QUANTITATIVE TRAIT VARIATIONS THROUGH CHEMICAL MUTAGENESIS IN SESAME (*Sesamum indicum. L*).

M.Sc. Thesis

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QUANTITATIVE TRAIT VARIATIONS THROUGH CHEMICAL MUTAGENESIS IN SESAME (*Sesamum indicum. L)*.

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As Thesis Research advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by Abraham Birara, entitled **Quantitataive trait variations through chemical mutagenesis in Sesame (*Sesamum indicum L)*.**. I recommend that it be submitted as fulfilling the *Thesis requirement*.

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DEDICATION

I dedicate this thesis manuscript to all of my beloved families, my wife Netsanet Ayanew, and my daughter Eden Abraham.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is the result of my own work and that all sources or materials used for this thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for an M.Sc. degree in Genetics at the Haramaya University and to be made available at the University's Library under the rules and regulations of the Library. I confidently declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
AT	Adenine- Thymine
GC	Guanine - cytocine
BMS	Butyl Methane Sulphonate
CSA	Central Statistical Authority
EMS	Ethyl Methane Sulfonate
EST	Expressed sequence tags
FAO	Food and Agricultural Organization
GCV	Genotypic Coefficient of Variation
GMO	Genetically Modified Organism.
HU	Haramaya University
IAEA	International Atomic Energy Agency
IPGRI	International Plant Genetic Resource Institute.
LAI	Leaf Area Index
LSD	Least Significant Difference
M1	First Generation
M3	Third Generation
MH	Maleic Hydrazide
Masl	Meters above sea level
PS	Presoaking
PIMM	Preferential Induction of Micro Mutation
TILLING	Targeted Induced local lesions in Genomes
RNAi	Ribonucleic acid interference
SESACO	Sesame Coordinators
T-DNA	Transfer DNA

BIOGRAPHICAL SKETCH

The author was born and raised in the Metropolitan Addis Ababa on April 5, 1985. He attended his elementary and secondary school education at Keraniyo Medihanialem Elementary and Junior Secondary School and Ayer Tena senior Secondary School in Addis Ababa, respectively.

In 2001, he joined the then Gondar College of Medical Sciences and Now University of Gondar and graduated with B. Sc. degree in Applied Biology in 2004. In 2005 , he was employed by the Amhara National regional state education bureau and served as a biology teacher for two consecutive years in Gojam ber preparatory and secondary school in eastern Gojam, Dejen woreda and Yifag secondary school in southern gondar zone, Libokemkem woreda respectively. Later on in 2007, he was recruited by the Mekelle University as a graduate assistant, after serving a single year, he joined the School of Graduate Studies, Haramaya University in October 2009

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QUANTITATIVE TRAIT VARIATIONS THROUGH CHEMICAL MUTAGENESIS IN SESAME (*SESAMUM INDICUM. L).*

ABSTRACT

*Sesame (*Sesamum indicum *L.) is considered to be an ancient oil seed crop to generate high quality edible oil having nutritional and health related value but still at an early stage in breeding. Its chromosome number is 2n=26 and it belongs to the Pedaliaceae family and having significant economic value globally as well as in Ethiopia.With the objective of broadening the gene pool of sesame an experiment was conducted in the genetics and molecular biology laboratory as well as agricultural research field of Haramaya University employing factorial CRD experimental setup. In this experiment Healthy and Dry seeds of sesame (*Sesamum indicum L.*), varieties, Abasena and Kelafo74, obtained from werer agricultural research institute were treated with sodium azide and hydroxyl amine hydrochloride at ascending mutagen concentrations of 0.01, 0.02,0.03,0.04 and 0.05 % targeted at determining the effects of the chemical mutagens to promote genetic variability in terms of the agro morphological parameters of sesame. Highly significant differences (P<0.01) were noticed in the varieties and treatments with respect to the traits under consideration (Germination percentage, root length ,shoot length, plant height, days to flowering ,days to maturity, internodes length, capsule length, number of capsules per plant, number of seeds per pod, hundred seed weight). Similarly Treatment and variety interactions were also highly significant (P<0.01) with respect to all traits. The variety Abasena exhibited better performance in comparison to Kelafo74. Upon the results obtained from computing the mutagenic efficiency of the chemicals, we can draw statistical inference that sodium azide could be utilized to induce genetic variability for the improvement of Sesame and it is more effective than hydroxyl amine hydrochloride. Since the produced mutants from first generation are not adequate for studying the genetic stability these traits should be investigated for the desired traits in subsequent generations and in the field conditions,developing sesame varieties resistant for different biotic and abiotic stresses and assisting the present work with the recent biomolecular techniquesshould be future prospects.*

Keywords: chemical mutagen, hydroxylamine hydrochloride, sesame. Sodium azide

1. INTRODUCTION

Sesame (*Sesamum indicum* L.) is considered to be an ancient oil seed crop (Brar and Ahuja, 1979). According to Oplinger *et.al*. (1990), it is an important oilseed to generate high quality edible oil and protein for low income peasants of major sesame growing countries including Sudan, Ethiopia, Uganda, Nigeria, Mexico, Venezuela, India, China, Pakistan, Turkey and Myanmar. India, with 2.5 million hectares under cultivation of this crop, is a leading sesame producer, accounting for 40% of the world's sesame area and 27% of world production .China is another major sesame growing country with about one million hectares under cultivation and 0.4 million tones of seed production per year (Zhang *et al.*, 1998).

The diploid chromosome number of sesame is 2n=26 and it belongs to the Pedaliaceae family. This family harbors 16 genera and 60 species and is a small family. The most important genus of this family is *Sesamum*. Sesame is typically an annual species. There are lots of varieties of *Sesamum indicum* L. according to the size, form and color of flowers, seed size, color and composition. Variation can also be manifested in such a way that some varieties are highly branched whereas others are unbranched (Peter, 2004).

The African continent is naturally gifted with suitable weather conditions that can enhance sesame production. The crop requires only 500-650 mm of rainfall per annum. Unfortunately, average world yield of sesame is still low at 0.46 ton ha^{-1} (FAO, 2004). Low yield had been attributed to cultivation of low yielding dehiscent varieties with low harvest index values, significant yield loss during threshing and shortage of agricultural inputs such as improved varieties, fertilizers and other agro-chemicals (Ashri, 1994, 1998; Weiss, 2000; Uzun and Cagirgam, 2006). However, non- dehiscent sesame varieties with yield potential of over 1 ton ha-1 and appropriate for mechanical combine harvest have been developed by Sesame Coordinators (SESACO) in USA (SESACO, 2007).

Ethiopia is known to be the origin of diversity for cultivated sesame (Frenge, 1983). Its seed harbors 50-60% oil and 25% protein with antioxidants lignans such as sesamolin, sesamin and has been used as active ingredients in antiseptics, bactericides, vermicides, disinfectants, moth repellants, anti-tubercular agents and considerable source of calcium, tryptophan, methionine and many minerals (Bidigian *et al*., 1985).

The work of Kindie (2007) implies that in 1997 the total area covered in ethiopia by sesame around metema area was about 128, 000 ha. In nearly ten years' time (up to 2007), the total area of sesame production has elevated by more than 200% to about 211,000 ha.

In general terms Sesame is unimproved and variety of collections have been generated of land races, with little or no genetic information that can lead to its application in breeding programs. A number of factors affecting sesame improvement programs have been identified. Firstly, the germplasm of sesame is not as large as in other crops (Ashri, 1982). Secondly, the genetic architecture of sesame is poorly adapted to mechanized farming system due to its indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Uzun and Cagirgan, 2006).

Mutations are the tools at hand exploited by the geneticist to study the nature and function of genes which are the basis of plant growth and development, hence producing raw materials for genetic improvement of economic crops (Adamu *et al*., 2007).

Primarily the advantage of mutational breeding is the probability of improving one or two characters without amending the rest of the genotype. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001).

Major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated take the advantage of induced mutations to improve their desired characteristics. Various mutagenic agents are employed to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens (Ahloowalia and Maluszynski, 2001).

Regarding many mutation breeding programs for seed propagated crops, the starting material for mutagenesis is usually seeds (Van Harten, 1998; Koornneef, 2002). However, other materials can also be used including whole plants, ex-plants like leaves or shoots, and gametes (pollen or egg cells) (Van Harten, 1998). Plant parts are usually treated for the case of vegetatively propagated plants (Koornneef, 2002).

A number of scholars have reported on the role of chemical mutagens in enhancing genetic variability in higher plants. The mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yields, increase stress tolerance, longer shelf life and reduced agronomic input (Ahloowalia & Maluszynski 2001).

Chemical mutagenesis is a simple approach to create mutation in plants for their improvement of potential agronomic traits. Mutation breeding methodology has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena (Van, Den-Bulk et al., 1990; Bertagne- Sagnard *et al*., 1996).

The successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in groundnut (Mensah and Obadoni, 2007), barley (Kleinhofs and Sander, 1975) and other crops (Avila and Murty, 1983; Routaray *et al.*, 1995).

Exploitation of hydroxylamine to induce mutants has been reported by a number of scientific scholars. The dynamics in the leaf number with alternating concentration of hydroxylamine (HA) over the experimental period was presented. The number of leaves increased with the dose of HA and plants treated with 0.09% produce high number of leaves.

The number of leaves was lower in the control and 0.01% treatment. The ability of chemical mutagen to increase the number of leaves has also been reported in *Trigonella foenumgraecum* using 0.25% of Ethylmethyl sulphonate treatment (Biswas and Datta, 1988).

It has been demonstrated by many workers that genetic variability for several desired characters can be enhanced successfully through mutations and its practical value in plant improvement programs has been well established. To date there is no published information about chemical induced mutation on Sesame in Ethiopia.

General objective

- To intentionally broaden the gene pool of sesame hence, leaving ample genetic resource for breeding foundation, increasing the productivity, and selection for desirable yield components.

Specific Objectives

- To identify the quantitative trait variations produced by induced chemical mutation
- To determine the effectiveness of mutagenic chemicals in sesame growth.

2. LITERATURE REVIEW

2.1 The Origin and History of Oilseed Sesame

Sesame is one of the most classical oil seed crops (Ashri., 1998). The Chinese used to burn sesame oil for light and to make soot for their ink blocks. African slaves brought sesame seeds, which they called benne seeds to America where it became a popular ingredient in Southern recipes. The English term sesame traces back to the Arabic simsim, Coptic semsem, and early Egyptian semsent. Updated botanical and archeological researches imply that domesticated sesame was derived from wild populations native to south Asia, western Indian peninsula, or the Punjab and parts of Pakistan. These wild populations have been alternatively named *Sesamum malabaricum* or *S. mulayanum*, which should be regarded as synonymous. This data also adds that cultivated sesame was established in North Western South Asia by the time of the Harappan civilization and had spread west to Mesopotamia before 2000 BC (Vates., 1940). Oplinger *et al*., (1990) have indicated it to be a highly prized oil crop of Babylon and Assyria about 4,000 years ago. However, some other studies indicate that sesame originated in sub-Saharan Africa (Ram *et al*., 1990).

A more detailed identification of the geographical regions of origin of sesame remains difficult, and requires further botanical research. One potential source of confusion is with regard to nomenclature as applied to probable wild progenitors of sesame. Bedigian *et al*., (1985) trace the origin of the crop to the domestication of *S .orientale* var. malabaricum (*S. malabaricum*). This was suggested to be a wild variety of sesame by John *et al*., (1950), although the nomenclature of these authors lacked typification and standard Latin description. Another potential difficulty to identify the origin of sesame is that this species is not fully domesticated. The capsules in sesame split as the seeds mature, leading to varying degrees of seed loss or unripe harvesting. This remains a conspicuous problem faced by sesame producers and breeders (Day, 2000).

2.2. Taxonomic and Botanical Description

Sesame belongs to the Family Pedaliaceae. Synonyms include; *Sesamum africanum*, *Sesamum brasiliense*, *Sesamum luteum* and *Sesamum malabaricum*. The flowers of sesame are typically self pollinated, although they may be cross pollinated by insects. It is widely naturalized in tropical regions around the world where it is cultivated for its edible seeds. It is an annual plant growing to a height of 50 to 100 cm, with opposite leaves that are 4 to 14 cm long with an entire margin. The leaves are broad lanceolate, 5 cm broad at the base of the plant, narrowing to just 1 cm broad on the flowering stem. The flowers are white to purple, tubular, 3 to 5 cm long. The stem is covered with fine hair. The fruit is a grooved capsule often containing more than 100 seeds. The seeds are small and flattened; they can be off-white, brown, grey or black. The growth habit of sesame is indeterminate: the plant continues to produce leaves, flowers and seed capsules through the warm summer months. The crop is grown primarily in the tropics by small land holders, of which 99.9% is produced in developing countries Sesame cultivation however stretches up to 40º N and 40º S. It is a warm weather crop and often grown under marginal or stressed conditions (Ashri, 1998).

2.3 Global Economic Importance of Sesame

According to FAO, in 2003, the reserved area for sesame planting in the world was 6.57 million ha; with a production rate of 3,096 million tons per year and an average yield of 471.2kg ha-1. Japan uses sesame seed as a health food and leads the world in sesame seed imports followed by Europe and the US. About 70% of the world's sesame seed is processed into oil and meal. Total annual consumption is about 65% for oil extraction and 35% for food. The food segment includes about 42% roasted sesame, 12% ground sesame, 36% washed sesame, and 10% roasted sesame seed with salt. People generally consume more than twice, as much white sesame as black sesame. Sesame oil is also referred to as teal oil or benne oil and is a pale yellow, oily liquid, and almost odorless with a bland taste. The oil consists of glycerides with about 43% each of oleic and linoleic acids, 9% palmitic, and 4% stearic acids. There are many foods in which sesame is an ingredient. Europeans sometimes use it as a substitute for olive oil.

Sesame oil is an excellent salad oil and is used by the Japanese for cooking fish. Sesame seeds undergo a special hulling process which produces a clear white seed. These seeds are then double washed, dried, and used on hamburger buns. (FAO, 2004).

Sesame seed has a nutty taste when the seed is roasted; Bread, breadsticks, cookies, chocolate, and ice cream are ideal products from roasted natural sesame seed. In Greece sesame seeds are used in cakes, while in Togo, the seeds are a main soup ingredient. Mechanically hulled sesame seed enriches baked goods and candies plus it is also the basis for the creamy, sweet wholesome tahini in the Middle East. Sesame seeds contain three times more calcium than a comparable measure of milk. African countries use the seeds as spice, for seed oil and frying vegetables and meat. In addition the seeds are eaten raw or fried, and used in confections such as candy and baked goods. They also use sesame to prepare perfumes. Sesame meal is an excellent feed for poultry and livestock (Oplinger *et al*., 1990).

Sesame contains several important secondary plant compounds including chlorosesamone, sesamin, sesamolin, lecithin, flavonoids and myristic acid. Myristic acid is used as an ingredient in cosmetics. It is also used as an antidiabetic, antitumor, antiulcer, cancer preventive, cardio protective, and laxative (Jellin *et al*., 2000). Sesamin has bactericide and insecticide activities plus it also acts as an antioxidant which can inhibit the absorption of cholesterol and the production of cholesterol in the liver. Chlorosesamone obtained from roots of sesame has antifungal activity (Begum *et al*., 2000). Sesamolin also has insecticidal properties (Beckstrom-Sternberg *et al*., 1994) and is used as a synergist for pyrethrum insecticides (Simon *et al*., 1984).

In the South East Asian countries including Japan, hardly is rice eaten without some sprinkles of sesame seeds. Sesame contains the lignan called sesamin which has antioxidant properties, and phytosterols, which block cholesterol production. (Watt and Breyer-Brandwijk, 1962).With the growing consciousness of healthy lifestyles, natural products with health inducing properties are becoming popular. *Moringa oleifera* for example has gained astronomical importance in Ghana within the last decade due to its phytochemical properties. (Fuglie, 1999).

2.4 Improvement of Crops through Induced Mutagenesis

Increasing crop yields to ensure food security is a major contemporary global challenge. Amongst the obstacles against this are the changing climate (increasing temperatures and more erratic rainfall) which most often compromise crop productivity (Parry *et al*., 2005) and the need to produce additional food and crops for bio-energy whilst minimizing the carbon costs of production (Powlson *et al*., 2005). There is therefore an urgent requirement for new higher yielding varieties (Parry *et al*., 2007; Reynolds *et al*., 2009) with improved nutrient (Lea and Azevedo, 2006) and water use efficiency (Richards, 2000).

Deliberately Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. Various mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens (Ahloowalia and Maluszynski, 2001).

In this century, there has been a dramatic increase in the amount of genome sequence data available for world major food crops, their pests and pathogens. Complete genome sequences have been reported for rice (Matsumoto *et al*., 2005) and sorghum (Paterson *et al*., 2009) and also for several crop pathogens (e.g. *Agrobacterium tumefaciens*, Wood *et al*., 2001; Phytoplasma, Oshima *et al*., 2004*; Fusarium graminearum*, Cuomo *et al*., 2007; *Magnaporthe grisea*, Dean *et al*., 2005). For the other major global crop, wheat, and other crop pests and pathogens the sequences of expressed sequence tags have become available.

The exploitation of these sequence data for crop improvement is limited by the complexity of many of the traits that determine agronomic performance (Parry *et al*., 2005; Parry and Reynolds, 2007). However, reverse genetics approaches allow progress to be made on the major challenge of linking sequence information to the biological function of genes and on determining their contribution to important characters and traits. Typically, these approaches rely on the disruption of candidate genes by mutagenesis, transposons, and T-DNA tagging or RNA interference (RNAi).

Induction of variability by mutagenic agents is of paramount importance in sunflower crop improvement when seed unfilling is a major problem. A crop plant can be improved in productivity; resistance to biotic and abiotic stress etc. when the genetic variability for the specific trait is available to the respective population or species. The induced variation and quantitative characters in the application of mutagenesis in plant breeding has been carefully investigated by several authors. Mutation breeding is the most useful and vital technology of sunflower (Jaya and Selva, 2003).

Conventional mutation techniques have often been used in order to improve yield, oil quality, disease, salt and pest resistance in crops, or to increase the attractiveness of flowers and ornamental plants. In some economically important crops (e.g barley, durum, wheat and cotton) mutant varieties nowadays occupy the majority of cultivated areas in many countries (Maluszynski *et al*., 1995).

Reddy *et al*. (1993) have compared the effectiveness and efficiency of chemical mutagens, sodium azide (SA), diethylsulphate (DES), and physical mutagen, gamma rays, on sunflower. According to these results, the chemical mutagens appeared to be more efficient for the sunflower mutagenesis than the physical treatment.

Osorio *et al*. (1995) has reported an increased variability in the fatty acid composition in oil of sunflower mutants, obtained from the seeds mutagenised with ethyl methanesulphonate (EMS). This chemical mutagen can induce GC-AT transitions in the DNA 10 - 100 fold more efficiently than AT-GC transitions (King, 1984).

Smolinska (1987) has pointed out the capability of EMS to induce point mutations not only in nuclear, but also in mitochondrial genomes of yeast. After mutagenesis with EMS or DES, new sunflower mutants with enhanced oil content and enhanced biomass production were obtained by Chandrappa (1982). Kübler (1984) has obtained sunflower mutants of M2 and M3 generations with high linoleic acid content for diet food and mutants with high oleic acid content for special purposes like frying oils after EMS mutagenesis.

This conventional approach of induced mutations in order to enhance the genetic variability of new plant mutants could be a promising alternative to genetic transformation for an improvement of metal accumulation potential in sunflower and other high yielding crops. In addition, the subsequent genetic analysis of mutants affected in metal uptake and accumulation potential could be a promising start to better understand mechanisms that govern metal accumulation (Salt *et al*., 1995). For instance, two mutants of *Arabidopsis thaliana* (pho1, P deficient and pho2, P accumulator) were used to assess uptake, translocation and accumulation of arsenic (As) in roots and shoots (Quaghebeur and Rengel, 2004).

In this context, sunflower mutants with enhanced metal accumulation and extraction properties have not yet been reported. However, in other plant species the classical mutagenesis has been used to get new mutant variants with enhanced metal accumulation traits. Mutant seedlings of *Arabidopsis thaliana* L. accumulate a 7.5 times higher amount of Mn and 4.6 times more Cu from the soil than the control (Delhaize, 1996). Zinc accumulation was enhanced by a factor of 2.8 and Mg by a factor 1.8 in the mutant variants.

Delhaize (1996) has also found out that this recessive mutation shows a positive correlation with ferric-chelate reductase activity. Howden *et al*. (1995) has reported about mutants of A. thaliana exhibiting a hypersensitivity to various combinations of Cd, Cu, Hg and other toxic metals. Navarro *et al*. (1999) have isolated two Cd-tolerant mutants, initially assessed by root growth, from the EMS mutagenised Arabidopsis seeds.

One mutant, cdht 1, shows an LD50 of 200 mM Cd versus an LD50 of 110 mM Cd for the control plants. Mutants cdht1 and cdht4 accumulate 2.3 times less Cd than control plants exposed to 150 mM CdCl2.

Nawrot *et al*. (2001) have used induced mutation for rapid creation of variability in Al tolerance in barley. Thirteen mutants with increased levels of tolerance to Al have been selected in M3 generation after mutagenic treatment of four barley varieties with N-methyl-N-nitroso urea (MNH) and sodium azide (NaN3).

An enhanced aluminium (Al) tolerance was also observed in barley cell lines obtained through mutagenesis by ethyl methanesulphonate, sodium azide and gamma ray (Zhu *et al*., 2003).

It is well established that mutation breeding served as significant tool for plant improvement (Larkin, 1998). For instance , the non-edible oil from linseed flax, *Linum usitatissimum,* was changed into an edible oilseed oil (linola) and a new industry in potential through induced mutations of the fatty acid biosynthesis pathway (Larkin, 1998). Much work on mutagenesis has been accomplished with many mutants of agronomic importance recorded as well reviewed by Natarajan (2005). More than 2000 mutant plant varieties have been released for cultivation, and faced none of the regulatory restrictions imposed on genetically modified material (Waugh *et al*. 2006).

Table 1. Various mutant crops produced by sodium azide (NaN3) treatment.

The plant species	**Improved Traits**	**Authors**
Zea mays (maize)	Resistant against pathogen *Striga*.	Kiruki *et al*., (2006)
Pisum sativum (pea)	ImprovedPyridoxin deficiency	Kumar (1988)
Hordeum vulgare (barley)	Chlorophyll mutant	Prina and Fevret (1983)
Musa spp. AAA	*oxysporum* f. sp. cubense Resistant against *Fusarium*	Bhagwat and Duncane (1988)
Hordeum vulgare (barley)	Mildew resistant	Molina-Cano *et al*., (2003)
Avena strigosa (oat)	Disease resistant	Papadopoulou *et al*.(,1999)

Arachis hypogaea L. (groundnut)	Yield traits	Menash and Obadoni (2007)
Vigna radiate L. (mungbean)	Quantitative traits	Samiullah *et al*., (2004)
Saccharum officinarum (sugarcane)	Red rot (*Colletotricum falcatum*) resistant	Ali *et al*., (2007)
Araches hypogea (groundnut)	Disease resistant	Mondal *et al*., (2007)
Lagerstroemia indica (crape myrtle)	Resistant to powdery mildew and leathery foliage	White and Carl (2004)
Spathoglottis plicata Blume	Improvedfloricultural significance	Roy and Biswas (2005)
Oryza sativa L. (rice)	Auxin resistant mutant	Chhun *et al*., (2003)
Lactuca sativa (lettuce)	Down mildew resistant	Okubara *et al*., (1994)
Hordeum vulgare (barley) deficient	Anthocyanins and proanthocynidins	Olsen *et al*.,(1993)

Oryza sativa L. (rice)	Enhanced amylase content	Suzuki *et al*.,(2008)
Halianthusannuus (sunflower)	Enhanced stearic acid content	Skoric *et al.,* (2008)
Halianthus annuus (sunflower)	Reduced triacylglycerol	Venegas-Caleron *et al.,* (2008)
Oryza sativa (rice)	Reduced amylase content	Jeng *et al.,* (2003)
Oryza sativa (rice)	Enhanced yield	Jeng *et al.,* (2006)
Zea Mays (maize)	Drought tolerant mutant	He *et al.,* (2009)
Triticum aestivum (durum wheat)	Salt tolerance	Agata *et al.,* (2001)
Oryza sativa (rice)	Silicon deficient mutant	Nakata *et al.*,(2008)
Hordeum vulgare (barley)	Reduced phytic acid content	Oliver *et al.,* (2009)
Glycine max (Soybean)	Enhanced fatty acid	Hammond and Fehr (1983*b*)
Phaseolus vulgaris (common bean)	Higher antioxidant activity	Jeng *et al.,* (2010)

2.5 Features and Attributes of Mutation

Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination (Hastings *et al.*, 2009). Mutations are changes in the DNA base sequence of a cell's genome caused by radiation, viruses, transposons and mutagenic chemicals, as well as errors that occur during DNA replication (Bertam *et al.*,2000).

Mutation represents a change in the hereditary material of a cell. It can be deletions, or molecular changes within the physical limits of the gene. It may involve the rearrangements, loss, or duplication of chromosome segments. Most mutations are deleterious, harmful and many are lethal (Milton and Sleper, 1995).

Mutation breeding in crop plants is an effective tool in hands of plant breeders especially in crops having narrow genetic base. Many mutants have been identified as donors of desirable traits in breeding program. Mutation breeding work in soybean crop has yielded in identification of many mutant lines with desirable traits like resistance to pod shattering habit, low linolenic and high oleic content (Rahman *et al.*, 1994, 1995) etc. Variations in M1 generation, though important in view of obtaining stable gene mutations, are often considered as indicator in measuring efficiency of mutagen treatments (Plesnik, 1993).

Mutation can be detected because of some phenotypic changes in plants. A visible change in a morphological characteristic such as, per carp color, leaf marking, chlorophyll deficiency, endosperm texture, spike density, etc, is most easily identified. Mutations that cause minute changes in quantitative plant characteristics, such as, physiological activity, chemical content, are more difficult to identify. Their effects may require precise measurements, often on a population of plants rather than a single plant (Milton and Sleper, 1995).

2.6 Mutagenic Agents and Mutation Breeding

Mutagenesis can be promoted through chemical, physical and biological agents (Koornneef, 2002). Both physical and chemical mutagens are known to act in different ways to cause DNA lesions (Chopra, 2005). They are known to induce a high frequency of mutations at random locations across the genome (Waugh *et al*., 2006). Among the chemical mutagens are the vegetable oils, alkylating agents including EMS, Butyl Methane sulphonate, (BMS) and arsenic (Natarajan, 2005). The most widely used physical mutagens are the ionizing radiations such as gamma and X-rays (Koornneef, 2002).

Many crops including wheat, rice, maize, barley and *Brassica* were mainly treated with radioactive isotopes (32P, 35S), X-rays or fast neutrons (Natarajan, 2005). Initial studies on induced mutations performed on a variety of species including wheat, barley, rice, tobacco, maize, *Brassica*, fruit crops and vegetables, were carried out for both physical and chemical mutagens and were directed towards finding an optimum combination of the mutagen and dose (Chopra, 2005).

Ionizing radiation and chemical mutagens have been principally employed to increase mutation frequency in plant. The radiation includes X-rays, neutrons, gamma rays, ultraviolet, and laser beams. X- Rays were used most extensively in early experiments because X-ray equipment was widely available and easily operated. Seeds, plants, or pollen could be treated with fairly accurate doses. There is a conspicuous parallelism between sterility and mutation rate, even for the different mutation types. Mutation frequency and degree of sterility are roughly proportional. A high fertility rate is connected to low mutability. The increased mutation rate is limited to the offspring of sterile plants, while the mutation rates of fertile plants remain constant (Gustafsson, 1940).

In the mid thirties the first "vital" mutations appeared. It was possible already at that time to distinguish two sub-groups:

[1] Morphological mutations, in which morphological characters are altered; as a rule they segregate sharply as qualitative characters in intercrossings or in crossings with the original line. [2] Physiological mutations, which can be clearly distinguished from the original ones, but only in properties with quantitative variation relating to straw-length, straw-stiffness, lateness, early maturity, tillering capacity, seed-size, seed-colour, and others (Gustafsson, 1941a).

Regarding the application of chemomutagens in the mid forties the following chemicals were included in experiments: Colchicine, Potassium cyanide, Hydrogen peroxide, Butter-yellow, Uranyl nitrate and Ferri sulphate. Some at that time unknown mutants were also discovered (D'Amato and Gustafsson, 1948; Gustafsson and Nybom, 1949). The exact ranging of these chemical mutagens has changed several times as the investigations have proceeded, so it is not certain that it is the definite one (Ehrenberg *et al*., 1956a; Ehrenberg, 1960; Gustafsson, 1960a).

Alkylating agents such as ethyl methane sulfonate, sodium azide and hydroxylamine hydrochloride etc, have been widely used in the past for producing mutagenized populations, which can then be used for forward genetic screens. It is applicable readily to most plant species, inducing single base pair G/C-to-A/T substitutions in nucleotides. Targeted iduced local lesions in genomes (McCallum *et al*., 2000 ; Colbert *et al*., 2001) is a reverse genetic tool that is used to identify these single base pair changes in target DNA sequences and is readily applicable to most plants (Henikoff and Comai, 2003). It is frequently carried out in parallel with forward screens, permitting pre-selection of deleterious mutations (Perry *et al*., 2003). Chemical mutagens are often preferred to the radiation, because they are simpler to apply and produce less damaging effects, producing more gene mutations and fewer chromosome disruptions (Milton and Sleper, 1995).

Over the last seven decades, more than 2250 varieties commercial mutants, used directly or through controlled crosses, have been created (Ahloowalia *et al*., 2004). Oat (*Avena sativa L.*) was introduced into Brazil, where the environmental conditions are completely different from the center of origin of the species. The existing genetic variability for traits of agronomic importance, such as plant vegetative cycle, is considered restricted. The narrowing of the genetic base in cultivated oat varieties can be a constraint on the efficacy of genotype selection in segregating generations (Carvalho and Federizzi 1989).

Genetic variability, indispensable for all effective natural and/or artificial selection, consists essentially of processes of evolution and plant improvement (Jennings *et al*., 1981). Aside from the predetermined genetic variability in the germplasm, variability can be added by means of artificial mutations, gene recombination, genetic transformation, and somaclonal mutations.

Table 2. List of some chemical mutagens

Chemical's name	Abbreviation
Methyl methanesulfonate	MMS
Methyl ethanesulfonate	MES
Ethyl methanesulfonate	EMS
Ethyl ethanesulfonate	EES
n-propyl methanesulfonate	PMS
Isopropyl methanesulfonate	iPMS
n-butyl methanesulfonate	BMS
sec-butyl methanesulfonate	sBMS
Isobutyl methanesulfonate	iBMS
Tert-butyl methanesulfonate	tBMS
Neopentyl methanesulfonate	NeoMS
Allyl methanesulfonate	AMS
2-chloroethyl methanesulfonate	CIEMS
2-methoxyethyl methanesulfonate	MOEMS

Low genetic variability in cultivated species hampers the selection of superior genotypes for breeding (Silva *et al.,* 1998). Mutations create genetic variability. They provide the raw material for the evolution process and are sometimes fundamental for improvement, whose success depends on the existence of variability. Variability in base population becomes essential when breeding objectives are more complex. Main interests of a plant breeder are quantitative traits, which are controlled by polygenic interactions. A series of experiments carried out with various crops have established that chemical mutagens induce polygenic variability (Rajput *et. al.,* 2001, Singh & Singh, 2001 and Khan *et al.,* 2004).

Usually before launching a mutation breeding programme, it is crucial that mutagen dose optimization experiments are conducted. This is because the dose and exposure time to the mutagen are important in determining the frequency and types of mutations. Optimization of dose, frequency of mutations and induction of genetic variability studies have been done in many crops including rice (Seetharami Reddi, 1984), grain sorghum (Seetharami Reddi and Prabhakar, 1983), chickpea (Shah *et al*., 2006), mungbean (Singh *et al*., 2005), common bean (Svetleva, 2004) and oats (Verhoeven *et al*., 2004).

In *Sorghum*, mutagenesis is recognized as one of the approaches that can be used to create genetic variability (Chantereau *et al*., 2001) though there is paucity of information on mutagenesis in sorghum. Brataudeau and Traore (1990) have shown that EMS has the potential to induce favorable mutations in sorghum with important mutants for drought and earliness being realized. Mutagens used in sorghum range from chemical agents such as ethyl methane sulfonate (EMS) to physical agents like gamma rays. The most popular agents used for sorghum are physical agents (Chantereau *et al*., 2001).

2.7 The Effects of Chemical Mutagenesis on Association of characters in Sesame

Seed yield is a multifaceted character that depends on interrelated characters. The components that influence the yield are best indices for selection. Hence, the knowhow of relationship between important yield traits and seed yield may help the researcher to identify favorable donors for a potential and successful breeding program. The estimation of character associations could identify the relative importance of independent characters contributing to dependent ones and suggest upon the character(s) that may be useful as indicator for one or more of other characters (Kumaresan and Nadrajan, 2002).

Since seed yield is a complex and polygenic trait, it is a total and converged expression of various factors. The knowledge of interrelationship among various developmental and productive traits is necessary for implementing an effective breeding program.

Knowledge of the association of component traits with yield may greatly succor in a precise selection for the improvement of yield. Selection based on yield components is expedient if different yield related traits have been well documented (Panse, 1957; Poehlman, 1991; Singh and Kakar, 1997; Sarwar *et al*., 2005).

2.7.1 Effect of mutagens on correlation coefficients of yield and its component characters

Research results evinced that number of capsules per plant exhibited highly significant and positive correlation coefficient with seed yield consistently in control population of all the experimental genotypes. Interestingly, mutant population exhibited change of relationship in a few correlated characters. It was observed that seed yield was positively and significantly correlated with plant height, number of branches per plant, number of capsules per plant, capsule length and number of seeds per capsule consistently in the mutated population of three genotypes, while flower duration, 1000- seed weight and days to maturity were also significantly and positively correlated with seed yield per plant but not consistently in the treated population of three genotypes.

Among the correlated characters highest magnitude of positive correlation coefficient was found between seed yield per plant and number of capsules per plant professing that number of capsules per plant was most important yield contributing character in both normal and mutant populations irrespective of the genotypes.

Similar significant positive correlation of seed yield per plant with number of capsules per plant in mutated population was also reported by Hassan *et al*. (2005) in chickpea; Govindarasu & Ramamoorthi (1998) and Sarwar *et al*. (2007) in sesame, while Geetha & Subramanian (1992), Anandakumar (1994), Arshad *et al*. (2003) and Atta *et al*. (2008) observed similar correlation in normal population.

On the other hand, Ramanathan & Rathinam (1983) observed negative association between number of pods and pod yield in M3 generation due to the induction of EMS in two varieties of groundnut. Combining all, it affirmed that plant height, number of branches per plant, number of capsules per plant, capsule length and number of seeds per capsule were important yield contributing characters consistently in all mutant populations.

The interrelationships of these characters depicted that number of capsules per plant was significantly and positively correlated with plant height in mutant population. Similarly, number of branches per plant and number of capsules per plant were significantly and positively interrelated. Such positive trend in correlation coefficients was also found in plant height and capsule length, plant height and number of seeds per capsule, capsule length and number of seeds per capsule. The results, therefore, inferred that number of capsules per plant would like to produce correlated response in plant height and number of branches per plant.

During selection procedure, a plant breeder always seeks minimum number of characters, which are effective in improving yield, as it becomes complicated to handle more number of characters. Therefore, number of capsules per plant should be considered as most important selection criteria for improving yield. Selection for more number of capsules per plant would obviously result in plant types with more seed yield.

Along with capsules per plant, plant height, number of branches per plant, capsule length and number of seeds per capsule were observed to be important yield components in the mutagen treated population. Thus restructuring or selection of plants with more number of capsules per plant, plant height, number of branches per plant, capsule length and number of seeds per capsule would likely to aid in evolving varieties with high yield.

2.7.2 Effect of mutagens on correlation coefficients between seed yield and biochemical characters

It was revealed that oil yield was significantly and positively correlated with seed yield but negatively correlated with protein content in both mutant and control populations. A contrasting relationship between oil content and seed yield as well as protein content and seed yield were observed by Pahlavani (2005) in safflower.

Sesame is cultivated chiefly for oil purpose in India. So, augmenting oil content against sacrifice of higher protein content would not be a disappointing proposition. Similar finding was also reported by Solanki & Gupta (2000) in sesame.

It is worth mentioning that the mutation distinctly spawned change of relationship between seed yield and some biochemical characters namely palmitic acid, stearic acid and linoleic acid as the correlation coefficients between seed yield with either of these unsaturated fatty acids were highly negative in control population but in mutant population the correlation coefficients were found to be significantly positive or positive .Similar result was also observed in interrelationship between linoleic acid and palmitic acid as well as linoleic acid and oleic acid.(Solanki & Gupta 2000) .

Similarly, in control population stearic acid was correlated negatively and significantly with oleic acid but in mutant population a significant positive relationship subsisted. Thus mutation appeared to break the negative linkage between characters. This obviously is enviable situation as selection for higher seed yield in mutant population would lead to high stearic acid, oleic acid and linoleic acid along with more oil content.(Solanki & Gupta 2000) .

Again in mutant population stearic acid was interrelated significantly and positively with both oleic acid and linoleic acid. Thus selection for high stearic acid will ameliorate both oleic acid and linoleic acid through correlated response. It is to be emphasized here that oleic acid and linoleic acid are the two important and beneficial unsaturated fatty acids in sesame (Sengupta & Das, 2003). If oil quality is also improved concomitant with oil content and seed yield, then that would be most rewarding situation or in other words such varieties would be the most demanding varieties in sesame cultivation.

2.8 Enhanced Sensitivity to Mutagens by Presoaking

One of the important aspects of chemical mutagenesis in any crop is to improve the efficiency of mutagens by certain modifications to enhance the sensitivity of the treated materials. Presoaking (PS) seeds prior to treatment are an effective manipulation, which minimizes quantity and cost of the chemicals and treatment time.This is well tested in many crops using different chemicals. The effective PS periods reported in various studies were highly variable depending on mutagens, their dose, characters and crops studied.

2.9 Mutagenesis as a Tool for Creating Genetic Variability

Availability of genetic diversity and genetic variation is the heart of any breeding program which plays a critical role in developing well-adapted and improved varieties. Mutation induction is an effective tool to enhance the genetic variation available to plant breeders, particularly for traits with a very low level of genetic variation (Szarejko and Forster, 2007). The high frequency with which certain radiations and chemicals can cause genes to mutate made it feasible to perform genetic studies that were not possible when only spontaneous mutations were available.

Consequently, much of our knowledge of genetics of higher organisms is based upon works utilizing induced mutations for analyzing gene function (McCallum *et al*., 2000). To date, several well documented examples of successful applications of mutation breeding to oilseed crops have been reported in the literature (Ahmad *et al*., 1991; Bacelis, 2001; Bhatia *et al*., 1999; Ferrie *et al*., 2008; Fowler and Stefansson, 1972; Kott *et al*., 1996; MacDonald *et al*., 1991; Newsholme *et al*., 1989; Osorio *et al*., 1995; Parry *et al*., 2009; Rowland, 1991; Sala *et*

al., 2008; Schnurbush *et al*., 2000; Spasibionek, 2006; Swanson *et al*., 1989; Velasco *et al*., 2008).

Induced mutations have been used mainly to generate variation that could rarely be found in germplasm collections. Mutation techniques have been applied to improve such traits as earliness, semidwarfness, lodging resistance, disease resistance, yield and quality (Bhatia *et al*., 1999; Newsholme *et al*., 1989; Osorio *et al*., 1995; Parry *et al*., 2009; Rowland, 1991; Schnurbush *et al*., 2000).

About 3088 mutant varieties have been developed according to FAO/IAEA mutant varieties database (FAO/IAEA, 2011). To date, 198 mutant cultivars of annual oilseed crops including soybean, sesame, canola, sunflower and linseed have been released (FAO/IAEA, 2011). Soybean with 155 mutant cultivars possesses the highest number of mutant cultivars, followed by sesame with 24 and canola with 15 cultivars. In canola, oil modification has been achieved by using seed and microspore mutagenesis (Ferrie *et al*., 2008; MacDonald *et al*., 1991; Velasco *et al*., 2008).

In spring canola, radiation treatment has been applied to the seeds of “Regent” cultivar and M5 lines selected with increased oleic acid contents varying from 63 to 79%. In winter canola, chemical mutagenesis was used to isolate two canola mutants of the cultivar “Winfield” with high oleic acid content (Wong and Swanson, 1991). Mutation breeding in canola has been also used to improve herbicide resistance (Ahmad *et al*., 1991; Sala *et al*., 2008; Swanson *et al*., 1988, 1989), disease resistance (Ahmad *et al*., 1991; MacDonald *et al*., 1991; MacDonald and Ingram, 1986; Newsholme *et al*., 1989), and lower glucosinolate content (Barro *et al*., 2002; Kott, 1998; Kott *et al*., 1996).

Chemical and physical mutagens are available for mutagenic treatment of crop plants. Nevertheless, several chemical mutagens have been applied of which ethyl methane sulfonate (EMS), Nitroso- N-methylurea (NMU), N-Nitroso, N-Ethylurea (ENU) and sodium azide are the preferred agents in plant mutation induction (Medrano *et al*., 1986; Szarejko and Forster, 2007). Alkylating agents are the most important chemical mutagens used in mutation breeding. They add ethyl or methyl groups to bases in the nucleotide structure, which leads to activating a silent gene, silencing an active gene, or altering a particular gene action (Snustad and Simmons, 2006).

Mutagenesis amends the genetic blueprint of plants through interference and modification of genes (Koornneef, 2002). Mutants with new alleles and genes are developed which enhances genetic variation (Koornneef, 2002; Singh and Kole, 2005). Production of heritable changes is an important aspect of many breeding programmes and breeders use mutations to produce these changes (Neuffer *et al*., 1997).

Mutagenesis is highly fundamental in plant biology to induce genetic variability to a great number of crops, mainly due to the fact that the technology is simple, relatively cost effective to perform, applicable to all plant species and equally usable on a small and large scale (Swaminatan, 1995, Siddiqui and Khan, 1999).

The frequency and saturation of mutations can be managed by fluctuating the mutagen dose (Jander *et al*., 2003; Menda *et al*., 2004) and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions (MacKenzie *et al*., 2005; Kim *et al*., 2006).

The application of mutation breeding is especially useful today to foster genetic variation in crops with narrowed genetic variability. For example, mutation breeding using EMS has been found effective in generating much needed variation for certain traits where the genetic variation was lacking (Yadav, 1987; Singh and Kole, 2005).

Various scholars (Mashenkov, 1986; Ricardo and Ando, 1998) have reported the role of chemo mutagens in enhancing genetic variability in higher plants since it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants (Kleinhofs *et al*., 1978). This variation can occur naturally or can be induced through mutations, using physical, biological or chemical mutagens and has attracted the interest of plant breeders for many decades. The mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yield and quality traits (Ahloowalia and Maluszynski, 2001).

In *Capsicum*, mutation studies have also shown that EMS mutagenesis increases the variation in many characters including leaf area, days to flowering, days to fruiting, and plant height. Such variation is essential to breed for desirable characters (Jabeen and Mirza, 2002).

In *maize*, the most efficient means of producing gene mutations has been found to be chemical mutagenesis and a rational protocol of chemical mutagenesis in this crop is well presented by Neuffer *et al*. (1997). Many agronomically significant mutations affecting plant and grain characters have been identified, including alteration of grain color, stem rust resistance, and earliness in wheat (Chopra, 2005). In oats, isolation and characterization of novel starch mutants has also been achieved (Verhoeven *et al*., 2004).

The success of mutation breeding in ornamentals and horticultural crops in India is impressive with 46 mutants commercially released (Chopra, 2005).

Jeya Mary and Jayabalan (1995) treated the seeds of sesame with EMS ranging from 10 µM to 50 µM at an interval of 10 µM each, induced variability in morphological characters. Some of the variants observed in response to EMS treatment are height mutants, branching mutants, leaf mutants, plant color and texture mutants, mutants for maturity period, floral and sterile mutants, capsule mutants and seed mutants. The analysis of their breeding behavior showed a dose dependent increase in the frequency of such mutations.

Kang *et al* (1996) released a variety Yangbackkae developed from Danbackkae seeds treated with 2 µM Sodium azide for 3h. Yangbackkae has not only a higher yield than its control variety but also improved oil quality. Another semi dwarf mutant Suwon 128, obtained after treatment with Sodium azide, has excellent lodging resistance and a good yield potential when planted in high density.

Six varieties and nine hybrids of sesame were subjected to gamma irradiation by Govindarasu *et al* (1997), the effects of irradiation on seven yield related traits were evaluated in the M2 and M3 generations. In general, irradiation of hybrids and varieties produced more or less similar patterns of mutations, with negative shift for most of the characters studied. Non alteration of population means for plant height and 1000 seed weight were observed in M2 and M3 generations. In F2M2 and F3M3 generations, 1000 seed weight remained unaltered. Most of the other characters which showed negative shifts in the F2M2 generation recorded non-alteration in the F3M3 generation.

Fourteen officially released cultivars have been produced through the use of induced mutations, 12 direct and 2 through hybridization. UMA, USHA and Kalika are important tin Orissa state in India while the remaining cultivars cover small areas (Ashri, 1998).

Govindarasu and Ramamoorthi (1998) irradiated nine F1 crosses in sesame with gamma rays at 20 and 30 kR doses using cobalt-60 gamma source. They studied the progenies of both irradiated and nonirradiated segregating populations for association of characters in the third generation. Only two component traits viz., branch number and capsule number maintained strong positive correlation with seed yield as well as among themselves in both the populations. They also had high positive direct effects on seed yield in the progenies of irradiated and non-irradiated hybrids, revealing that these two are the most important traits in the determination of seed yield. The other character pairs viz., plant height, capsule length, seed number per capsule and 1000 seed weight expressing significant correlation in the untreated segregating progenies did not maintain the same level of association in gamma irradiated segregating progenies.

Seeds of six varieties and nine hybrids in sesame were treated with gamma rays by Govindarasu *et al.* (1998). Macro-mutations affecting chlorophyll, plant size, leaf characters, capsule characters and sterile plants were studied in M2 and F2M2 populations. Frequencies of deviations were more in F2M2 than M2 for all these characters, except plant size, indicating the efficiency of irradiation of hybrids in producing major deviations. The plants of F2 and F2M2 generations were studied for frequency distribution for seed yield, branch number and capsule number. Trans aggressive distribution was observed towards the positive direction for all three characters. Irradiated hybrid progenies showed a better symmetry in distribution with positive transgression, suggesting the better chance for selection of high yielding genotypes.

In an attempt to create better male sterile material which can be used directly in sesame breeding work, Li-Ying De, *et al*. (1998) irradiated dry seeds of the good variety Yuzhi 4 with 60Co- gamma rays at 300, 500 and 700 Gy. From 60,000 M2 plants, 10 male sterile mutants were obtained, which were used for further investigation in the M3 and M4. Six mutants were selected which exhibited full male sterility and full female fertility. The male sterility shown to be genetic sterility and controlled by one pair of recessive genes.

Shahin (1998) studied the effect of different factors including gamma radiation on the growth and aflatoxin production of *Aspergillus flavus* in sesame seeds. Sesame seeds were inoculated with aflatoxigenic *Aspergillus flavus*, and then irradiated at different doses. Irradiation decreased aflatoxin production in the seeds and the decrease was proportional to the irradiation dose. Aflatoxin production was completely prevented in inoculated sesame seeds by an irradiation dose of 2.5 k Gy, even under conditions optimum for fungal growth.

Sorour *et al* (1999) irradiated the seeds of sesame cultivars Giza 25 and Giza 32 with 100, 200, 300 and 400 Gy of gamma-radiation. M1 plants irradiated with 400 Gy had lower means than the parent plants in all the characters studied, but had much higher coefficients of variation. In addition, highly significant variation was observed in the M2, allowing selection of number of useful mutants.

In general, all doses except 100 Gy were effective for selection in M2 and M3 generations. Mutants with more capsules, long capsules, multi-capsules per leaf axil, semi-shattering capsules and early maturity were selected.

Yuzhi11 was developed from a single mutant plant of CV. Yuzhi 4 found in 1991 by Wei-Wenxing *et al* (1999). In trials (1994-98) the average seed yield of Yuzhi 11 was 1.01 t per ha which was 7.93 per cent higher than the yield of Yuzhi 4. Yield of Yuzhi 11 was also more stable than that of Yuzhi 4, seed oil content was 56.66 per cent, which was slightly higher than that of Yuzhi 4, 1000-seed weight was 2.7-3.0 gm and seeds were white. Yuzhi 11 showed high resistance to *Fusarium oxysporum* f.sp. sesami, *Macrophomina phaseolina* and *Cercospora sesami*.

Govindarasu (2000) irradiated the seeds of 3 varieties TMV 3, TMV 4 and TMV 6 with gamma rays at LD50 value of 20 kR and crossed individually with RJS 199. The mean performance of F2 in all the three crosses was significantly better than M2 of all the three varieties for seed yield, branch number, capsule number and 1000 seed weight. A similar trend was also noticed in F3 and M3 populations. Genotypic variability was relatively of higher magnitude in F2's and F3's than M2's and M3's for seed yield, branch number and capsule number. Estimates of heritability and genetic advance also showed the same trend.

The effects of sesamol, a phenolic compound responsible for the high resistance of sesame oil to oxidative deterioration as compared with other vegetable oils, were investigated by Kaur *et al* (2000), after mutagen treatment in various strains of *Salmonella typhimurium*. Mutagenicity was induced by the generation of oxygen radicals by tert-butyl hydroperoxide (t- B00H) or hydrogen peroxide (H_2O_2); the antimutagenic property of sesamol was attributed to its antioxidant properties.

Sheeba *et al*. (2003) subjected two sesame varieties viz., SVPR 1 and Co 1 to gamma irradiation (30, 40, 50, 60 and 70 kR) and EMS (0.8, 1.0, 1.2, 1.4 and 1.6%) treatments. They observed comparatively higher GCV for capsule length followed by number of seeds per capsule in both the varieties, 1000 seed weight in Co1 and single plant yield in SVPR

registered high GCV. In SVPR 1, maximum heritability was recorded by 40 kRad (99.4%) for number of capsules per plant in gamma rays treated progenies and by 1.0per cent (98.4%) for single plant yield in EMS treated progenies. In Co 1, 60 kRad (99.9%) of gamma rays and 1.4 per cent (99.8%) of EMS registered maximum heritability for number of branches per plant and capsule length respectively.

Narrow leaf mutants were identified by Sonali and Animeshkumar (2005) in the M2 generation of sesame, cultivar B-67 treated with nitrous acid (0.25%, 2h-6.25% and 0.25%, 6h-2.50%) and hydrogen peroxide (0.25%, 2h-6.25%; 1.0%, 4h-0.63%; 0.25%, 6h-4.69% and 1.0%, 6h-3.28%). The estimated frequency of the mutant plant over the M2 population (6137 plants scored) was 0.28 per cent. The leaves of the mutant plants were narrow and oblong to lanceolate in shape with entire to undulated margins. The narrow leaf mutant plants have higher number of capsules per plant, and on the main axis and smaller distance from base to first branching compared to controls.

2.10 Application and Integration of Induced Mutations in Biotechnology

Despite the fact that classical mutation breeding has lost its preeminent position, induced mutations continue to be in great demand for several applications in biotechnology. The methods of mutation induction and analyses of mutants have witnessed great changes in recent years. For a brief period during the 1980s and 90s, when it was discovered that in vitro culture of plant cells gives rise to genetic variation (Larkin, P. J. and Scowcroft, S. C., 1981) somaclonal variation was widely tested as an alternative to traditional mutation breeding. Variations were found in plants regenerated through tissue culture for almost all traits.

People were successful in developing an improved variety of *Brassica juncea* 'Pusa Jai Kisan' through this approach (Katiyar, R. K. and Chopra, V. L., A. 1995,). Nevertheless, it is now concluded that somaclonal variation does not offer special advantage over existing methods of inducing genetic variation.

Induced mutations are playing a major role in basic studies especially for the elucidation of biochemical and plant developmental pathways. For example, identification of key genes involved in floral organ development, which ultimately led to the construction of ABC model of flower development, was made possible through the isolation and molecular characterization of floral mutants of *Arabidopsis* and *Antirrhinum* (Coen, and Meyerowitz, 1991).

The genome sequencing projects have given a new fillip to mutagenesis. With the availability of complete genome sequences of major organisms including plants like *Arabidopsis thaliana* and *Oryza sativa*, scientists are now trying to find biological functions of various putative gene sequences. While computational approaches have been found useful in gene annotations, the true biological meaning can be established only by empirical experiments. Induced mutations are expected to play a key role in such functional genomic analyses. Today scientists are interested in locating and identifying the exact genetic change at the nucleotide level. Traditional forward genetic approach 'from phenotype to genotype to gene' is very cumbersome and hence conventional mutagenesis is not considered ideal for molecular analysis.

The availability of plant transformation techniques has provided novel ways to create and screen specific mutations. Mutations can now be induced through transposon or T-DNA insertion via transformation (i.e. lines carrying foreign DNA insertions into host chromosomes) can be isolated based on the presence of foreign DNA sequence. Thus one can avoid screening based on an altered phenotype. Further, using the sequence information of the transposon or T-DNA, the mutated gene can be easily tagged and cloned through inverse PCR or other techniques. (Resminath, *et .al.*, 2005).

Reverse genetic approaches (i.e. proceeding from gene sequence information to phenotype) have also become available in recent years. For example, antisense suppression or silencing of genes is now feasible through transgenic approach. Thus any test sequence can now be employed for gene silencing via antisense or RNAi approach to elucidate its biological function. However, transformation is still not very routine and easy in many plant species.

Hence traditional forward genetic approach is still being followed. (Waterhouse, and Helliwell, 2003).

One of the chief advantages of traditional mutagenesis is that it can give rise to many different mutant alleles with different degree of trait modification. This variation in expression is very useful in many basic studies, such as identification of amino acid residues critical for enzyme activity. In contrast, transposon or T-DNA mutagenesis generally leads to loss of function through gene disruption.

Therefore, conventional mutagenesis is still favored for basic studies. New molecular approaches have greatly simplified forward genetic approach with conventionally derived mutants. Saturated molecular maps are now being constructed in most crop plants. Using such maps, the mutant locus is first delimited using molecular markers. In the next step, the gene is cloned through positional cloning or chromosome walking (Brown, *et al*., 2003).

Site-directed-mutagenesis leading to specific nucleotide modification within a gene sequence is now feasible in vitro. Similar efforts at introducing specific changes in vivo have proved successful in mouse (Yu, and Bradley, 2001) and fruit fly (Rong, *et al*., 2001). In plants, gene replacement experiments through homologous recombination with introduced DNA sequences have met with limited success. Therefore, conventional mutagenesis will continue to be important in molecular-genetic studies of plants. (Hohn, and Puchta, 2003).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The research was carried out at Haramaya University in genetics and biotechnology laboratory as well as Rarre agricultural research field, eastern Hararghe. The research field is 1980 m above sea level and at a geographical coordinate of latitude 9° 26 ' N and longitude 42 ° 3 ' E. It receives a total annual rainfall of 790 mm of rainfall with a mean temperature of 17 °C. The rainy season displays a bimodal fashion i.e. the short rainy season stretches from March to May and the main rainy season from July to September (Kebede, 2002).

3.2. Study Design

The experimental design employed was factorial CRD with three replications for each treatment and control. The design of the study involved laboratory as well as greenhouse experiments. The study utilized two varieties of sesame i.e. Abasena and Kelafo 74.The basis of variety selection was their economic importance. The laboratory study was based on bioassay aimed to assess the seed germination percentage and seedling growth. The greenhouse experiment was mainly focused on identifying the quantitative trait variations and mutagenic efficacy of the chemicals in the selected varieties of Sesame.

3.3. Laboratory Examination

The induced chemical mutagenesis study utilized two mutagenic chemicals, namely sodium azide and hydroxylamine hydrochloride. The selection of the mutagenic chemicals was based on their effectiveness, availability and inspiration from previous works.

3.3.1. Preparation of mutagenic agents

Ascending concentration gradients of Sodium azide (NaN_3) and Hydroxylamine hydrochloride ($NH_2OH.HCL$) with (0.01, 0.02, 0.03, 0.04 and 0.05 %) V/V was prepared in plastic beaker using distilled water as a solvent as it was recommended by (Dhanavel *et al*.2008).

3.3.2. Methods of seed mutagenesis

Seeds of Sesame variety Abasena and Kelafo 74 were used for inducing mutation by sodium azide (NaN_3) and hydroxylamine hydrochloride ($NH_2OH.HCL$). For each variety 440 dry and normal uniform seeds were used. The seeds of selected varieties were surface sterilized with 0.1% mercuric chloride for 1 minute to remove the fungal spores on the surface of the seeds.

Then the seeds were washed with distilled water several times to remove the mercuric chloride. After this treatment seeds were pre soaked in plastic beaker which contained distilled water for six hours and then treated with mutagenic agent of sodium azide and hydroxylamine hydrochloride at different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05%) for 6 hrs using methods of Dhanavel *et al.*(2008).

3.3 .3. Bioassay studies

The bioassay studies were carried out following the method of Heisey (1990). Five treated seeds were placed on whatman filter paper in Petri plates (9cm X 2cm). Each petriplate was moistened with 2ml/ plate of distilled water and the controls were normal seeds and incubated at room temperature. The germination percentage, plumule and radical length were measured after 8 days. The germination percentage was calculated using the following formula.

$$\text{Total germination} = \frac{\text{Number of seeds germinated} \times 100}{\text{Number of seeds placed for germination}}$$

3.3.4. Greenhouse pot experiments

The seeds that were treated with chemical mutagenic agents in the laboratory were sown in the earthen pots (24 cm x 24 cm) in greenhouse using factorial CRD design with three replications along with their respective control. Then the experimental plants were grown under the greenhouse condition in Haramaya University. The control plants were grown from untreated seeds.

The following quantitative traits were measured in order to infer reliable data. Trait selection and measurement techniques were as per the IPGRI descriptors of sesame.

- **Plant height:** Mean height of plants from ground level to the apex of the main stem.
- **Number of capsules per plant:** Mean of total number of capsules on both primary and secondary branches.
- **Capsule length:** Distance from the base to the tip of capsules.
- **Internodes length:** The Distance between any two successive nodes on the main axis.
- **Days to 50% flowering**: Numbers of days from seed sowing until 50% plants have at least one flower in each accession.
- **Days to 90% maturity**: Number of days from seed sowing until 75% of plants reaching physiological maturity.
- **Number of seeds per pod**: Mean number of seeds of pods from.
- **Hundred seed weight**: Weight of 100 randomly dried seeds.

3.3.5. Evaluation of the effectiveness of mutagenesis in sesame

The effectiveness of mutation was determined according to the formula suggested by Konzak *et al*. (1965).

$$\text{Mutagenic } Effectiveness = \frac{MF}{C\ X\ t}$$

MF: Mutation frequency (%)

C: Concentration of chemical mutagen

t: Period of treatment with chemical mutagen

3.4. Analysis of Data

Statistical Inference and analysis were made on the collected data. Analysis of Variance (ANOVA) was done for comparing the treatments with the respective controls using SAS software Program. Mean separation was carried out using least significant difference (LSD) at 1% and 5% level of significance.

4. RESULTS AND DISCUSSION

4.1. Bioassay Studies on Sesame

4.1.1. The Effects of Sodium Azide on seed germination and seedling growth of Sesame varieties (Abasena and kelafo 74).

Table 3 shows the germination percentage, length of root, and shoot of sodium azide treated plants. The decrease in germination showed a negative correlation with the increased mutagen concentration in both varieties of sesame. In Abasena variety Maximum reduction in seed germination was 47.37 % and it was observed in 0.05% concentration in comparison to the control and other treatments and highly significant difference was observed at ($P<0.01$) level. Such similar results were reported by Siddiqui *et al. (*2007). Mutagens such as sodium azide decrease the germination percentage and increase chromosomal aberrations in root tip mitotic cells of plants in a dose-dependent fashion. The reduction in seed germination in mutagenic treatments has been explained due to the delay or inhibition of physiological and biological processes necessary for seed germination including enzyme activity (Kurobane *et al*., 1979), hormonal imbalance (Chrispeels and Varner, 1967) and inhibition of mitotic process (Ananthaswamy *et al*., 1971). Root growth was stimulated in 0.02% concentration, where as the maximum reduction in root growth was observed in 0.05% concentration. Highly significant increase was observed in shoot length in 0.02% concentration in comparison with other treatments and the control. The rest treatments displayed a negatve mean shift in shoot length.

The inhibitory effect on the seed germination by sodium azide on kelafo 74 was also in a similar trend with that of abasena and the effect was drastical at 0.05% concentration. Yusuf and Nair (1974) also inferred that gamma irradiation interfered with the synthesis of enzymes and at the same time accelerated the degradation existing enzymes involved in the formation of auxins and thus reduces the germination of seeds. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009).

These findings are in close agreement with the earlier reports of Kumar *et al.* (1993) in Faba bean. In both varieties negative correlation was observed between germination percentage and ascending mutagen concentration. The root and shoot length was stimulated in 0.02% and 0.03% concentrations and the effect was highly significant in comparison to the control and the remaining mutagen concentrations.

Table 3: Bioassay study of Sodium Azide on seed germination and seedling growth of Sesame (Abasena and kelafo 74 variety).

Treatment	**Germination (%)**		**Root Length(cm)**		**Shoot Length(cm)**	
	Abasen	**kelafo 74**	**Abasen**	**kelafo 74**	**Abasena**	**kelafo 74**
Control	95.00a	93.90a	4.53b	3.96c	6.60b	5.91c
0.01%	82.20b	80.00b	3.03d	3.50d	4.47c	5.50d
0.02%	80.07bc	67.90c	5.03a	4.50a	7.00a	6.50a
0.03%	72.50d	67.80c	3.09cd	4.00b	3.87c	6.00b
0.04%	59.70e	65.90d	2.25f	3.50d	3.90d	4.5e
0.05%	52.63f	59.20e	1.89g	2.50e	3.72f	3.5f
CV (%)	0.20	0.12	0.37	0.43	7.11	0.30
LSD	0.19	0.11	0.016	0.020	0.45	0.020

4.1.2. The Effect of Hydroxylamine hydrochloride on seed germination and seedling growth of Sesame (Abasena and Kelafo 74 variety).

Hydroxylamine hydrochloride exhibited inhibitory effect on seed germination in all of the concentrations as compared with control (Table 4). A gradual decrease was observed in seed germination percentage along the increasing mutagen concentrations. The significant reduction in seed germination was observed at higher concentration when compared with control at ($P<0.01$). The similar findings were also reported by Singh and Kole (2005).

Root growth was inhibited in all the concentrations, but the effect was highly significant in 0.04 and 0.05% concentrations as compared with control plants. Regarding the shoot growth, it was stimulated at 0.01 concentration when compared to the control plants and the stimulatory effect was significant over the control plants (Table 4).

Table 4: Bioassay Study of Hydroxylamine hydrochloride on seed germination and seedling growth of Sesame (Abasena variety).

Treatment	**Germination (%)**		**Root Length(cm)**		**Shoot Length(cm)**	
	Abasena	**kelafo74**	**Abasena**	**kelafo74**	**Abasena**	**kelafo74**
Control	93.87a	94.9a	4.53a	3.96a	6.60a	5.91b
0.01%	80.07c	80.07b	3.66b	3.84b	6.89b	6.50c
0.02%	80.00d	79.80c	3.30cd	3.64c	3.66c	5.61a
0.03%	3.90f	3.00d	3.30cd	3.60d	3.24d	4.83d
0.04%	61.30i	60.00e	1.92cg	3.54e	3.18d	4.59e
0.05%	52.03k	52.90f	1.89dg	3.2f	3.00e	3.63f
(CV %)	0.64	0.1	1.02	0.51	1.020	0.21
LSD	0.025	0.09	0.058	0.02	0.058	0.014

This study also showed that the effect of Hydroxylamine hydrochloride on seed germination of kelafo74 variety was concentration dependent. A maximum reduction in seed germination of 52.90 % was observed in 0.05% concentration compared to the control (Table 4). Similar results have been recorded in soya bean by Raut *et al. (*1982) and Patil *et al*. (1985). The reduction in growth was probably due to higher doses. It may also be attributable to the increase in destruction of growth inhibitors, the increase in growth promoters, the sudden increase in metabolic status of seeds at certain levels of dose, or it may be due to the induced chromosomal aberrations. These findings are in close agreement with the earlier works of Wang and Yu (1988), Solanki and Sharma (1999), Solanki and Sharma (2002), Kumar and Selvaraj (2003).

The root growth was inhibited in all the concentration. The inhibitory effect was significant as compared to control.

The gradual decrease in shoot length was observed in all the treatment except in 0.01% concentration when compared with the control plants. The stimulatory effect was observed in 0.01% concentration and it was highly significant (Table 4).

In this investigation an attempt has been made to study the mutagenic effects of sodium azide and hydroxylamine hydrochloride on two varieties of Sesame i.e Abasena and kelafo 74, based on bioassay studies. The above results reflected that sodium azide and hydroxylamine hydrochloride displayed significant inhibitory effect on seed germination and seedling growth. Similar results have been reported by Adamu and Aliyu (2007) through induced chemical mutagenesis of tomato. The authors observed the mutagenic effect on tomato which was treated with sodium azide was very effective in eliciting mutations with respect to seed germination percentage, root and shoot growth.

Table 5 ANOVA of the effects of Sodiumazide and hydroxylamine hydrochloride on seed germination percentage of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	7.57	7.57	592.98	0.0001
Replication	2	0.014	0.00722	0.57	0.5760
Treatment	11	12342.68	1122.062	87939.6	0.0001
Replication*Variety	2	0.01566	0.0078	0.61	0.5504
Treatment*Variety	11	383.322	34.85	2731.10	0.0001
Rep*Treatment	22	0.2495	0.01134	0.89	0.6075
Error	22	0.281	0.01276		
Total	71	12734.14			

Table 6 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on root length of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	9.43	9.42	65.76	0.0001
Replication	2	2.61	1.30	9.11	0.0013
Treatment	11	44.77	4.07	28.39	0.0001
Replication*Variety	2	2.74	1.37	9.55	0.001
Treatment*Variety	11	8.69	0.79	5.51	0.0003
Replication*Treatment	22	3.21	0.14	1.02	0.4843
Error	22	3.16	0.14		
Total	71	74.58			

Table 7 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on shoot length of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	5.77	5.77	182.13	0.0001
Replication	2	0.023	0.01	0.36	0.7023
Treatment	11	92.55	8.41	265.37	0.0001
Replication*Variety	2	0.053	0.03	0.84	0.4446
Treatment * Variety	11	27.18	2.47	77.92	0.0001
Replication*Treatment	22	0.71	0.03	1.01	0.4890
Error	22	0.70	0.03		
Total	71	126.97			

4.2. Green house studies

4.2.1. The Effects of Sodium Azide and Hydroxylamine hydrochloride on the phenological parameters of Sesamum indicum L.

4.2.1.1. Effects of Sodium Azide on the phenological parameters of Sesame (Abasena variety)

The results indicated that plant height and internodes length was significantly increased at 0.02 % concentration of sodium azide treated plants(Table 7). The effect was highly significant at P<0.01 level, when compared with the control and other treatments. Such a similar result on plant height has been reported by Selim *et al.* (1974). The maximum plant height (Table 7) at maturity of 66cm cm was recorded in 0.02 % of sodium azide treatment while minimum plant height was observed in 0.05m % sodium azide (55 cm). In the present study, sodium azide treatment in the lower concentrations has shown stimulatory effect as compared to control and the rest of the treatments. Again in the lower doses of mutagenic treatments, days required for flowering and days required for maturity were found to be significantly decreased. The significant changes in terms of days were observed in 0.02% concentration of sodium azide at P < 0.01 level than the control and the rest of treatments plants. Days to first flowering, ranged from 44, 44, 40, 44, 45, and45 respectively, among control and treatments. A minimum decrease in days to first flowering (40), was recorded in 0.02% when compared to control and other treatments of sodium azide. The plants germinated from seeds which were treated with0.0 4 and 0.05 % sodium azide took the longest duration for corresponding traits of abasena variety (Table 7)

The number of capsule per plant was observed. Significant increase in capsule number (P<0.05) was observed at 0.01%, 0.02% and 0.03% concentration of sodium azide treatments when compared to the control and the rest of treated plants. Since capsules are seed bearing structures their increase has a positive corelation with the yield of a seed. Similar result was cited by Surejon and Sharma (2000). The hundred seed weight was increased significantly at (0.02% concentration) at P < 0.01 level in comparison to the control and other treatments.

The number of seeds per pod of treated and untreated plants was counted. Maximum number of seeds per pod (68) was observed in 0.02 % sodium azide among. A minimum number of seeds per pod was (45) and recorded in 0.05 % sodium azide, respectively.A low concentration of sodium aside (0.02 %) was shown to increase seed number when compared to other mutagen treatments.

Table 8: Effects of Sodium Azide on the phenological parameters of sesame (Abasena variety).

Treatment	PH	IL	DM	CL	NCPP	HSW	NSPP	DF
Control	60a	11e	85b	2c	15d	3d	50d	44b
0.01%	61a	12d	85b	2.1b	18b	3.2c	55c	44b
0.02%	66b	15a	80c	2.5a	20a	3.7a	68a	40c
0.03%	61a	14b	85b	2c	17c	3.5b	60b	44b
0.04%	60a	13c	90a	1.8d	15d	3d	50d	45a
0.05%	55a	12d	90a	1.8d	13e	2.8e	45e	45a
(CV %)	22.15	0.34	0.023	0.40	0.013	0.021	0.021	0.043
LSD	16.39	0.056	0.024	0.011	0.027	0.015	0.015	0.024

PH= Plant Height (cm), IL= Internodes length (cm), DM= Days to Maturity, CL = Capsule Length, (cm), NCPP = Number of Capsule per plants, HSW= Hundred Seed Weigh (gram)t, DF= Days of Flowering, NSPP = Number of seeds per pod.

4.2.1.2 Effects of Hydroxylamine hydrochloride on the phenological parameters of sesame (Abasena variety)

A comparison of plant height and internodes length among the treatments and control, showed that the mean values increase at 0.01,and 0.02% concentration (Table 8). But the stimulatory effect was highly significance at 0.01% concentration when compared with control and other mutagen treatments. A similar trend was observed by Mensah *et al.* (1990), where the low dosage of hydroxylamine hydrochloride led to increased plant height in *Vigna unguiculata*, while the higher dosage decreased plant height significantly. Significant changes were observed in number of capsules per plant especially at 0.01% concentrations when it was compared with control plants and treatments.

The result indicated that the increase in internodes length and capsules per plant per plants was highly significant ($P<0.01$) at 0.01% concentration. An encouraging increase was observed at 0.01%, 0.02 and 0.03% concentration for number of capsules per plant and at 0.01%, 0.02 for internode length. ($P<0.05$).

In the higher doses of mutagenic treatments, days required for flowering and days required for maturity were found to be significantly increased , especially in 0.05% concentration it was highly significant ($P<0.01$) than control. Similar result was reported in presoaked seeds of chili variety that were treated with methyl methane sulphonate (Neeraj and Anis 1995). In addition to this use of induced mutations for obtaining early maturing cultivars has been a frequent breeding objective (Micke, 1979).

The hundred seed weight was highly significant at ($P<0.01$) in 0.01% concentration when compared with control and other concentrations. The number of capsules per plant was increased significantly ($P<0.01$) at 0.01%, 0.02% and 0.3% concentration when compared with the control. The result of this finding is similar with Singh and Raghuvanshi (1987).

Table 9: Effects of Hydroxylamine hydrochloride on the phenological parameters of Abasena variety.

Treatment	PH	IL	DM	CL	NCPP	HSW	NSPP	DF
Control	60a	11c	85b	2c	15d	3.00d	50d	44b
0.01%	64b	14a	80c	2c	17b	3.80c	50d	40c
0.02%	61a	12bc	85b	1.9c	16a	2.50a	48c	44b
0.03%	60a	11c	90d	1.8b	16a	2.40b	46c	44b
0.04%	58c	10c	92cd	1.5d	15d	2.40b	44a	45a
0.05%	56bc	9c	95e	1.5d	14d	2.20e	42e	45a
CV	0.06	0.32	0.53	11.23	0.07	0.99	0.58	0.69
LSD	0.08	0.046	0.60	0.26	0.014	0.032	0.035	0.39

PH= Plant Height (cm), IL= Internodes length (cm), DM= Days to Maturity, CL = Capsule Length, (cm), NCPP = Number of Capsule per plants, HSW= Hundred Seed Weigh (gram), DF= Days of Flowering, NSPP = Number of seeds per pod.

4.2.2. The Effects of Sodium Azide and Hydroxylamine hydrochloride on the Phenological parameters of Kelafo 74 variety.

4.2.2.1. Effects of Sodium Azide on the Phenological parameters of Kelafo 74 variety

The effects of sodium azide on the plant height of Kelafo 74 variety was observed at 0.01, 0.02% and 0.03 concentrations and highly significant difference at ($P<0.01$) was observed when compared with other concentrations and control plants. The inhibitory effects were concentration dependent (Table 10)

There was no significant difference observed in internodes length of different concentration mutagenic treatments and control and that of number of capsules per plant, number of seeds per pod, capsule length and hundred seed weight exhibited totally a negative mean shift.
But Singh et *al*. (2000) reported increase in variability for number of pods per plant following mutagenic treatments of sodium azide in *Vigna mungo*.

The experimental results showed favorable effects on days to flowering and days to maturity was decreased in 0.02 and 0.01% respectively and highly significant changes was observed at ($P<0.01$) .It gives a clue for selecting early maturing mutants.

Table 10: Effects of Sodium Azide on the phenological parameters of Kelafo 74 variety.

Treatment	PH	IL	DM	CL	NCPP	HSW	NSPP	DF
Control	86d	9c	85e	1.8a	12a	2.5a	45a	40c
0.01%	88c	9cd	87d	1.6b	10ab	2.4ab	45a	40c
0.02%	90b	9.5b	90c	1.6b	10ab	2.4ab	43b	38d
0.03%	95a	9a	93b	1.5c	9bc	2.3bc	40c	40c
0.04%	85d	9c	95a	1.4d	8c	2.2c	39d	42b
0.05%	80e	8.5d	95a	1.3e	6d	2d	35e	44a
CV	0.70	2.95	0.38	2.99	10.54	3.46	1.79	0.90
LSD	0.78	0.35	0.45	0.06	1.23	0.10	0.94	0.47

PH= Plant Height, IL= Internodes length, DM= Days to Maturity, CL = Capsule Length, NCPP = Number of Capsule per plants, HSW= Hundred Seed Weight, DF= Days of Flowering, NSPP = Number of seeds per pod .

4.2.2.2. Effects of Hydroxylamine hydrochloride on Phenological parameters of Kelafo 74 variety

The effects of hydroxylamine hydrochloride to induce polygenic variability with respect to plant height, was observed in all the treatments,and it was significant at 0.02% of mutagen treatment. Athwal *et al*., (1970) created variability in plant height in chickpea through gamma radiation. Variability in plant height was observed through EMS treatments in *Capsicum annum* (Jabeen and Mirza, 2002) which supports the present study. Jamil and Khan (2002) found that the radiation doses of 5, and 10 Krad has slightly reduced plant height while other dose had no considerable effect on plant height. Chen and Gottschalk (1970) and Okuno and Kawai (1978) have reported that mutations were affecting the plant height. Wheras Days to maturity has shown a negative mean shift in all the treatments while the days to flowering show a significant positive mean shift Only at 0.02% in comparison to the control and the rest of the treatments.The capsule length, number of capsule per plant and hundred seed weight was decreased with increasing the concentration of the treatment. The results indicated that mutagens could cause both positive and negative genetic variability in plant height and the rest of polygenic parameters. (Table 11).

Table 11: Effects of Hydroxylamine hydrochloride on phenological parameters of Kelafo 74 varieties.

Treatment	PH	IL	DM	CL	NCPP	HSW	NSPP	DF
Control	86a	9c	85d	1.8a	12a	2.5a	45a	40c
0.01%	86a	9c	87e	1.6b	10ab	2.4ab	45a	40c
0.02%	90c	9.5b	90a	1.6b	10ab	2.4ab	45a	36d
0.03%	85b	9c	90a	1.4e	8c	2.2bc	39b	41c
0.04%	83e	8.7a	92b	1.4e	7cd	2.2bc	39b	43bc
0.05%	80d	8.5ab	93bc	1.2c	7cd	2.0d	35e	45a
CV	0.24	3.85	0.18	5.12	3.38	2.02	0.48	0.50
LSD	0.27	0.45	0.20	0.10	0.40	0.06	0.26	0.27

PH= Plant Height, IL= Internodes length, DM= Days to Maturity, CL = Capsule Length, NCPP = Number of Capsule per plants, HSW= Hundred Seed Weight, DF= Days of Flowering, NSPP = Number of seeds per pod.

Table 12 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on plant height of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	12186.46	12186.47	110514	0.0001
Replication	2	0.97	1.94	8.79	0.0016
Treatment	11	67.99	747.99	616.66	0.0001
Replication*Variety	2	0.86	1.74	7.85	0.0027
Treatment*Variety	11	14.03	154.42	127.30	0.0001
Replication*Treatment	22	0.10	2.40	0.99	0.5101
Error	22	0.11	2.43		
Total	71		13097.38		

Table 13 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on internode length of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	155.76	155.76	3229.84	0.0001
Replication	2	0.37	0.19	3.86	0.0366
Treatment	11	70.88	6.44	133.60	0.0001
Replication*Variety	2	0.3257333	0.16	3.38	0.0526
Treatment*Variety	11	37.28	3.39	70.26	0.0001
Replication*Treatment	22	1.21	0.05	1.13	0.3856
Error	22	1.061	0.04		
Total	71	266.87			

Table 14 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on days to maturity of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	68.41	68.41	529.25	0.0001
Replication	2	0.60	1.21	4.66	0.0205
Treatment	11	52.25	574.80	404.28	0.0001
Replication*Variety	2	0.17	0.34	1.29	0.2959
Treatment*Variety	11	61.07	671.80	472.51	0.0001
Replication*Treatment	22	0.14	3.071	1.08	0.4294
Error	22	0.13	2.84		
Total	71		1322.45		

Table 15 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on capsule length of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	2.77	2.77	249.62	0.0001
Replication	2	0.01	0.0023	0.10	0.9025
Treatment	11	0.25	2.79	22.87	0.0001
Replication*Variety	2	0.04	0.078	3.47	0.0488
Treatment*Variety	11	0.06	0.71	5.77	0.0002
Replication*Treatment	22	0.01	0.29	1.20	0.3387
Error	22	0.011	0.24	0.012	
Total	71		6.88		

Table 16 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on number of capsules per plant of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	847.35	847.35	3180.42	0.0001
Replication	2	1.66	3.33	6.24	0.0071
Treatment	11	15.37	169.16	57.72	0.0001
Replication*Variety	2	1.54	3.09	5.80	0.0095
Treatment*Variety	11	5.89	64.82	22.12	0.0001
Replication*Treatment	22	0.27	6.06	1.03	0.4693
Error	22	0.27	5.86		
Total	71		1099.67		

Table 17 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on hundred seed weight of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	6.12	6.11	2444.49	0.0001
Replication	2	0.0001	0.005	0.02	0.9802
Treatment	11	5.27	0.48	191.71	0.0001
Replication*Variety	2	0.00023	0.0001	0.05	0.9545
Treatment*Variety	11	2.58	0.23	93.82	0.0001
Replication*Treatment	22	0.052	0.0023	0.94	0.5565
Error	22	0.055	0.0025		
Total	71	14.062			

Table 18 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on the number of seeds per pod of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	1619.75	1619.76	11068.5	0.0001
Replication	2	0.0044	0.0089	0.03	0.9704
Treatment	11	141.22	1553.46	965.04	0.0001
Replication*Variety	2	0.014	0.0289	0.10	0.9065
Treatment*Variety	11	67.20	739.29	459.26	0.0001
Replication*Treatment	22	0.14	3.02	0.94	0.5590
Error	22	0.15	3.22		
Total	71		3918.78		

Table 19 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on days to flowering of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of Squares	F- value	P- value
Variety	1	153.13	153.13	1873.36	0.0001
Replication	2	0.45	0.90	5.50	0.0116
Treatment	11	20.85	229.38	255.11	0.0001
Replication*Variety	2	0.13	0.27	1.63	0.2192
Treatment*Variety	11	7.23	79.38	88.28	0.0001
Replication*Treatment	22	0.06	1.34	0.74	0.7565
Error	22	0.082	1.80		
Total	71		466.17		

Table 20 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on plant height of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	12186.46	12186.47	110514	0.0001
Replication	2	0.97	1.94	8.79	0.0016
Treatment	11	67.99	747.99	616.66	0.0001
Replication*Variety	2	0.86	1.74	7.85	0.0027
Treatment*Variety	11	14.03	154.42	127.30	0.0001
Replication*Treatment	22	0.10	2.40	0.99	0.5101
Error	22	0.11	2.43		
Total	71		13097.38		

Table 21 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on plant height of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	155.76	155.76	3229.84	0.0001
Replication	2	0.37	0.19	3.86	0.0366
Treatment	11	70.88	6.44	133.60	0.0001
Replication*Variety	2	0.3257333	0.16	3.38	0.0526
Treatment*Variety	11	37.28	3.39	70.26	0.0001
Replication*Treatment	22	1.21	0.05	1.13	0.3856
Error	22	1.061	0.04		
Total	71	266.87			

Table 22 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on days to maturity of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	68.41	68.41	529.25	0.0001
Replication	2	0.60	1.21	4.66	0.0205
Treatment	11	52.25	574.80	404.28	0.0001
Replication*Variety	2	0.17	0.34	1.29	0.2959
Treatment*Variety	11	61.07	671.80	472.51	0.0001
Replication*Treatment	22	0.14	3.071	1.08	0.4294
Error	22	0.13	2.84		
Total	71		1322.45		

Table 23 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on capsule length of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	2.77	2.77	249.62	0.0001
Replication	2	0.01	0.0023	0.10	0.9025
Treatment	11	0.25	2.79	22.87	0.0001
Replication*Variety	2	0.04	0.078	3.47	0.0488
Treatment*Variety	11	0.06	0.71	5.77	0.0002
Replication*Treatment	22	0.01	0.29	1.20	0.3387
Error	22	0.011	0.24	0.012	
Total	71		6.88		

Table 24 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on number of capsules per plant of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	847.35	847.35	3180.42	0.0001
Replication	2	1.66	3.33	6.24	0.0071
Treatment	11	15.37	169.16	57.72	0.0001
Replication*Variety	2	1.54	3.09	5.80	0.0095
Treatment*Variety	11	5.89	64.82	22.12	0.0001
Replication*Treatment	22	0.27	6.06	1.03	0.4693
Error	22	0.27	5.86		
Total	71		1099.67		

Table 25 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on hundred seed weight of sesame varieties.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F- value	P- value
Variety	1	6.12	6.11	2444.49	0.0001
Replication	2	0.0001	0.005	0.02	0.9802
Treatment	11	5.27	0.48	191.71	0.0001
Replication*Variety	2	0.00023	0.0001	0.05	0.9545
Treatment*Variety	11	2.58	0.23	93.82	0.0001
Replication*Treatment	22	0.052	0.0023	0.94	0.5565
Error	22	0.055	0.0025		
Total	71	14.062			

Table 26 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on the number of seeds per pod of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of squares	F- value	P- value
Variety	1	1619.75	1619.76	11068.5	0.0001
Replication	2	0.0044	0.0089	0.03	0.9704
Treatment	11	141.22	1553.46	965.04	0.0001
Replication*Variety	2	0.014	0.0289	0.10	0.9065
Treatment*Variety	11	67.20	739.29	459.26	0.0001
Replication*Treatment	22	0.14	3.02	0.94	0.5590
Error	22	0.15	3.22		
Total	71		3918.78		

Table 27 ANOVA of the effects of sodiumazide and hydroxylamine hydrochloride on days to flowering of sesame varieties.

Sources of variation	Degrees of freedom	Mean square	Sum of Squares	F- value	P- value
Variety	1	153.13	153.13	1873.36	0.0001
Replication	2	0.45	0.90	5.50	0.0116
Treatment	11	20.85	229.38	255.11	0.0001
Replication*Variety	2	0.13	0.27	1.63	0.2192
Treatment*Variety	11	7.23	79.38	88.28	0.0001
Replication*Treatment	22	0.06	1.34	0.74	0.7565
Error	22	0.082	1.80		
Total	71		466.17		

4.3 Effectiveness of the Chemical Mutagens

4.3.1 Effectiveness of the chemical mutagens on Abasena variety

The mutagenic effectiveness was found to be the highest at lower concentration with all the mutagenic treatments in germination percentage of Abasena varieties. The maximum effectiveness was also observed at 0.01% of sodium azide and hydroxylamine hydrochloride at (1370 and 1334.5 respectively) followed by 0.02% of sodium azide (667.25)(Table 28). The mutagenic effectiveness also found to be highest at lower concentration with all the mutagenic treatment in (days of flowering, days of maturity, plant height, and number of capsule per plant, hundred seed weight and internode length).The maximum effectiveness was observed at 0.01% of sodium azide at (1370) followed by 0.01% of hydroxylamine hydrochloride (1334.5) in germination percentage. In days of maturity the maximum effectiveness was observed in sodium azide at (1416.67) followed by hydroxylamine hydrochloride by (1333.33). This was in confirmation with the findings of Packiaraj (1988) in cowpea and Sharma *et al*. (2005) in black gram.

Table 28: The Mutagenic effectiveness of sodium azide and hydroxylamine hydrochloride on selected Biometrical traits of Abasena variety

Treatments	G (%)	DF	DM	PH	NCPP	HSW	IL
S.A 0.01%	1370	733.33	1416.67	1016.67	300	53.33	200
0.02%	667.25	333.33	666.67	550	166.67	30.83	125
0.03%	402.78	244.44	472.22	338.89	94.44	19.44	77.78
0.04%	248.75	187.5	375	250	62.5	12.5	54.17
0.05%	175.43	150	300	183.33	43.33	9.33	40
HA 0.01%	1334.5	666.67	1333.33	1066.67	283.33	46.67	200
0.02%	666.67	366.67	708.33	508.33	133.33	20.83	125
0.03%	410.56	244.44	500	333.33	88.89	13.33	77.78
0.04%	255.42	187.5	383.33	241.67	62.5	10	54.17
0.05%	173.43	150	316.67	186.67	46.67	7.3	40

SA = Sodium azide
HA = Hydroxylamine hydrochloride
G = Germination
NCPP = Number of capsules per pod
DF = days to flowering
HSW = Hundred seed weight
DM = Days to maturity
IL = Intenode length
PH = Plant height

4.3.2. Effectiveness of the chemical mutagens on Kelafo 74 variety

The values of mutagenic effectiveness for hydroxylamine hydrochloride and Sodium Azide follow a dose related decreasing fashion in kelafo 74 varieties. Accordingly the effectiveness of germination percentage, days to maturity, number of pods per plants, hundred seed weight and number of capsules per pod were found to be the highest at lower concentration with all the mutagenic treatments. Accordingly, the maximum mutagenic effectiveness for germination percentage was observed in hydroxylamine hydrochloride at 0.01% (1333.33) followed by sodiumazide at 0.01% (1334.5). In the case of days to maturity and pods per plant, the maximum effectiveness was observed in both chemicals for days to maturity at 0.01 % (1450), and in number of capsule per plants similar result was observed in both sodium azide at 0.01 % (166.67). Regarding hundred seed weight the maximum effectiveness was seen in hydroxyl amine hydrochloride at 0.01% by 40. (Table 29)

Many workers have recorded the effectiveness/efficiency values to be higher at lower dose of gamma rays, EMS and HZ (Gaul, 1962; Siddiq and Swaminathan, 1968; Nerkar, 1977; Hakande, 1992, More 1992, Satpute 1994). Panchabhaye (1997), Kashid (2004) and Khadke (2005) proposed that the relative higher efficiency at lower concentration/dose of the mutagen could be ascribed to the lesser percentage of injury at such doses.

General decrease in effectiveness with increasing doses of Gamma rays has been reported in foxtail millet (Gupta & Yashvir 1975), lentil (Sharma 1990) and mungbean (Solanki 1999).

In the present study, it was also observed that effectiveness reduced with an increase in concentration in both the varieties of sesame. Higher mutagenic effectiveness and efficiency were observed in *Lathyrus sativus* at lower concentrations of EMS treatments by Waghmare and Mehra (2001) and Kumar *et al*. (2003).

Table 29: The Mutagenic effectiveness of Sodium azide and Hydroxylamine hydrochloride on selected Biometrical traits of Kelafo 74 variety

Treatments	G (%)	DF	DM	PH	NCPP	HSW	IL
S.A 0.01%	1333.33	666.67	1450	1466.67	166.67	40	150
0.02%	565.83	316.67	750	750	83.33	20	79.17
0.03%	376.67	222.22	516.67	527.78	50	12.78	55.56
0.04%	274.58	175	395.83	354.17	33.33	9.17	37.5
0.05%	197.33	146.67	316.67	266.67	20	6.67	28.33
HA 0.01%	1334.5	666.67	1450	1433.33	166.67	40	150
0.02%	665	300	750	750	83.33	20	79.17
0.03%	405.56	227.78	500	472.22	44.44	12.22	50
0.04%	250	179.17	354.17	345.83	29.17	9.17	36.25
0.05%	176.33	150	276.67	266.67	23.33	6.67	28.33

SA = Sodium azide
HA = Hydroxylamine hydrochloride
G = Germination
DF = Days to flowering
IL = Internode length
PH = Plant height
DM = Days to maturity
HSW = Hundred seed weight
NCPP = Number of capsules per pod

Kundi *et al*. (1997) reported differential sensitivity within crop and even genotype. Such difference in effects of mutagen on different materials might be due to seed metabolism and onset of DNA synthesis. It was opined that the sensitivity depends upon the genetic architecture and mutagens employed (Blixt, 1970) besides the amount of DNA, its replication time in initial stages and degree of heterochromatin. The efficiency of a mutagenic agent is of complex nature, as it not only depends on reactivity of agent with the material and on its applicability through which physiological damage, chromosomal aberrations and pollen sterility gets induced in addition to mutation (Tariq 2008).

5. SUMMARY AND CONCLUSION

Sesame (*Sesamum indicum L.*), is one of the most important oilseed crops because of its nutritional composition and health related value. Its production is dispersed all over the world and throughout Ethiopia. In the contemporary scenario of globally urgent demand for increasing food production which is still impeded by different biotic and abiotic factors, mutation breeding or (the Non – GMO Approach) is optimistic genetic tool at hand in achieving the goal of ample food production. Also in the Ethiopian context one of the core and timely crucial policy areas is food security with the aim to transform the nation into food self- sufficiency.

With this connection a research was carried out in the laboratory as well as a green house to perform germination test and screening for the variations in agro morphological parameters and evaluating the effects of the chemo mutagens employed. The experiment was laid out in a factorial completely randomized design with three replications. Two varieties i.e. Abasena and Kelafo 74 and two chemicals i.e. Sodium azide and hydroxylamine hydrochloride were utilized for the experiment.

Analysis of variance in Bioassay studies showed highly significant difference in germination percentage of abasena and kelafo 74 varieties under the treatment of sodium azide and hydroxyl amine hydrochloride compared to the respective controls. In Abasena variety the root and shoot growth was stimulated in 0.02 and 0.01% of sodium azide respectively and regarding hydroxylamine hydrochloride only in 0.01 %. Whereas in the variety kelafo both the root and shoot were stimulated in 0.02% of sodium azide and 0.01 % in the application of hydroxyl amine hydrochloride.

On the other side statistical inference done on the quantitative traits in a greenhouse study reflected that in the variety Abasena plant height , number of seeds per pod, internode length, capsule length and number of capsules per plant were stimulated at 0.02% of sodium azide and the days to maturity and flowering were reduced at this same concentraton.

Whereas in hydroxylamine hydrochloride hundred seed weight was increased, plant height, internode length and number of capsules per plant were stimulated and the days to maturity and flowering were reduced at 0.01% against the respective controls.

In the variety kelafo 74 internode length and plant height were stimulated at 0.02 and 0.03% of sodium azide respectively and the days to flowering was reduced at 0.02% and using hydroxylamine hydrochloride plant height and internode length were stimulated at 0.02% and at this same concentration the days to flowering was significantly reduced.

Regarding the effectiveness of the chemo mutagens, in the bioassay studies of abasena variety treated with sodium azide the maximum effectiveness was observed in 0.01% at (1370) and that of hydroxylamine hydrochloride was also in the same concentration at (1334.5). Concerning the quantitative parameters under consideration i.e. Plant height, days to flowering , days to maturity, number of capsules per plant, hundred seed weight and internode length the maximum is at 0.01% at (733.33, 1416.67,300,53.33 and 200) respectively.

Again in the kelafo 74 variety treated with sodium azide in the germination test the upper limit was found in the 0.01% at (1333.33) and also considering the same quantitative traits mentioned above maximum effectiveness was exhibited in 0.01% at (1334.5). The same trend was followed with the treatment of hydroxylamine hydrochloride except the difference in magnitude (1334.5 in the upper limit and 176.33 for the lower limit).

Hence it can be concluded that utilizing these chemo mutagens it is possible to foster genetic variability in a tangible way and here sodium azide is more efficacious than hydroxylamine hydrochloride. Generally speaking the mutagenic efficiency is inversely proportional to the concentration gradient of the mutagens.

Overall results indicated the possibility of obtaining sesame varieties which display more agrometrical traits variation than their parents. But the produced mutants from first generation are not adequate for studying the genetic stability, so these traits should be investigated for the desired traits in subsequent generations and in field conditions.

The mutagen tretments that enhanced a positive mean shift in polygenic traits need to be selected and investigated further.

Also, Inheritance of all the mutants has to be studied for their exploitation in improvement of sesame and all the obtained mutants can be used as genetic stocks.

Further, mutation breeding of sesame tolerant for the tremendous biotic and abiotic stresses, enhancing the biochemical properties of its seed,incorporation of isolated mutants into cross breeding programs and support and integration of the present work with the recent biomolecular techniques should be future prospects.

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