67^{abc}
Check-1	1.32 ± 0.09^{abc}	4.61 ± 1.05^{ab}	88.76 ± 5.68^{ab}	1.87 ± 0.13^{a}	33.92 ± 4.50^{abc}
ML-4	1.28 ± 0.17^{abc}	4.95 ± 0.66^{ab}	74.62 ± 2.47^{a}	1.97 ± 0.32^{ab}	26.46 ± 6.04^{a}
ML-5	1.25 ± 0.15^{ab}	5.09 ± 0.90^{ab}	98.70 ± 1.17^{bcd}	2.53 ± 0.35^{abcde}	24.50 ± 2.78^{a}
ML-6	1.84 ± 0.18^{d}	7.59 ± 1.20^{b}	123.83 ± 4.88^{e}	2.93 ± 0.07^{bcde}	41.30 ± 10.84^{c}
ML-7	1.51 ± 0.02^{bcd}	6.61 ± 1.36^{ab}	107.00 ± 1.62^{bcde}	3.27 ± 0.37^{e}	43.07 ± 3.22^{d}
Check-2	1.51 ± 0.12^{bcd}	5.60 ± 1.26^{ab}	114.85 ± 7.71^{cde}	3.13 ± 0.41^{cde}	36.38 ± 1.39^{cd}
ML-8	1.66 ± 0.14^{cd}	6.77 ± 1.22^{ab}	121.59 ± 4.39^{e}	3.20 ± 0.30^{de}	31.31 ± 4.17^{abcd}
ML-9	1.04 ± 0.12^{a}	5.04 ± 0.51^{ab}	94.60 ± 3.33^{abc}	2.87 ± 0.07^{bcde}	33.35 ± 6.51^{abc}
ML-10	1.42 ± 0.10^{abc}	5.21 ± 0.21^{ab}	115.66 ± 6.14^{cde}	2.27 ± 0.27^{abcd}	41.12 ± 10.91^{c}
ML-11	1.26 ± 0.08^{ab}	5.95 ± 1.02^{ab}	102.57 ± 4.83^{bcde}	2.67 ± 0.07^{abcde}	$31.03 \pm 3.^{87abcd}$
Check-3	1.45 ± 0.04^{bc}	4.88 ± 1.10^{ab}	104.72 ± 5.94^{bcde}	2.53 ± 0.07^{abcde}	34.83 ± 6.20^{bcd}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P < 0.05$.
SH=Seedling height (two weeks after planting), JH= Juvenile height (five weeks after planting), HM= Height at maturity, BPP= Branches per plant, HFC= Height of first capsule of main stem.

Plate I: Leaf morphology of M_4 gamma-irradiated *Sesamum indicum*. A. Ovate leaf with entire leaf margin. B. Narrowly cordate leaf with serrate leaf margin. C. Ovate leaf with serrate leaf margin. D. Narrowly cordate leaf with entire leaf margin. E. Leaf with medium lobe incision. F. Lanceolate leaf.

4.1.2 Yield parameters of M_4 lines of gamma irradiated sesame

4.1.2.1 Days to first flowering

The least number of days to the appearance of first flower was observed in ML-2 (31.07 ± 2.15 days) and was not significantly different ($p > 0.05$) from ML-3 (32.13 ± 0.68 days), ML-5 (31.87 ± 3.40 days), ML-6 (32.80 ± 2.77 days), ML-8 (32.13 ± 0.58 days), ML-10 (32.67 ± 1.85 days) and ML-11 (32.60 ± 2.66 days) (Table 4.2). The highest number of days to first flowering was observed in Check-1 (41.47 ± 2.67 days). Significant decreases were observed in number of days to first flowering of mutants ML-2 (31.07 ± 2.15 days), ML-3 (32.13 ± 0.68 days) and ML-6 (32.80 ± 2.77 days) (Table 4.2) indicating a probable induction of early maturity in the mutant lines.

4.1.2.2 Number of flowers per plant and flower morphology

Most of the M_4 lines showed significant variation in number of flowers (Table 4.2). The highest number of flowers per plant was observed in ML-8 (90.67 ± 3.48). The least number of flowers per plant was observed in ML-2 (25.67 ± 3.71). All the mutant lines showed significant enhancement in number of flowers produced per plant except for ML-2 (25.67 ± 3.71), ML-4 (32.33 ± 7.42), ML-11 (52.00 ± 4.04) and ML-10 (61.67 ± 4.37) that showed a significant decrease over their check varieties (Table 4.2).

Young flowers were observed to be yellowish in colour with profuse hairs and change colours as they develop in all the mutant lines. Matured flowers appeared as white (ML-3, ML-8, Check-2 and Check-3), white with light pink shading (ML-4, ML-6, ML-7, ML-9 and ML-11) or white with dark pink shading (Check-1, ML-1, ML-2, ML-5 and ML-10) (Plate II).

4.1.2.3 Days to 50 % flowering

No significant difference was observed in number of days to 50 % flowering (Table 4.2).

4.1.2.4 Days to physiological maturity

The highest number of days to physiological maturity was observed in ML-4 (123.67 ± 6.44 days) and was not significantly different ($p > 0.05$) from ML-2 (120.33 ± 1.33 days) and Check-1 (121.00 ± 3.06 days) (Table 4.2). The least number of days to physiological maturity was observed in ML-5 (101.33 ± 2.33 days) and was not significantly different ($p > 0.05$) from ML-6 (109.00 ± 3.46 days) ML-7 (108.67 ± 0.88 days) and ML-10 (106.00 ± 6.25 days). Mutants with significant lower number of days to attain physiological maturity comprised of ML-5 (101.33 ± 2.33 days), ML-10 (106.00 ± 6.25 days), ML-7 (108.67 ± 0.88 days) and ML-6 (109.00 ± 3.46 days) (Table 4.2).

4.1.2.5 Capsules per plant

The average least number of capsules per plant was observed in Ml-1 (46.22 ± 2.61) and was not significantly different ($p > 0.05$) from ML-4 (51.00 ± 5.29). Highest number of capsules per plant was observed in ML-8 (163.00 ± 5.57) and was not significantly different ($p > 0.05$) from ML-7 (151.00 ± 11.27) (Table 4.2). Mutant lines showing significant enhancement in number of capsules per plant over their checks comprised of ML-2 (77.30 ± 9.24), ML-3 (105.33 ± 5.84), ML-7 (151.00 ± 11.27) and ML-8 (163.00 ± 5.57) (Table 4.2).

4.1.2.6 Capsule length and capsule width

The least capsule length was observed in ML-8 (2.40 ± 0.10 cm) (Table 4.2). Check-2 and ML-3 were observed to have the highest capsule length (3.07 ± 0.09 cm). All mutants

derived from Check-1 and Check-3 showed significant increment in capsule length except ML-8 (2.40 ± 0.10 cm) and ML-10 (2.73 ± 0.18 cm). No significant increase was observed in mutant lines obtained from Check-2 (Table 4.2).

The highest width of capsule was observed in ML-9 (3.33 ± 0.03 cm) and was not significantly different ($p > 0.05$) from ML-11 (3.27 ± 0.23 cm). Mutants with significant increment in capsule width comprised of ML-1 (2.83 ± 0.12 cm), ML-5 (3.00 ± 0.23 cm), ML-6 (2.43 ± 0.09 cm), ML-7 (3.00 ± 0.06 cm), ML-9 (3.33 ± 0.03 cm), ML-10 (2.83 ± 0.03 cm) and ML-11 (3.27 ± 0.23 cm) (Table 4.2).

4.1.2.7 Fresh capsule weight and dry capsule weight

The line ML-3 had the least fresh capsule weight (1.29 ± 0.06 g). The highest fresh capsule weight was observed in ML-7 (2.26 ± 0.05 g). Significant increase was observed in the fresh capsule weight of all the mutant lines over their respective checks except in ML-3 (1.29 ± 0.06 g) and ML-4 (1.47 ± 0.14 g) (Table 4.2).

The highest dry capsule weight was observed in ML-8 (2.07 ± 0.51 g) (Table 4.2). The least dry capsule weight was observed in ML-1 (0.42 ± 0.00 g).

4.1.2.8 Seeds per capsule

The highest number of seeds per capsule was observed in ML-11 (103.33 ± 1.33) (Table 4.2). The least number of seeds per capsule was observed in ML-2 (66.00 ± 2.00). Significant enhancement was observed in number of seeds per capsule in all mutant lines derived from Check-2 and Check-3 varieties and ML-1 obtained from Check-1 (Table 4.2).

4.1.2.9 Hundred seed weight

ML-4 was observed to have the highest weight of hundred seeds (0.54 ± 0.05 g). The least weight of hundred seeds was observed in ML-2 (0.18 ± 0.01 g). Mutants with enhanced increment in hundred seed weight comprised of ML-1 (0.37 ± 0.02 g), ML-3 (0.31 ± 0.02 g), ML-4 (0.54 ± 0.05 g), ML-6 (0.28 ± 0.00 g) and ML-7 (0.26 ± 0.01 g).

Table 4.2: Yield Parameters of M_4 Generation of Irradiated Sesame Lines

Mutant Lines	DFF	NF	D50%F	DPM	CPP	CL (cm)	CW (cm)	FCW (g)	DCW (g)	SPC	HSW (g)
ML-1	33.66±5.20^{ab}	47.33±1.60^{cd}	43.33±8.19^{a}	111.33±2.60^{ab}	46.22±2.61^{a}	2.67±0.17^{abcd}	2.83±0.12^{bc}	1.62±0.07^{bcd}	0.42±0.00^{a}	78.00±8.00^{bc}	0.37±0.02^{d}
ML-2	31.07±2.15^{a}	25.67±3.71^{a}	42.00±1.00^{a}	120.33±1.33^{b}	77.30±9.24^{bc}	2.93±0.09^{bcd}	2.50±0.12^{ab}	1.61±0.06^{abcd}	0.60±0.02^{a}	66.00±2.00^{a}	0.18±0.01^{a}
ML-3	32.13±0.68^{a}	72.00±4.04^{fg}	45.33±0.67^{a}	112.67±5.49^{ab}	105.33±5.84^{d}	3.07±0.09^{d}	2.43±0.09^{ab}	1.29±0.06^{a}	0.46±0.02^{a}	76.00±2.00^{abc}	0.31±0.02^{c}
Check-1	41.47±2.67^{c}	36.67±2.60^{abc}	45.00±2.00^{a}	121.00±3.06^{b}	66.67±6.33^{ab}	2.60±0.12^{ab}	2.53±0.15^{ab}	1.38±0.08^{abc}	0.47±0.03^{a}	70.67±1.76^{abc}	0.28±0.04^{bc}
ML-4	33.03±3.57^{ab}	32.33±7.42^{ab}	43.67±1.86^{a}	123.67±6.44^{b}	51.00±5.29^{a}	2.60±0.12^{ab}	2.33±0.09^{a}	1.47±0.14^{abc}	0.61±0.05^{a}	76.00±2.00^{abc}	0.54±0.05^{e}
ML-5	31.87±3.40^{a}	46.00±6.03^{bcd}	44.00±0.58^{a}	101.33±2.33^{a}	78.00±8.39^{bc}	2.63±0.12^{abc}	3.00±0.23^{cd}	1.81±0.05^{de}	0.61±0.01^{a}	75.33±2.67^{abc}	0.24±0.02^{abc}
ML-6	32.80±2.77^{a}	71.67±5.20^{f}	42.66±2.40^{a}	109.00±3.46^{a}	65.33±12.84^{ab}	3.03±0.18^{cd}	2.43±0.09^{ab}	1.66±0.03^{cd}	0.52±0.04^{a}	72.00±1.15^{abc}	0.28±0.00^{bc}
ML-7	33.03±3.82^{ab}	84.33±4.9^{gh}	44.00±4.16^{a}	108.67±0.88^{a}	151.00±11.27^{e}	2.77±0.09^{abcd}	3.00±0.06^{cd}	2.26±0.05^{f}	0.88±0.06^{ab}	93.33±4.67^{d}	0.26±0.01^{bc}
Check-2	34.13±3.70^{ab}	37.00±2.65^{abc}	43.00±3.61^{a}	113.67±1.76^{ab}	96.00±5.13^{cd}	3.07±0.09^{d}	2.27±0.09^{a}	1.35±0.12^{abc}	0.57±0.02^{a}	70.00±1.15^{ab}	0.23±0.02^{ab}
ML-8	32.13±0.58^{a}	90.67±3.48^{h}	43.00±0.58^{a}	114.67±4.63^{ab}	163.00±5.57^{e}	2.40±0.10^{a}	2.43±0.09^{ab}	1.45±0.05^{abc}	2.07±0.51^{b}	72.00±1.15^{abc}	0.27±0.02^{bc}
ML-9	33.67±4.17^{ab}	71.00±9.85^{f}	42.00±4.93^{a}	118.67±3.18^{ab}	103.00±4.73^{d}	2.93±0.15^{bcd}	3.33±0.03^{d}	2.05±0.17^{ef}	0.59±0.02^{a}	81.33±3.71^{c}	0.22±0.02^{ab}
ML-10	32.67±1.85^{a}	61.67±4.37^{ef}	42.00±0.58^{a}	106.00±6.25^{a}	113.67±6.57^{d}	2.73±0.18^{abcd}	2.83±0.03^{bc}	1.36±0.14^{abc}	0.56±0.06^{a}	72.67±1.76^{abc}	0.21±0.01^{ab}
ML-11	32.60±2.66^{a}	52.00±4.04^{de}	42.33±1.76^{a}	114.33±7.62^{ab}	89.33±5.78^{bc}	2.97±0.07^{bcd}	3.27±0.23^{d}	2.09±.016^{ef}	1.08±0.33^{ab}	103.33±1.33^{e}	0.28±0.02^{bc}
Check-3	36.93±4.57^{bc}	67.33±3.39^{f}	44.67±3.18^{a}	116.33±4.06^{ab}	104.67±6.39^{d}	2.80±0.12^{abcd}	2.47±0.09^{ab}	1.30±0.06^{ab}	0.67±0.01^{a}	70.00±3.46^{ab}	0.27±0.01^{bc}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P < 0.05$.
DFF= Days to first flowering, NF= Number of flowers per plant, D50%F= Days to 50 % flowering, DPM= Days to physiological maturity, CPP=Capsule per plant, CL=Capsule length, CW= Capsule width, FCW= Fresh capsule weight, DCW= Dry capsule weight, SPC= Seeds per capsule, HSW= Hundred Seeds Weight.

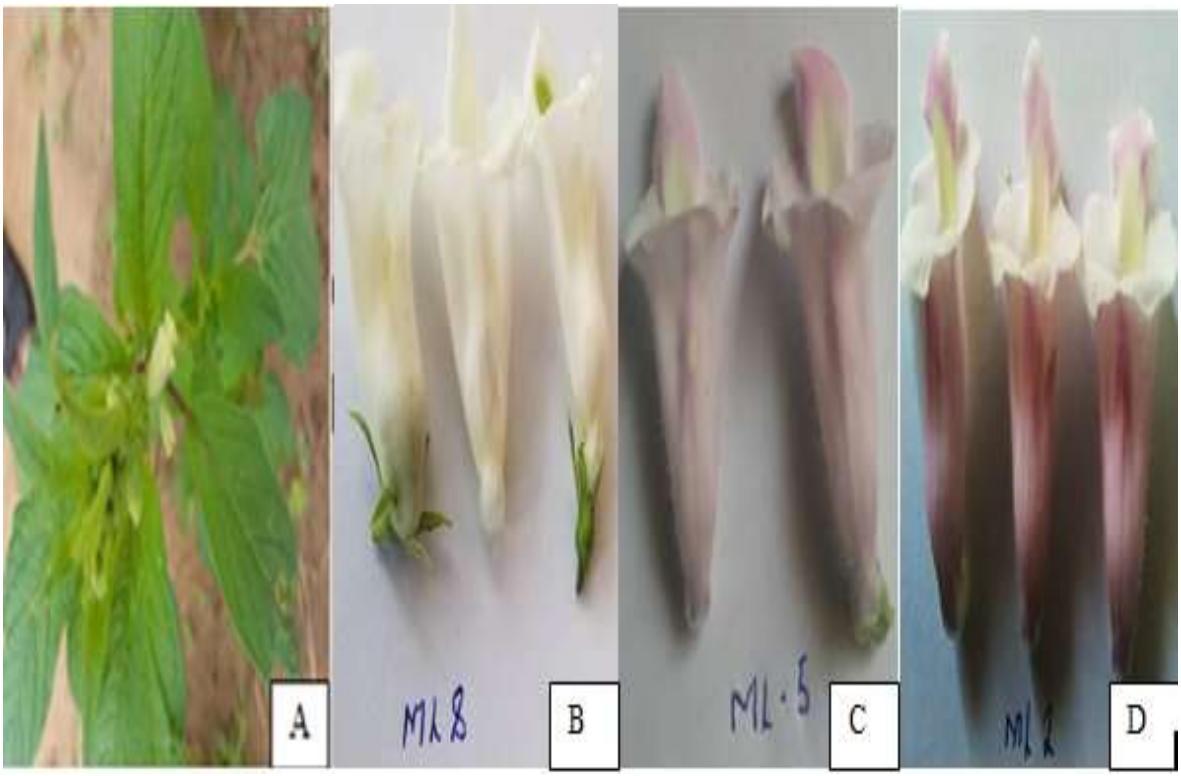

Plate II: Flower morphology of M_4 gamma-irradiated *Sesamum indicum*. A. Young flowers. B. Matured white flower. C. Matured white flower with light pink shading. D. Matured white flower with deep pink shading.

4.1.3 Capsule characteristics of gamma-irradiated M_4 lines of sesame

Notable differences were observed in some of the capsule characteristics (Table 4.3). All the bicarpellate capsules were broad oblong. Capsule hairiness ranged from weak (3) to medium (5) with ML-1 and Check-1 having weak capsule hairs (Table 4.3). Capsule arrangement ranged from monocapsular capsules (ML-1, ML-7, ML-8 and Check-3) to multi capsules per leaf axils (ML-2, ML-3, ML-4, NL-5, ML-6, ML-9, ML-10, ML-11, Check-1 and Check-2). Colour of sun-dried capsules was observed to be brown/tan except in ML-11 and Check-3 which had yellow/straw sundried capsules (Table 4.3).

Great variations were observed in the capsule morphology (Plate III). Some of the M_4 mutant lines had bicarpellate single capsules (ML-1, ML-8), some lines had bicarpellate multicapsule (ML-2, ML-3, ML-4, ML-6, ML-10), ML-7 had multicarpellate single capsules while ML-5, ML-9 and ML-11 had multicarpellate multicapsule with varying number of locules per capsule (4-8), length, width and number of seeds enclosed (Plate IV).

Notable disparities were observed in capsule arrangement ranging from monocapsular (opposite or alternate) to multicapsular (whorl) arrangements with capsules having sparse to medium hairs. Colour of sun-dried capsules ranged from brown/tan to yellow/straw while fresh capsules were green (Plate V).

Notable differences were also observed in capsule angle on main stem (Plate VI). Some of the mutant lines showed mixed or segregating capsules (ML-2, ML-3, Check-3, ML-4, ML-7 and ML-11), some showed capsules that are narrow/snug on the stem (ML-8, ML-9 and ML-10). Mutant lines ML-1 and ML-6 and Check-1 and Check-2 showed medium capsule angle (30^o) while ML-5 showed wide capsule angle (45^o).

Table 4.3: Capsule Characteristics of Gamma-Irradiated M_4 Lines of Sesame

Mutant lines	Bicarpellate capsule shape	Capsule hairiness	Capsule arrangement	Capsule angle on stem	Colour of sun-dried capsule
ML-1	2	3	1	5	3
ML-2	2	5	2	1	3
ML-3	2	5	2	1	3
Check-1	2	3	2	7	2
ML-4	2	5	2	7	3
ML-5	**	3	2	7	3
ML-6	2	5	2	1	3
ML-7	**	5	1	3	3
Check-2	2	5	2	5	3
ML-8	2	5	1	1	3
ML-9	**	5	2	5	3
ML-10	2	5	2	1	3
ML-11	**	5	2	5	2
Check-3	2	5	1	3	3

Capsule shape; tapered at the apex (1), narrow oblong (2), broad oblong (3) or square (4) and multicapsulate (**)
Capsule hairiness; glabrous (0), weak (3), medium (5) and strong (7).
Capsule arrangement; mono-capsular (1) or multi capsular (2).
Colour of sun-dried capsule: Green (1), Straw/yellow (2), Brown/tan (3) and Purple (4)
Capsule angle on stem: Segregating or mixed (1), narrow or snug on stem (3), Medium about 30^o (5), Wide about 45^o (7).

Plate III: Capsules per leaf axil of M_4 gamma-irradiated *Sesamum indicum*. A. ML-2 showing 2-3 capsules per leaf axil. B. ML-6 with 2 capsules per leaf axil. C. ML-9 showing leaf axils with single and 2 capsules. D. ML-10 showing 2-3 capsules per leaf axil. E. ML-11 showing leaf axils with 1-3 capsules.

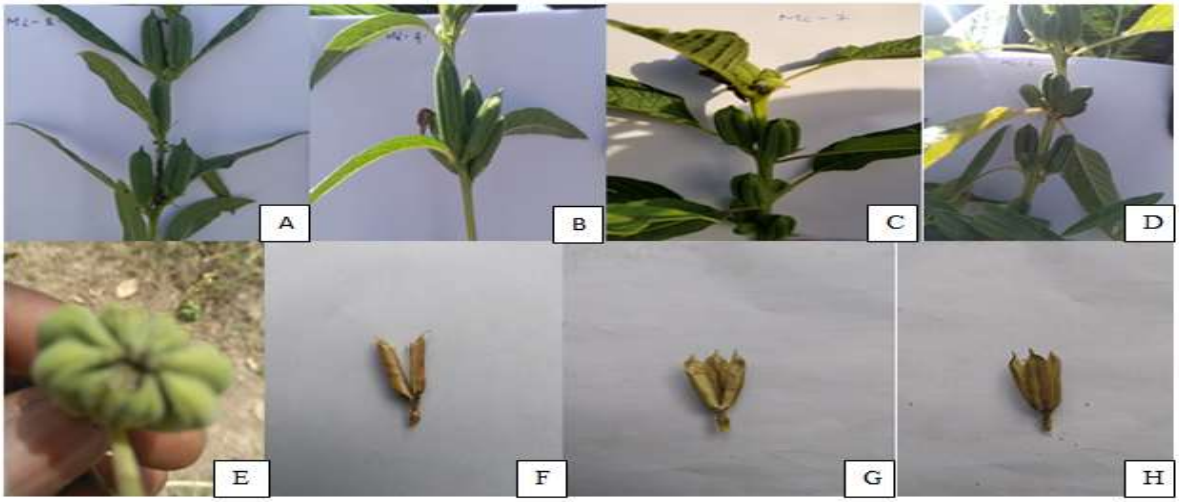

Plate IV: Capsule arrangement and locules of M_4 gamma-irradiated *Sesamum indicum*. A. Bicarpellate single capsules. B. Bicarpellate multicapsule. C. Multicarpellate single capsules. D. Multicarpellate multicapsule. E. Capsule with seven carpels. F. Bicarpellate capsule with four locules. G. Multicarpellate capsule with six locules. H. Multicarpellate capsule with eight locules.

Plate V: Colour of sun-dried capsules of M_4 gamma-irradiated *Sesamum indicum*. A. Multicarpellate capsules with brown/tan sundried capsules. B. Multicarpellate capsules with yellow/straw sundried capsules with brown patches. C. Bicarpellate capsules with brown/tan sundried capsules. D. Bicarpellate capsules with yellow/straw sundried capsules with brown patches. E. Bicarpellate capsules progressing to yellow before physiological maturity.

Plate VI: Angle of capsule on stem of M_4 lines of *Sesamum indicum*. A. segregating or mixed capsules. Black arrow shows a snug capsule while red arrow shows a wide capsule (45^o) and the blue arrow shows a medium capsule (30^o). B. capsules that are narrow/snug on the stem. C. Medium capsule angle (30^o). D. Wide capsule angle (45^o).

4.1.4 Seed characteristics of gamma-irradiated M_4 lines of sesame

Notable disparities were observed in the morphological characters of seeds of gamma-irradiated M_4 lines. Though the seeds were tiny and the seed coat textures were smooth, the seed coat colour however, ranged from brick red to dark brown, medium brown, light brown to cream to white and with varying weights (Plate VII).

Plate VII: Seeds of gamma irradiated M_4 lines. A=ML-1: cream, B=ML-2: cream, C=ML-3: medium brown, D=Check-1: white, E=ML-4: medium brown, F=ML-5: dark brown, G=ML-6: medium brown, H=ML-7: cream, I=Check-2: cream, J=ML-8: tan, K=ML-9: light brown, L=ML-10: light brown, M=ML-11: light brown and N=Check-3: cream.

Table 4.4: Description and Characteristics of M_3 and M_4 Lines

Mutant lines	Mutant Name	Parental Description	New M_4 Lines
ML-1	$04E450G_{1-3}$	3 carpels per capsule, single capsule per leaf axil	3 carpels per capsule, single capsule per leaf axil
ML-2	$04E450G_{2-3}$	2 carpels per capsule, single capsule per leaf axil	2 carpels per capsule, 2-3 capsules per leaf axil
ML-3	$04E450G_{3-3}$	2 carpels per capsule, 2-3 capsules per leaf axil	2 carpels per capsule, 2-3 capsules per leaf axil
ML-4	$01M350G_{2-22}$	2 carpels per capsule, 2-3 capsules per leaf axil	2 carpels per capsule, 1-3 capsules per leaf axil
ML-5	$01M350G_{1-21}$	3-4 carpels per capsule, 2-3 capsules per leaf axil	3-4 carpels per capsule, 2-3 capsules per leaf axil
ML-6	$01M550G_{2-2}$	2 carpels per capsule, single capsule per leaf axil	2 carpels per capsule, 1-2 capsule per leaf axil
ML-7	$01M350G_{1-2}$	3 carpels per capsule, single capsule per leaf axil	3 carpels per capsule, single capsule per leaf axil
ML-8	$03L550G_{1-2}$	2 carpels per capsule, single capsule per leaf axil	2 carpels per capsule, single capsule per leaf axil
ML-9	$03L450G_{2-2}$	3 carpels per capsule, single capsule per leaf axil	3 carpels per capsule, 1-2 capsules per leaf axil
ML-10	$03L250G_{1-1}$	2 carpels per capsule, single capsule per leaf axil	2 carpels per capsule, 2-3 capsules per leaf axil
ML-11	$03L250G_{1-11}$	3 carpels per capsule, single capsule per leaf axil	3 carpels per capsule, 1-3 capsules per leaf axil

Notable differences were observed in number of capsules per leaf axil between M_3 and M_4 generations. Some of the mutants previously described to possess single capsules per leaf axil in M_3 generation were observed to be multicapsulate with 2-3 capsules per leaf axil in the M_4 generation (Table 4.4). This indicates that the mutant lines are still segregating and are yet to be stable in terms of this trait. Mutants with enhancement in number of capsule per leaf axil comprise of ML-2 (2-3 capsules per leaf axil), ML-6 (1-2 capsules per leaf axil), ML-9 (1-2 capsules per leaf axil), ML-10 (2-3 capsules per leaf axil) and ML-11 (1-3 capsules per leaf axil), all of which were previously reported to produce single capsules per leaf axil in M_3 generation.

4.1.5 Pollen parameters of M_4 lines of gamma irradiated sesame lines

4.1.5.1 Pollen viability

The highest pollen viability (Plate VIIIa) was observed in ML-10 (97.56 ± 0.89 %) which was not significantly different ($p > 0.05$) from ML-7 (95.61 ± 1.03 %), Check-2 (95.60 ± 1.18 %) and ML-8 (95.01 ± 1.69 %) (Table 4.5). The least pollen viability was observed in ML-1 (86.70 ± 3.80 %). No significant difference was observed in number of semi-viable pollens among the mutant lines (Table 4.5).

4.1.5.2 Pollen shape and size

A few elliptic pollens in polar view (Plate VIIIb) were observed in all the mutant lines except in ML-7 and the check groups. The elliptic pollens had relatively smaller pollen diameter. The highest grain size was observed in Check-1 (169.52 ± 1.76 μm). The least grain size was observed in ML-7 (106.52 ± 0.33 μm). The least pollen diameter of elliptic pollens was observed in ML-5 (99.00 ± 0.58 μm). The pollen grains were suboblate in

shape (Plate VIIIc) in all the mutant lines investigated. No variations were observed in pollen shape of the mutant lines and check groups.

4.1.5.3 Pollen germinability

The least pollen germinability at 10 % sucrose concentration was observed in Check-1 (11.46 ± 0.24 %) (Plate IXa) and was not significantly different ($p > 0.05$) from ML-1 (11.65 ± 2.26 %) and ML-4 (12.15 ± 3.35 %). The highest pollen germinability at 10 % sucrose concentration was observed in ML-8 (22.81 ± 1.66 %) (Table 4.5). At 20 % sucrose concentration, highest pollen germinability was observed in ML-7 (39.70 ± 4.75 %) while the least (24.23 ± 1.55 %) was observed in Check-2 (Plate IXb). At 30 % sucrose concentration, the least pollen germinability was observed in the control Check-1 (12.81 ± 2.14 %) and ML-10 (13.15 ± 2.00 %) (Plate IXc).

Table 4.5: Pollen Parameters of M_4 Mutants of Gamma-Irradiated Sesame

Mutant Lines	Pollen Physical Parameters				Pollen Germinability Percentage		
	VP (%)	SVP (%)	PDC (µm)	PDE (µm)	10 % sucrose	20 % sucrose	30 % sucrose
ML-1	86.70±3.80^{a}	4.28±0.27^{a}	138.02±0.88^{b}	117.00±2.00	11.65±2.26^{a}	32.11±3.29^{abc}	19.05±1.76^{b}
ML-2	94.53±0.79^{ab}	1.06±0.05^{a}	144.00±1.15^{ab}	109.49±0.88	19.80±5.26^{b}	36.03±1.98^{bc}	19.16±4.81^{b}
ML-3	93.26±2.63^{ab}	1.87±0.10^{a}	156.02±1.45^{abc}	109.49±0.88	15.29±3.01^{ab}	38.23±0.81^{bc}	20.83±1.18^{b}
Check-1	92.62±2.24^{ab}	1.83±0.31^{a}	169.52±1.76^{c}	**	11.46±0.24^{a}	24.67±2.40^{a}	12.81±2.14^{a}
ML-4	92.97±2.84^{ab}	1.86±0.12^{a}	165.02±1.20^{bc}	108.00±1.15	12.15±3.35^{a}	34.13±3.70^{abc}	20.58±1.92^{b}
ML-5	90.63±2.46^{ab}	1.44±0.09^{a}	156.02±1.45abc	99.00±0.58	15.17±1.84^{ab}	35.77±1.13^{bc}	16.05±4.43^{ab}
ML-6	91.90±3.04^{ab}	0.77±0.08^{a}	139.50±0.58^{b}	117.00±1.15	15.60±7.06^{ab}	30.57±3.13^{abc}	14.02±1.68^{ab}
ML-7	95.61±1.03^{b}	0.97±0.06^{a}	106.52±0.33a	**	15.00±1.40^{ab}	39.70±4.75^{c}	21.24±7.80^{b}
Check-2	95.60±1.18^{b}	0.96±0.14^{a}	138.02±0.67^{b}	**	15.31±1.83^{ab}	24.23±1.55^{a}	15.63±3.70^{ab}
ML-8	95.01±1.69^{b}	1.55±0.27^{a}	138.02±0.67^{b}	117.00±1.15	22.81±1.66^{b}	38.71±4.48^{bc}	19.71±1.30^{b}
ML-9	89.35±1.33^{ab}	2.21±0.12^{a}	151.52±0.88^{abc}	117.00±1.15	20.36±5.18^{b}	38.84±1.32^{bc}	15.37±0.76^{ab}
ML-10	97.56±0.89^{b}	0.57±0.07^{a}	157.50±3.05^{abc}	121.50±0.57	18.98±2.70^{ab}	29.05±5.94^{abc}	13.15±2.00^{a}
ML-11	91.77±4.63^{ab}	2.07±0.15^{a}	147.02±1.20^{ab}	111.02±1.20	16.39±2.39^{ab}	28.19±4.42^{ab}	18.70±2.08^{b}
Check-3	92.12±3.48^{ab}	1.75±0.10^{a}	163.49±2.33^{bc}	**	14.55±1.73^{ab}	24.40±0.50^{a}	15.60±2.40^{ab}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P \leq 0.05$.

VP=Viable pollen, SVP= semi viable pollen, PDC= Pollen diameter of circular pollens, PDE= Pollen diameter of elliptic pollens.

**: No elliptic pollen structure

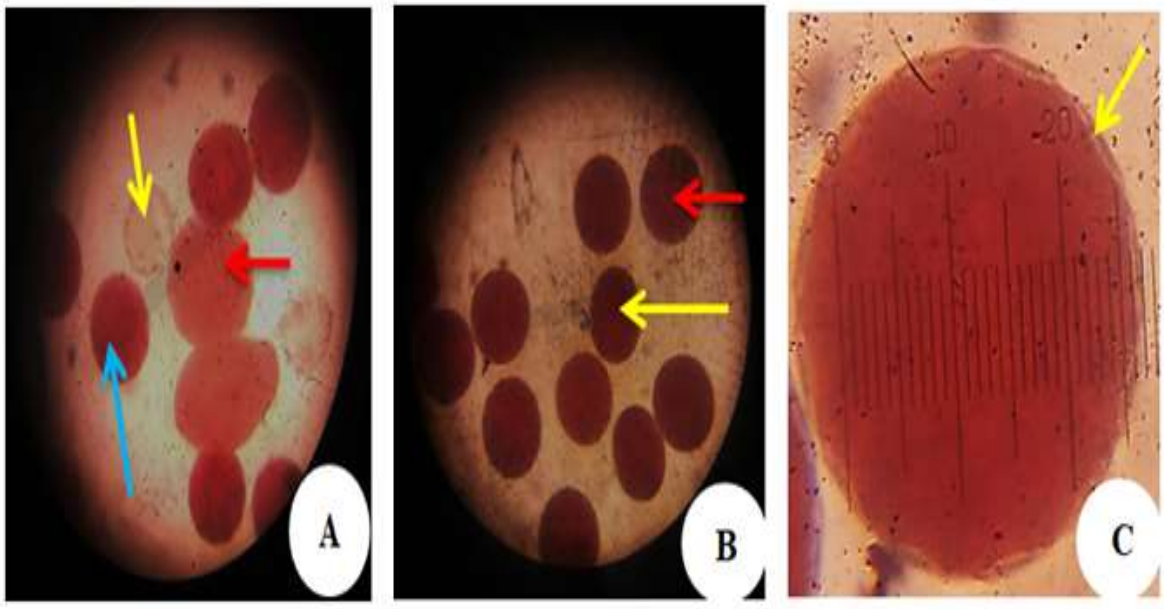

Plate VIII: Pollen viability and pollen shape of M_4 gamma-irradiated *Sesamum indicum*.

A. Pollen viability: yellow arrow showing non-viable pollen, red arrow showing a semi-viable pollen and blue arrow showing viable pollen. B. Pollen shape in polar view: red arrow showing a circular pollen and yellow arrow showing elliptic pollen in polar view. C. Pollen shape described as suboblate with yellow arrow showing the colpi.

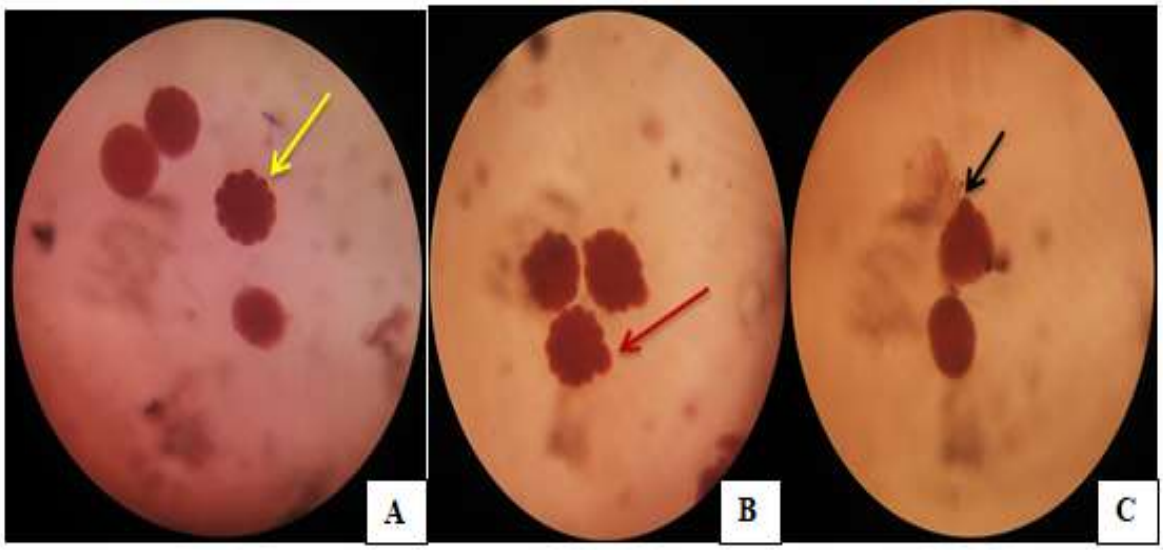

Plate IX: Pollen germinability of M_4 gamma-irradiated *Sesamum indicum*. A. Pollen germinability at 10 % sucrose concentration, yellow arrow showing protrusions for tube growth B. Pollen germinability at 20 % sucrose concentration, red arrow showing pollen tube growth C. Pollen germinability at 30 % sucrose concentration, black arrow showing pollen tube growth

4.1.6 Correlation between yield parameters of M_4 lines of gamma irradiated sesame

Both positive and negative correlations were observed in yield parameters of M_4 lines (Table 4.6). Positive and negative correlations were observed between capsule per plant and hundred seed weight but were not significant. A perfect positive correlation was observed between seeds per capsule and hundred seed weight in ML-10 (1.00*). High positive correlations were observed between capsule per plant and seeds per capsule in ML-11 (0.97*) and between capsule per plant and seeds per capsule in ML-8 (0.99*) (Table 4.6).

A perfect positive correlation was observed between capsule length and seeds per capsule in ML-1 (1.00**). High positive correlation was observed between number of flowers and capsule per plant in ML-7 (0.95*) and check-3 (0.88*) while a high negative correlation was observed in ML-9 (-0.82*). A perfect positive correlation was also observed between branches per plant and capsules per plant in ML-3 (1.00**).

Table 4.6: Correlation between yield parameters of M_4 lines

PARAMETERS	ML-1	ML-2	ML-3	CHECK 1	ML-4	ML-5	ML-6	ML-7	CHECK 2	ML-8	ML-9	ML-10	ML-11	CHECk-3
CPP/HSW	0.08	0.08	-0.84	-0.91	0.08	0.48	0.48	0.84	-0.44	-0.25	0.16	-0.42	-0.43	-0.99
SPC/HSW	-0.36	-0.76	0.00	-0.95	-059	-0.90	0.87	0.14	-0.78	0.16	0.94	1.00**	-0.66	0.00
CPP/SPC	-0.20	-0.79	0.94	0.01	-0.56	-0.76	-0.22	0.95	-0.94	-0.99*	0.48	-0.42	0.97*	0.17
CL/SPC	1.00**	-0.19	0.95	0.98	0.87	-0.69	-0.66	0.54	-0.98	0.00	0.27	0.93	-0.50	0.50
NOF/CPP	0.22	0.71	-0.48	-0.55	-0.59	0.52	-0.82	0.95*	-0.57	-0.78	-0.82*	0.74	0.03	0.88*
BPP/CPP	-0.99	0.86	1.00**	-0.33	0.84	0.90	0.52	-0.58	0.82	0.09	-0.64	-0.94	-0.71	0.34

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).
CPP=Capsule per plant, CL=Capsule length, SPC= Seeds per capsule, HSW= Hundred Seeds Weight, NOF= Number of flowers per plant, BPP= Branches perplant

4.1.7 Correlation among morphological and yield parameters of M_4 lines of gamma irradiated sesame

Correlations among the yield parameters ranged from moderate to low in all the mutant lines and their respective checks. Moderate positive correlations were observed between plant height and capsules per plant (0.58*), capsule width and fresh capsule weight (0.65*), capsule width and seeds per capsule (0.57**) and between fresh capsule weight and seeds per capsule (0.66**) (Table 4.7).

Moderate negative correlations were observed between plant height and hundred seed weight (-0.54**), plant height and days to first flowering (-0.45**), , capsule per plant and days to first flowering (-0.42**), capsule per plant and days to physiological maturity (-0.37*), branches per plant and days to first flowering (-0.61**), and between branches per plant and days to physiological maturity (-0.53**) (Table 4.7), this indicates that an increase in one trait leads to a decrease in the other.

Low positive correlations observed between capsule per plant and dry capsule weight (0.44*), branches per plant and plant height (0.37*), branches per plant and fresh capsule weight (0.35*), branches per plant and dry capsule weight (0.35*), branches per plant and capsule per plant (0.46**) and between branches per plant and number of flowers per plant (0.33*).

Table 4.7: Pearson's Linear Correlation among Morphological and Yield Parameters of M_4 Lines

PARAMETERS	PH	CL	CW	FCW	DCW	SPC	HSW	DFF	DF	DPM	CPP	BPP	NF
PH	1.00												
CL	0.30	1.00											
CW	-0.11	-0.02	1.00										
FCW	0.02	0.11	0.65**	1.00									
DCW	0.14	-0.26	0.07	0.10	1.00								
SPC	-0.15	0.20	0.57**	0.66**	0.08	1.00							
HSW	-0.54**	-0.25	-0.20	-0.11	-0.03	0.05	1.00						
DFF	-0.45**	-0.12	-0.28	-0.25	-0.26	-0.19	0.20	1.00					
DF	0.05	0.16	-0.19	-0.21	-0.04	-0.06	-0.06	0.27	1.00				
DPM	-0.20	0.01	0.09	-0.09	-0.17	-0.08	0.03	0.35*	0.04	1.00			
CPP	0.58*	-0.20	0.20	0.14	.44**	0.25	-0.23	-0.42**	-0.17	-0.37*	1.00		
BPP	0.37*	0.19	0.09	0.35*	0.35*	0.18	-0.27	-0.61**	-0.26	-0.53**	0.46**	1.00	
NF	0.08	-0.23	0.30	0.15	0.31*	0.30	-0.17	-0.22	-0.16	-0.25	0.88**	0.33*	1.00

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

PH; plant height, CL: capsule length, CW: capsule width, FCW: fresh capsule weight, DCW: dry capsule weight, SPC: seeds per capsule, HSW: hundred seed weight, DFF: days to first flowering, DF: days to 50% flowering, DPM: days to physiological maturity, CPP: capsules per plant, BPP: branches per plant and NF: Number of flowers per plant.

4.1.8 Proximate composition of gamma irradiated M_4 lines of sesame

4.1.8.1 Moisture content

The least moisture content was observed in check-2 (3.26 ± 0.02 %) (Table 4.8). The highest moisture content was observed in ML-1 (5.46 ± 0.05 %). All the M_4 mutants showed increased moisture content over their check groups with the exception of ML-8 (3.38 ± 0.01 %) (Table 4.8).

4.1.8.2 Percentage ash

The least percentage ash was observed in ML-9 (3.34 ± 0.36 %) (Table 4.8). Check-2 (5.97 ± 0.12 %) was observed to have the highest percentage ash and was significantly the same ($p > 0.05$) with ML-2 (5.77 ± %), Check-1 (5.85 ± 0.01 %), Check-3 (5.85 ± 0.04 %) and ML-1 (5.85 ± 0.52 %). Significant decrease was observed in ash contents of ML-3 (5.33 ± 0.40 %), ML-4 (4.33 ± 0.29 %), ML-5 (5.29 ± 0.05 %), ML-6 (4.85 ± 0.01 %), ML-7 (3.89 ± 0.03 %), ML-8 (4.78 ± 0.01 %), ML-9 (3.34 ± 0.36 %) and ML-11 (4.90 ± 0.03 %) over their check groups (Table 4.8).

4.1.8.3 Crude fibre

ML-2 (3.23 ± 0.12 %) had the least percentage crude fibre (Table 4.8). The highest percentage crude fibre was observed in ML-10 (4.08 ± 0.17 %). Mutants that showed enhancement in crude fibre content over their control groups comprise of ML-1 (4.01 ± 0.05 %), ML-5 (4.02 ± 0.08 %), ML-8 (4.04 ± 0.04 %) and ML-10 (4.08 ± 0.17 %).

4.1.8.4 Crude protein

The highest percentage crude protein was observed in ML-7 (32.83 ± 1.50 %) (Table 4.8). The least percentage crude protein was observed in Check-2 (19.93 ± 0.03 %). Mutants

obtained from Check-1 and Check-2 showed significant enhancement in protein contents. However, mutants obtained from Check-3 showed no significant increase in protein contents of the M_4 generation (Table 4.8).

4.1.8.5 NFE/CHO Kg/g

The least NFE/CHO kg/g was observed in ML-6 (15.98 ± 1.17 kg/g) and was significantly the same ($p > 0.05$) with ML-5 (17.10 ± 0.67 kg/g) (Table 4.8). The highest NFE/CHO kg/g was observed in ML-1 (38.44 ± 0.58 kg/g). Mutants exhibiting enhanced NFE/CHO comprise of ML-1 (38.44 ± 0.58 kg/g), ML-4 (28.17 ± 0.87 kg/g), ML-8 (29.59 ± 1.00 kg/g), ML-9 (27.03 ± 0.62 kg/g) and ML-11 (25.77 ± 1.06 kg/g) (Table 4.8).

4.1.8.6 Energy kj/kg

The highest energy kj/kg was observed in ML-6 (586.86 ± 5.37 kj/kg) and was not significantly different ($p > 0.05$) from energy value observed in ML-5 (579.27 ± 2.83 kj/kg) and Check-2 (572.58 ± 5.72 kj/kg). The least energy value was observed in ML-1 (455.73 ± 6.35 kj/kg). Mutants 2 (527.01 ± 4.04 kj/kg), ML-9 (541.50 ± 7.51 kj/kg), ML-10 (539.81 ± 4.08 kj/kg) and ML-11 (544.46 ± 6.42 kj/kg) showed significant increase in energy value over their respective controls (Table 4.8).

4.1.8.7 Oil percentage

ML-6 (48.28 ± 0.58 %) had the highest percentage oil and was statistically the same ($p > 0.05$) with ML-5 (46.31 ± 1.56 %) and Check-2 (45.10 ± 1.10 %). The least percentage oil was observed in ML-1 (23.41 ± 1.15 %). Mutants revealing significant enhancement in seed oil content comprised of ML-2 (40.32 ± 1.75 %) and ML-10 (40.05 ± 0.55 %) (Table 4.8).

Table 4.8: Proximate Composition of M_4 Mutants of Gamma-irradiated Sesame Seed

Mutant Lines	Moisture content %	Ash content %	Crude fibre %	Crude protein %	CHO kg/g	Energy value kj/kg	Oil (%)
ML-1	5.46±0.05^{j}	5.85±0.52^{e}	4.01±0.05^{b}	22.83±0.98^{abc}	38.44±0.58i	455.73±6.35^{a}	23.41±1.15^{a}
ML-2	5.12±0.01^{i}	5.77±0.29^{e}	3.23±0.12^{a}	28.63±1.56^{e}	20.39±1.02^{bc}	527.01±4.04bc^{de}	40.32±1.75^{d}
ML-3	4.56±0.05^{g}	5.33±0.40^{de}	3.50±0.03^{ab}	27.41±1.10^{de}	25.49±2.02^{def}	513.75±3.75^{b}	33.71±2.25^{b}
Check-1	3.76±0.03^{d}	5.85±0.01^{e}	3.44±0.08^{ab}	20.31±1.50^{ab}	31.17±1.21^{h}	525.15±5.54^{bcd}	35.65±0.70^{bc}
ML-4	5.39±0.01^{j}	4.33±0.29^{bc}	3.65±0.05^{ab}	23.72±0.01^{bc}	28.17±0.87^{fg}	520.17±4.33^{bc}	34.73±2.14^{bc}
ML-5	3.79±0.03^{d}	5.29±0.05^{de}	4.02±0.08^{b}	22.85±1.10^{abc}	17.10±0.67^{a}	579.27±2.83^{g}	46.31±1.56^{e}
ML-6	3.90±0.02^{e}	4.85±0.01^{cd}	3.54±0.46^{ab}	23.41±1.44^{abc}	15.98±1.17^{a}	586.86±5.37^{g}	48.28±0.58^{e}
ML-7	4.89±0.05^{h}	3.89±0.03^{ab}	3.33±0.05^{a}	32.83±1.50^{f}	18.32±0.63^{ab}	535.26±0.11^{cdef}	36.74±0.98^{bcd}
Check-2	3.26±0.02^{a}	5.97±0.12^{e}	4.00±0.07^{b}	19.93±0.03^{a}	21.74±0.68^{c}	572.58±5.72^{g}	45.10±1.10^{e}
ML-8	3.38±0.01^{b}	4.78±0.01^{cd}	4.04±0.04^{b}	23.73±0.81^{bc}	29.59±1.00^{gh}	523.60±1.15^{bc}	34.48±1.85^{bc}
ML-9	4.97±0.02^{h}	3.34±0.36^{a}	3.72±0.25^{ab}	24.99±1.30^{cd}	27.03±0.62^{efg}	541.50±7.51^{ef}	37.50±0.68^{bcd}
ML-10	4.21±0.03^{f}	5.88±0.07^{e}	4.08±0.17^{b}	25.50±1.25^{cde}	22.59±0.64^{cd}	539.81±4.08^{def}	40.05±0.55^{d}
ML-11	3.66±0.03^{c}	4.90±0.03^{cd}	3.50±0.40^{ab}	24.63±0.90^{cd}	25.77±1.06^{ef}	544.46±6.42^{f}	38.65±0.66^{cd}
Check-3	3.45±0.03^{b}	5.85±0.04^{e}	3.65±0.03^{ab}	25.31±0.46^{cde}	24.85±0.26^{de}	532.46±2.88^{cdef}	36.97±1.22^{bcd}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P < 0.05$.

4.1.9 Anti-nutritional factors of gamma irradiated M_4 lines of sesame

4.1.9.1 Tannin content (%)

The highest tannin content was observed in ML-10 (0.303 ± 0.001 %) (Table 4.9). The least tannin content was observed in ML-11 (0.170 ± 0.001 %). Significant increase was observed in tannin content of ML-2 (0.281 ± 0.008 %), ML-9 (0.299 ± 0.002 %) and ML-10 (0.303 ± 0.001 %). However, no significant increase was observed in tannin contents of mutants obtained from Check-2 (Table 4.9).

4.1.9.2 Flavonoid and alkaloid content (%)

Check-2 was observed to have the highest flavonoid content (5.445 ± 0.675 %). The least flavonoid content was observed in ML-8 (0.460 ± 0.020 %) (Table 4.9). Mutants with significant increment in flavonoid content comprise of ML-1 (2.645 ± 0.112 %), ML-2 (2.015 ± 0.005 %), Ml-3 (3.005 ± 0.115 %), ML-9 (3.500 ± 0.030 %) and ML-11 (4.620 ± 0.100 %). The least alkaloid percentage was observed in ML-1 (0.265 ± 0.045 %) (Table 4.9). The highest alkaloid content was observed in check-2 (1.500 ± 0.030 %). Mutants with significant increase in alkaloid content comprise of ML-2 (1.480 ± 0.040 %), ML-3 (0.620 ± 0.120 %) and ML-8 (1.096 ± 0.024%) (Table 4.9).

4.1.9.3 Oxalate (mg)

The highest oxalate content was observed in ML-11 (12.014 ± 0.089 mg) (Table 4.9). Mutants ML-1 (2.545 ± 0.005 mg) and ML-3 (2.570 ± 0.070 mg) showed significantly least oxalate content. Mutants with significant reduction in oxalate content comprise of ML-1 (2.545 ± 0.005 mg), ML-2 (4.837 ± 0.004 mg), ML-3 (2.570 ± 0.070 mg), ML-9 (3.522 ± 0.002 mg) and ML-10 (4.522 ± 0.020 mg).

4.1.9.4 Phytate (%)

Check-2 had the significant least phytate content (0.532 ± 0.002 %). The highest phytate content was observed in ML-2 (1.655 ± 0.003 %) (Table 4.9). All mutants derive from Check-3 revealed significant reduction in phytate content.

4.1.9.5 Hydro cyanide (mg/kg)

The highest hydro cyanide content was observed in ML-10 (50.236 ± 0.135 mg/kg). The least hydro cyanide content was observed in ML-11 (24.137 ± 4.011 mg/kg). Mutants with significant enhancement in hydro cyanide content comprise of ML-2 (47.980 ± 0.168 mg/kg), ML-4 (46.300 ± 0.630 mg/kg), ML-5 (46.298 ± 0.370 mg/kg), and ML-10 (50.236 ± 0.135 mg/kg) (Table 4.9).

Table 4.9: Anti-nutritional Factors of M_4 Mutants of Gamma-Irradiated Sesame Seed

Mutant lines	Tannin (%)	Flavonoid (%)	Alkaloid (%)	Oxalate (mg)	Phytate (%)	Hydro cyanide (mg/kg)
ML-1	0.242±0.003^{c}	2.645±0.112^{cd}	0.265±0.045^{a}	2.545±0.005^{a}	1.278±0.002^{gh}	43.219±0.003^{cd}
ML-2	0.281±0.008^{e}	2.015±0.005^{bc}	1.480±0.040^{f}	4.837±0.004^{d}	1.655±0.003^{j}	47.980±0.168^{efg}
ML-3	0.264±0.002^{d}	3.005±0.115^{de}	0.620±0.120^{c}	2.570±0.070^{a}	1.048±0.011^{e}	43.708±0.004^{cd}
Check-1	0.267±0.001^{d}	1.520±0.010^{b}	0.540±0.010^{bc}	5.790±0.070^{e}	0.981±0.011^{d}	44.177±0.263^{cd}
ML-4	0.201±0.003^{b}	3.495±0.235^{ef}	1.005±0.025^{de}	2.750±0.352^{ab}	1.101±0.070^{ef}	46.300±0.630^{def}
ML-5	0.278±0.002^{e}	4.030±0.030^{fg}	0.510±0.010^{bc}	3.306±0.005^{c}	1.319±0.001^{h}	46.298±0.370^{def}
ML-6	0.248±0. 004^{c}	1.437±0.115^{b}	0.505±0.005^{bc}	2.961±0.013^{b}	1.120±0.002^{f}	40.925±0.184^{c}
ML-7	0.194±0.002^{b}	5.195±0.305^{hi}	1.495±0.005^{f}	2.860±0.048^{ab}	0.687±0.015^{b}	32.464±0.130^{b}
Check-2	0.286±0.003^{ef}	5.445±0.675^{i}	1.500±0.030^{f}	2.770±0.002^{ab}	0.532±0.002^{a}	44.332±0.113^{cde}
ML-8	0.269±0.002^{d}	0.460±0.020^{a}	1.096±0.024^{e}	7.826±0.054^{g}	0.861±0.002^{c}	48.180±0.032^{fg}
ML-9	0.299±0. 002^{gh}	3.500±0.030^{ef}	0.870±0.110^{d}	3.522±0.002^{c}	0.953±0.025^{d}	48.633±0.998^{fg}
ML-10	0.303±0.001^{h}	1.480±0.020^{b}	0.500±0.030^{bc}	4.552±0.020^{d}	0.949±0.017^{d}	50.236±0.135^{g}
ML-11	0.170±0.001^{a}	4.620±0.100^{gh}	0.420±0.030^{ab}	12.014±0.089^{h}	1.228±0.003^{g}	24.137±4.011^{a}
Check-3	0.294±0.0005^{fg}	1.995±0.025^{bc}	0.925±0.095^{de}	7.258±0.003^{f}	1.529±0.002^{i}	48.898±0.009^{fg}

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P < 0.05$

4.1.10 Fatty acid profiling of the gamma irradiated M_4 lines of sesame oils

4.1.10.1 Free fatty acid (%)

The least free fatty acid percentage was observed in ML-8 (1.13 ± 0.03 %) (Table 4.10). Highest free fatty acid was observed in ML-5 (3.08 ± 0.02 %). All mutants derived from Check-1 and Check-3 varieties showed a significant increase in free fatty acid except ML-8 (1.13 ± 0.03 %) (Table 4.10).

4.1.10.2 Acid value

Mutant line five (ML-5) was observed to have the highest acid value (6.16 ± 0.45 mg KOH/g) (Table 4.10). The least acid value was observed in ML-8 (2.24 ± 0.04 Mg HOH/g). Significant increment was observed in acid values of ML-1 (4.68 ± 0.14 Mg KOH/g), ML-2 (5.38 ± 0.10 Mg KOH/g), ML-3 (5.72 ± 0.20 Mg KOH/g), ML-5 (6.16 ± 0.45 Mg KOH/g), ML-10 (5.12 ± 0.05 Mg KOH/g) and ML-11 (5.50 ± 0.68 Mg KOH/g) (Table 4.10).

4.1.10.3 Iodine value

No significant difference ($p > 0.05$) was observed in the iodine value of the mutant lines and control groups (Table 4.10).

4.1.10.4 Peroxide value

The highest peroxide value was observed in ML-1 (10 ± 0.65 $MeqO_2/g$) and was significantly the same ($p > 0.05$) with ML-4, ML-7, ML-10 and Check-2. Check-1 had the least peroxide value (7.00 ± 0.22 $MeqO_2/g$) and was significantly different ($p < 0.05$) from other mutant lines. Mutants derived from Check-1 varieties showed significant increase in peroxide values (Table 4.10).

4.1.10.5 Saponification value

Mutant line ML-11 had the significantly highest saponification value (195.42 ± 0.95 Mg KOH/g). The least saponification value was observed in ML-11 (183.54 ± 1.37 Mg KOH/g) and was significantly the same ($p > 0.05$) with ML-6 (183.76 ± 1.89 Mg KOH/g) but significantly different ($p < 0.05$) from that of other mutant lines. Significant decreases were observed in saponification values of ML-6 (183.76 ± 1.89 Mg KOH/g), ML-7 (184.66 ± 0.68 Mg KOH/g), ML-8 (185.90 ± 1.88 Mg KOH/g), ML-9 (184.22 ± 0.16 Mg KOH/g) and ML-11 (183.54 ± 1.37 Mg KOH/g).

4.1.10.6 Refractive index and pH value

No significant difference ($p > 0.05$) was observed in the refractive index of the mutant lines and control groups (Table 4.10). Mutant line ML-3 had the highest pH value (6.38 ± 0.23) while ML-1 (5.60 ± 0.02) had the significant lowest pH value. Mutant lines exhibiting significant decrease in pH comprise of ML-1 (5.60 ± 0.02), ML-4 (5.80 ± 0.01), ML-6 (5.90 ± 0.03), ML-8 (6.40 ± 0.04), ML-9 (6.20 ± 0.12) and ML-10 (6.50 ± 0.04) (Table 4.10).

Table 4.10: Physical Properties of M_4 Mutants of Gamma-Irradiated Sesame Seed Oil

Mutant Lines	Free fatty Acid (%)	Acid Value (Mg KOH/g)	Iodine value (g of I_2 100/g)	Peroxide value (Meq O_2/g)	Saponification value (Mg KOH/g)	Refractive index	pH
ML-1	2.32±0.01^{f}	4.68±0.14^{def}	119.18±3.93^{a}	10.00±0.65^{c}	187.40±1.88^{abcd}	1.469±0.04^{a}	5.60±0.02^{a}
ML-2	2.69±0.01^{i}	5.38±0.10^{efg}	118.20±2.19^{a}	8.00±0.58^{ab}	188.02±1.82^{bcd}	1.472±0.13^{a}	6.00±0.02^{abcd}
ML-3	2.86±0.00^{j}	5.72±0.20^{fg}	118.15±3.29^{a}	9.00±0.46^{bc}	184.98±0.84^{ab}	1.474±0.02^{a}	6.83±0.23^{g}
Check-1	2.06±0.01^{d}	4.14±0.13^{cd}	118.41±4.41^{a}	7.00±0.22^{a}	185.08±4.76^{ab}	1.472±0.01^{a}	5.90±0.04^{abc}
ML-4	2.24±0.03^{e}	4.48±0.17^{de}	119.40±2.71^{a}	10.33±0.76^{c}	186.21±0.58^{abcd}	1.472±0.02^{a}	5.80±0.01^{ab}
ML-5	3.08±0.02^{k}	6.16±0.45^{g}	108.12±1.15^{a}	8.00±0.44^{ab}	189.95±0.62^{d}	1.471±0.00^{a}	6.30±0.05^{cdef}
ML-6	1.68±0.02^{c}	3.36±0.24^{bc}	104.09±3.18^{a}	8.00±0.21^{ab}	183.76±1.89^{a}	1.472±0.03^{a}	5.90±0.03^{abc}
ML-7	2.49±0.03^{g}	4.98±0.18^{def}	119.16±1.88^{a}	10.00±0.21^{c}	184.66±0.68^{ab}	1.474±0.02^{a}	6.07±0.08^{bcd}
Check-2	2.46±0.00^{g}	4.92±0.26^{def}	119.32±1.58^{a}	10.00±00^{c}	189.11±00^{cd}	1.472±0.05^{a}	6.00±0.26^{abcd}
ML-8	1.13±0.03^{a}	2.24±0.04^{a}	119.78±3.55^{a}	9.00±0.47^{bc}	185.90±1.88^{abc}	1.471±0.00^{a}	6.40±0.04^{def}
ML-9	1.62±0.03^{c}	3.24±0.06^{abc}	118.16±1.15^{a}	8.00±0.39^{ab}	184.22±0.16^{ab}	1.472±0.02^{a}	6.20±0.12^{bcde}
ML-10	2.56±0.01^{h}	5.12±0.05^{defg}	119.23±1.03^{a}	10.00±0.36^{c}	195.42±0.95^{e}	1.471±0.00^{a}	6.50±0.04^{efg}
ML-11	2.75±0.04^{i}	5.50±0.68^{efg}	120.09±6.27^{a}	8.00±0.51^{ab}	183.54±1.37^{a}	1.471±0.02^{a}	6.63±0.20^{fg}
Check-3	1.28±0.03^{b}	2.61±0.78^{ab}	120.00±2.12^{a}	8.00±0.20^{ab}	187.23±1.16^{abcd}	1.471±0.00^{a}	6.63±0.19^{fg}
CODEX	3.00	≤6.00	104.00 – 120.00	≤10.00	186.00 -195.00	1.469 - 1.479	-

Values are mean ± standard error of mean. Values followed by different superscript along the same column are significantly different at $P<0.05$

4.2 Discussion

4.2.1 Vegetative parameters of M_4 gamma irradiated sesame lines

4.2.1.1 Plant height

Morphological characters varied among the M_4 lines. The variation could be an indication that gamma irradiation can cause both positive and negative genetic variability in morphological parameters of M_4 gamma irradiated sesame. This can be corroborated by the work of Abdul *et al.* (2018) who reported that gamma irradiation can either result in gene reshuffling corresponding to healthy results or genomic damages leading to growth abnormalities in crop plants.

The significant decrease observed in seedling height and juvenile height in some of the mutant lines could be attributed to physical injuries caused by gamma irradiation. Similar reductions have been reported in shoot lengths of young seedlings of gamma irradiated sesame (LTK-4 local variety) using six doses of gamma irradiation viz; 150 Gy, 300 Gy, 450 Gy, 600 Gy, 750 Gy and 900 Gy by Kumari *et al.* (2016) and were attributed to the progressive injury caused by ionizing radiation on the seedlings. However, significant increase in plant height of some mutant lines (ML-2, ML-3, ML-6, ML-8, ML-9, ML-10 and ML-11) over the checks at maturity could be due to the improved cell division rate and stimulation of growth hormones as a result of gamma irradiation. This result is in conformity with the report of Dhakshanamoorthy *et al.* (2011) who has reported that gamma irradiation could result in possible acceleration of cell division in meristematic tissues which could contribute to plant growth.

Mustafa *et al.* (2015) opined that previous researches on sesame breeding have demonstrated that plant height has a significant and positive correlation with yields per

plant at both genotypic and phenotypic levels and as such, is a good criterion for selection for future breeding programmes.

4.2.1.2 Branches per plant

The significant increase observed in number of branches per plant in most of the mutants over their checks may be due to the stimulatory effects of gamma irradiation. Similar findings have been reported by Aristya *et al.* (2018) in gamma-irradiated M_4 generation of white seeded sesame irradiated with eight doses (100 - 800 Gy) of Co-60 and in gamma-irradiated pepper by Abu *et al.* (2020). The number of branches per plant observed in the M_4 lines (1.86 ± 0.24 to 3.27 ± 0.37) is quite lower from that previously reported by Mohammed (2019) in M_3 lines (2.40 - 5.80) of these sesame varieties. This variation could be attributed to the DNA aberrations caused by ionizing radiation. This can be corroborated by the reports of Micco *et al.* (2011) who have reported genetic aberrations leading to different phenotypic development as a result of gamma irradiation. In sesame improvement programmes, the number of branches per plant is one of the most important selection criteria because a higher number of branches will enable more capsule bearing ability thereby increasing yield (Baydar, 2005). The significant increase in number of branches in some lines could increase the seed yield of the crop.

4.2.1.3 Height of first capsule on main stem

Significant increase and decrease observed in the height of first capsule on main stem has been previously reported by Aristya *et al.* (2018) in M_4 gamma-irradiated sesame. According to Disowja *et al.* (2020), correlation studies on sesame has revealed that yield per plant expressed positive and significant correlation with height to formation of first

capsule, Hence, the selection of genotypes based on height to formation of first capsule will be quite effective in aiding yield improvement in further generations.

4.2.1.4 Leaf morphology

The fluctuations observed in morphological characters of the M_4 lines especially in leaf morphology might be influenced by DNA repair mechanism after damage over successive generations. Similar claims have been made by Aliyu *et al.* (2017).

4.2.2 Yield parameters of M_4 gamma irradiated sesame lines

4.2.2.1 Days to first flowering

The results on yield attributes indicate highly significant differences among the eleven mutant lines and control groups. Significant decrease observed in number of days to appearance of first flower in some mutant lines may be attributed to the effect of gamma irradiation on biochemical pathway which assists in synthesis of flower inducing substances, hence conferring earliness to flowering. Similar result of reduction in number of days to flowering has been reported in rice by Okasa *et al.* (2020).

4.2.2.2 Number of flowers and flower morphology

The significant variations observed in number of flowers per plant and flower morphology in the M_4 lines could be due to the ionizing effect of gamma irradiation causing disturbances at the genetic level. Similar findings of variations in number of flowers have been reported in M_3 generation of gamma irradiated sesame lines by Mohammed (2019).

4.2.2.3 Days to 50 % flowering

No delay was observed in days to 50 % flowering. This result is in conformity with reports of Pradhan and Paul (2019). However, gamma ray has been reported to delay the days to 50 % flowering in black gram irrespective of treatment level by Yasmin and Arulbalachandran (2016).

4.2.2.4 Days to physiological maturity

Significant decrease observed in number of days to physiological maturity is in line with reports of Saha and Paul (2017a) on M_1 generation of sesame. Reduction in the number of days to physiological maturity is a good trait for sesame that could be grown in areas with reduced duration for precipitation.

4.2.2.5 Capsule per plant

Notable increase observed in number of capsules per plant in some mutant lines could be attributed to the stimulatory effect of gamma irradiation on capsule formation. Similar findings have been reported by Ravichandran and Jayakumar (2015) in M_2 and M_3 generations of sesame. The production of high number of capsules is one of the most important traits in defining the ideal type of sesame plant (Masoudi, 2019) as this will consequently increase yield. The significant decrease observed in some lines however, maybe due to formation of sterile capsule manifestation previously reported by Ravichandran and Jayakumar (2014) and Mohammed (2019) in M_1 and M_3 lines of sesame respectively.

4.2.2.6 Capsule length and width

Significant increase observed in capsule length and width of some mutant lines could be due to increased physiological activities at the genetic level. Similar trend was observed by Mohammed (2019) in capsule length of M_3 generation of these lines. Contrary to the capsule length range of 2.85 - 3.63 cm previously reported in M_3 generation of these sesame lines, lower capsule length was observed ranging from 2.40 ± 0.10 to 3.07 ± 0.09 cm. These variations may be as a result of the cumulative effect of ionizing radiation.

4.2.2.7 Fresh and dry capsule weight

The significant increase observed in fresh and dry capsule weight in some mutants may be attributed to the beneficial effect of gamma irradiation. This result is in conformity with reports of Mensah *et al.* (2007) who reported a significant increase in capsule weight with increase in concentration of sodium azide and colchicine in sesame. Similarly, Mohammed (2019) has reported a significant increase in capsule weight of some M_3 lines.

4.2.2.8 Seeds per capsule

Significant increase in number of seeds per capsule could be attributed to the antioxidant defense system of sesame plant which enables the toxic free radicals to perform useful biological functions without too much damage (Karuppanapandian, 2011; Aliyu *et al*., 2017). These results were similar to the report of Aliyu *et al.* (2018) and Mohammed (2019). Contrarily, Ravichandran and Jayakumar (2018) have reported a decrease in number of seeds per capsule of irradiated sesame. This might be due to the differences in the doses of irradiation used and the mutant generation being examined.

4.2.2.9 Hundred seed weight

The significant increase observed in seed weight of some mutant lines over the control groups is in conformity with reports of Singh *et al.* (2020) who reported increase seed weight with increasing dose of gamma irradiation. Contrary to the hundred seed weight previously reported by Mohammed (2019) in M_3 generation (0.38 - 0.53 g), hundred seed weight ranging from 0.18 ± 0.01 to 0.54 ± 0.05 g was observed in the M_4 lines. The values observed were however higher than (0.25 - 0.42 g) previously reported by Pham *et al.* (2010). The significant differences observed in M_4 lines indicate the existence of high genetic variability among the mutant lines for yield and yield components at M_4 generation. Similar claims have been made by Begum and Dasgupta (2010).

4.2.3 Capsule characteristics

Notable variations observed in capsule characteristics especially in number of capsules per leaf axil and capsule angle on main stem could be due to the ionizing effect of gamma rays and suggests that these mutants are still segregating. Similar findings have been reported by Muhammad (2018) in M_2 generation of gamma irradiated sesame lines and were attributed to the positive effects of gamma irradiation.

4.2.4 Seed characteristics

The result on seed characteristics showed notable disparities in seed colour ranging from brick red to dark brown, medium brown, light brown to cream to

white. These variations could be due to the accumulated effects of gammar rays on seed colour. This result is in conformity with reports of Mohammed (2019) on M_3 generation of gamma irradiated Nigerian sesame.

4.2.5 Pollen parameters of M_4 gamma irradiated sesame lines

4.2.5.1 Pollen viability

The results on pollen viability depicts that the pollens of irradiated M_4 lines of sesame have a high level of pollen viability varying from 86.70 % to 97.56 %. This might be due to the stimulatory effect of gamma irradiation on the irradiated seeds. Similar findings have been previously reported by Falusi *et al.* (2001) in *Sesamum radiatum* and *Sesamum indicum*. Also, Abejide *et al.* (2013) who studied three sesame cultivars observed high pollen viability in irradiated sesame cultivars. On a contrary, Singh *et al.* (2018) has reported up to 50 % reduction in pollen viability of M_1 irradiated sesame. The differences in pollen viability percentages could be attributed to genotypic differences brought about by the gamma irradiation. The fertility of male plants is dependent on its pollen viability and germinability (Tunistra and Wedel, 2000) and a monoecious plant with high pollen viability have greater tendencies of producing high seed set percentage. This finding also further explain that sesame mutant seeds still maintain their pollen viability and that certain doses of gamma-irradiation viz (350 Gy, 450 Gy and 550 Gy) did not hinder pollen viability.

4.2.5.2 Pollen shape and size

Notable disparities were however observed in range of pollen grain size. The pollen grains were large and had different units ranging from 106.52 μm- 169.52 μm with number of colpi varying from 10 - 13 and were either circular or elliptic in polar view. This is in line with

findings of Akhila and Beevy (2015) who reported colpi range of 11 - 13 and circular and elliptic pollens as characteristics of *Sesamum indicum*. No variations were observed in pollen shape of the mutant lines and control groups. This finding is in line with the work of Damaiyani *et al.* (2020) who reported gamma irradiation as high as 600 Gy does not affect the pollen morphology of sesame plants.

4.2.5.3 Pollen germinability

Significant differences were observed in pollen germinability of the M_4 lines. The high germinability percentages observed in all the M_4 mutant lines at 20 % concentration relative to the check groups could be a result of the stimulatory effect of gamma irradiation on sesame pollen germination. This finding is in line with the reports of Falusi *et al*. (2013) who reported an increase in pollen germinability percentages following Fast Neutron Irradiation (FNI) in three Nigerian sesame cultivars. On the contrary, Bashir *et al.* (2013), Kumari *et al.* (2016) and Ariharasutharsan *et al.* (2019) reported a reduction in pollen fertility of irradiated sesame, similar to the works of Ariraman *et al.* (2018) on *Cajanus cajan* and Khah and Verma (2020) on Barley. Although results on pollen germinability were lower when compared to pollen viability, 20 % sucrose concentration proved to be the optimum concentration for pollen germinability test in this study. This is contrary to the work of Liqin *et al.* (2007) who reported high germinability of up to 58 % at 35 % sucrose concentration but in line with reports of Abejide *et al.* (2013). This could be due to the close similarities in the genotypes studied.

The generally low pollen germinability observed in all the mutant lines and check groups in all sucrose concentrations in this study could be attributed to pollen tube rupture, environmental factors and differences in the composition of the germination medium as

against the *in vivo* environment. Similar reports have been made by Gilissen (1978) who reported low pollen germination in vitro due to possible differences in germination medium and stigmatic exudates between the *in vivo* and *in vitro* environment.

Based on the observations from this study, it was noted that pollen germinability and pollen tube growth rate were not synchronous. Similar findings have been reported by Gaaliche *et al.* (2013) who observed heterogeneous pollen germination rate in *Ficus carica* L. cultivars cultured in the same media. Varying germination rates observed indicates that germinability is influenced by various conditions such as nutrition conditions of the plants, suitable in vitro conditions and other environmental factors such as temperature and humidity. Similar claims have been made by Abejide *et al.* (2013). This implies that gamma irradiation tends to increase the germinability of the pollens and could lead to a better pollinizer of the genotype over their non-irradiated counterparts.

4.2.6 Correlation among yield parameters

The phenotypic correlation coefficient estimated to determine the strength and direction of the relationship between yield and yield contributing traits showed significant negative and positive correlations. The significant positive correlations observed implies that an increase in one variable will consequently result in an increase in the other while the significant negative correlations implies that an increase in one will consequently result in a decrease in the other. This can be used to identify the principal yield components, and the selection

for these traits in a positive manner in the mutant lines may be useful in improving seed yield in sesame (Kumar *et al.*, 2019).

The significant positive correlations observed between branches per plant and capsules per plant in ML-3 and between capsules per plant and capsule weight are in line with reports of Kumar *et al.* (2019) and Disowja *et al.* (2020).

4.2.7 Correlation among morphology and yield parameters

Negative correlations observed between capsules per plant and seeds per capsule are in conformity with reports of Saravanan *et al.* (2020). Negative correlations also observed between plant height and days to first flowering, capsules per plant and days to physiological maturity, branches per plant and days to physiological maturity are in line with findings of Singh *et al.* (2020).

4.2.8 Proximate composition of M_4 gamma irradiated sesame lines

The proximate composition analysis of M_4 sesame seeds (*Sesamum indicum*) revealed the presence of significant quantities of oil, carbohydrate, crude protein, crude fibre, ash and moisture. The presence of these constituents gives an indication of the nutritional qualities obtainable from sesame seeds.

4.2.8.1 Moisture content

Significant increase observed in the moisture content of the M_4 lines over the control groups could be due to the negative effect of the irradiation. Similar findings have been reported by Maraei and Hammoud (2019) in date seeds. Mutant lines with lower moisture content would be suitable for conventional threshing (Nobre *et al.,* 2019) while those with higher moisture content can be used to obtain higher cake recovery of up to 74 % (Ishola *et al*., 2020). High moisture content encourages the growth of microbes (Afolabi, 2008). This implies that mutant lines with lower moisture content will be better stored and have a longer shelf life.

4.2.8.2 Ash content

Significant decrease observed in percentage ash of the irradiated M_4 lines over the control could be attributed to the negative effect of ionizing irradiation. This result is in conformity with the reports of Abu *et al.* (2020) in two Nigerian peppers. The ash content observed in this study (3.34 ± 0.36 to 5.97 ± 0.12 %) was higher than that reported by Ogbonna and Ukaan (2013) in Sesame ranging from (1.2 - 2.8 %) but was similar to reports of Ahmed *et al.* (2020) who reported a range of (4.41 - 5.42 %) in sesame cultivars. This variation might be attributed to varietal differences. Ash content is the inorganic residue remaining after the organic matter and water are removed by heating and is important in determining the total mineral composition in foods (Sanni *et al*., 2008; Zubair *et al.*, 2020)

4.2.8.3 Crude fibre

The crude fibre content observed in this study (3.23 ± 0.13 to 4.08 ± 0.17%) was in conformity with reports of Gadade *et al.* (2017) but was however lower than reports of Aliyu *et al.* (2018) and higher than reports of Ogbonna and Ukaan (2013). Bello *et al.* (2013) opined that fibre in diet helps to maintain human health by lowering cholesterol level in the body. It has also been proven to help prevent chronic diseases such as cancer, diabetes and cardiovascular diseases but lower or higher intake more result in bowel irritation and possibly, colon cancer (Christian *et al*., 2019).

4.2.8.4 Crude protein

Significant increase observed in crude protein of irradiated lines is in line with findings of Rizki *et al.* (2015) but contrary to reports of Abu *et al.* (2020). This non uniformity might be attributed to the differences in the doses of irradiation. The crude protein observed in this study (19.93 ± 0.03 to 32.83 ± 1.50 %) is in close range with the reports of Kaur (2018) who reported a crude protein range of (20 - 30 %) in sesame seeds. Ogbonna and Ukaan (2013) opined that protein is a vital part of human nutrition and is one of the nutrients that are mostly low in plant products.

4.2.8.5 NFE/CHO content

Both significant increase and decrease were observed in the NFE/CHO %. The NFE/CHO values observed (15.98 ± 1.17 - 38.44 ± 0.58 %) was much higher than that reported by Prasad *et al.* (2012) in sesame cultivars ranging from (13 -14 %). This variation might also be due to ionizing effect of the irradiation or varietal differences.

4.2.8.5Oil content

Notable differences were observed in oil quantity and properties of gamma-irradiated M_4 lines. Significant increase observed in oil percentage of some of the mutant lines over the control groups could be due to the positive effect of the irradiation. Similar reports have been made in some M_4 mutants of gamma-irradiated by Begum and Dasgupta (2015). Contrary to the reports of Mohammed (2019) who reported higher oil percentages and an insignificant difference in oil percentage of M_3 lines of these mutants, the oil percentages observed in the M_4 lines were quite lower ranging from (23.41 ± 1.15 to 48.28 ± 0.58 %). This could be due to the accumulative effect of the irradiation or differences in environmental conditions such as climate during oil accumulation.

4.2.9 Anti-nutritional factors of M_4 gamma irradiated sesame lines

4.2.9.1 Tannin content

The result obtained revealed significant differences in anti-nutritional factors of M_4 gamma irradiated sesame seed. Significant increase observed in tannin content of some mutants could be caused by elevated rates of reactions due to gamma irradiation. Tannin in sesame accounts for its anti-bacterial and anti-fungal properties (Shittu *et al*., 2007) and higher tannin contents observed in some lines could increase its anti-bacterial and anti-fungal activities.

4.2.9.2 Flavonoid and alkaloid content

Notable enhancements observed in flavonoid and alkaloid contents are in conformity with reports of Rizki *et al.* (2015). The presence of alkaloids and flavonoids in sesame accounts for its antioxidant property (Ramesh *et al*., 2005) and indicates that its consumption is good for the management of cardiovascular diseases and oxidative stress. The presence of these natural antioxidants increases stability to sesame oil.

4.2.9.3 Oxalate content

Similar significant reductions were observed in oxalate contents of M_4 lines. Oxalate contents were also lower than that previously reported in the M_3 lines showing that the mutant lines are not yet stable. Low/zero oxalate and phytate contents are considered as part of major sesame breeding objectives for value addition as high contents of oxalate and phytate result in kidney stones, lowers mineral absorption and increased risk of zinc and iron deficiency in humans.

4.2.9.4 Phytic acid content

Significant reduction observed in phytic acid content of some mutants is in line with reports of Rahman and Das (2001) and (El-adawy *et al*., 2005) on gamma irradiated sesame but contrary to reports of Mohammed (2019) on M_3 generation of these lines. The phytic acid content observed in M_4 generation (0.532 ± 0.002 to 1.529 ± 0.002 %) was much lower than what was observed in M_3 generation (2.009 - 7.582 %).

4.2.10 Fatty acid profile of M_4 gamma irradiated sesame seed oil

4.2.10.1 Free fatty acid

Significant increases were also observed in free fatty acid and acid value of some mutant lines over their control groups. This finding is in conformity with reports of Bhatti *et al.* (2010) in gamma irradiated pea nut. Free fatty acid is a vital parameter that confirms the stability of oil. High level of free fatty acid indicates poor quality of oil as it gives a bad taste (Dayrit *et al.*, 2007). The free fatty acid observed in this study (1.13 ± 0.03 to 3.08 ± 0.02 %) is higher than that previously reported in M_3 lines of these mutants but falls within the acceptable limits (Codex, 2001).

4.2.10.2 Acid value

The acid value is mostly used as a general indication of the condition and edibility of the oil. According to FAO recommendations, the permissible level of acid for all edible oil should be below 6 MgKOH/g. Thus, the acid values obtained in these mutant lines signify good oil quality and as such, fit for human consumption.

4.2.10.3 Iodine value

No significant difference was observed in iodine values and refractive index of gamma irradiated M_4 lines. This result is in conformity with reports of Al-Bachir (2015) and Mohammed (2019). High iodine values are mostly desired by oil processors and it indicates the presence of unsaturated fatty acid and can also be used to quantify the amounts of double bonds present in the oil which signifies the susceptibility of the oil to oxidation (Paul, 2013) while lower iodine values is an indication of lower oil quality (Tesfaye and

Abebaw, 2016). The iodine values observed in this study falls within the acceptable range for sesame oil (Codex, 2001).

4.2.10.4 Peroxide value

Peroxide value determination is often used as a measure for taste and smell. Vegetable oils with elevated peroxide values show bad oil quality (Chabiri *et al*., 2009; Mohammed, 2019). It is also used as an indication for the ability of the oil to get rancid due to hydrogen absorption during storage or processing and it increases with storage time (Paul, 2013). The peroxide values obtained in this study (7.00 ± 0.22 to 10.00 ± 0.36 meq O_2/kg) were higher than that previously reported in M_3 lines of these mutants but were however, similar to the reports of El-kheir (2008) and Sa'ed and Shola (2015) but fall within Codex acceptable range for sesame oil (≤ 10.00 meq O_2/kg). The increase in peroxide values might be due to oxidation and preferential cleavage of bonds in the oil and could also be attributed to interaction of gamma irradiation with fat molecules which triggered oxidation, dehydration and polymerization reactions (Evren and Gulden, 2008; Bhatti *et al*., 2010).

4.2.10.5 Saponification value

Saponins in high concentration impart a bitter taste but reduce the risk of heart diseases in human. They control plasma cholesterol, preventing peptic ulcer, osteoporosis (Gemede and Ratta, 2014). High saponification value indicates high level of triacylglycerol and ester values showing the oil's potential to be used in cosmetic industry. Significant increase observed in saponification values of some of the mutant lines is in line with reports of Bhatti *et al.* (2010) who reported a significant increase in saponification values of gamma

irradiated pea nut. The saponification values obtained in this study falls within the acceptable range for sesame oil (186 -195 Mg KOH/g) recommended by Codex (2001) and as such fit for consumption.

5.0 CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The variations observed in vegetative and yield attributes especially in capsule characteristics of these mutants suggests that these mutants are not yet stable and are still segregating.

This study has revealed that all the M_4 mutant lines had high percentage pollen viabilities which could be beneficial in the crop's improvement and that 20 % sucrose concentration is the most suitable concentration for pollen germinability in sesame.

Variability in seed nutritional composition, oil quantity and Anti nutritional factors reflects the existence of genetic diversity and suggests potential genetic improvements.

Result on oil composition revealed that the physical properties of oil obtained from the mutant lines are within the acceptable limits by Codex and as such, fit for human consumption.

5.2 Recommendations

The following recommendations are made based on the findings:

i. On-site evaluation and multi-location trials of the mutant lines.

ii. The positive changes in capsule characteristics of ML-2, ML-6, ML-9, ML-10 and ML-11 could be exploited for increasing the productivity of sesame and for further improvement of the crop.

iii. Investigations should be carried out on any changes in the mutant's chromosome or allele frequency.

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