INFLUENCE OF MUTATION ON STABILITY ANALYSIS AND PERFORMANCE OF SOYBEAN (*Glycine max* L.) VARIETIES FOR YIELD AND QUALITY TRAIT UNDER PUNJAB CONDITION

Thesis Submitted for the award of the Degree of

DOCTOR OF PHILOSOPHY

in

Botany

By

Anas Hamisu

Registration Number: 12116810

Supervised by
Dr. Bhupendra Koul
ID:18673
Department of Botany
Professor
Lovely Professional University

Co-Supervised by
Dr. Anita Jaswal
ID:27139
Department of Agronomy
Assistant Professor
Lovely Professional University

Transforming Education Transforming India

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2026

Dedicated
to my beloved
Late Father and Entire
Family

DECLARATION

I, hereby declare that the present work in the thesis entitled "**Influence of mutation on stability analysis and performance of Soybean (*Glycine max* L.) varieties for yield and quality trait under Punjab condition"** in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Bhupendra Koul**, working as Professor, in the Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

Signature of Scholar

Name of the scholar: Anas Hamisu

Registration No.: 12116810

Department/school: Botany/Bioengineering and Biosciences

Lovely Professional University, Punjab, India

Date: 5/3/2026

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Influence of mutation on stability analysis and performance of Soybean (*Glycine max* L.) varieties for yield and quality trait under Punjab condition"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in Botany, School of Bioengineering and Biosciences, is a research work carried out by **Anas Hamisu**, reg. no.: 12116810, is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

Signature of Supervisor

Name of supervisor: Dr. Bhupendra Koul
Designation: Professor
Department of Botany
School of Bioengineering & Biosciences,
Lovely professional University, India
Date: 5/3/2026

ACKNOWLEDGEMENTS

First and foremost, all praises are due to Allah, the Lord of all and cherisher of the universe, who in his infinite grace and mercy bestowed me with health and capacity to carry out this research work. My profound gratitude and deep appreciations go to my supervisor, **Dr. Bhupendra Koul,** Professor, Department of Botany, Lovely Professional University, Jalandhar, Punjab, for his wisdom, patience, and intellectual guidance have been indispensable throughout this process. From the early conceptualization of this research to the final stages of writing, he has provided invaluable advice and mentorship. Your unwavering support, constructive feedback, and belief in my potential have pushed me to expand my thinking and produce work of which I am truly proud. I am fortunate to have had the opportunity to work under his supervision.

This page cannot be complete without mentioning the marvelous assistance and contributions of my co-supervisor **Dr. Anita Jaswal**, Assistant professor, Department of Agronomy, School of Agriculture, LPU, which made this work a success.

I wish to convey my sincere thanks to **Dr. Joydeep Dutta,** Professor & Head, Department of Botany and Zoology, **Dr. Neeta Raj Sharma**, Professor & Dean, School of Bioengineering and Biosciences, for her kind support. I would also like to thank **Dr. Ashok Mittal**, Chancellor, LPU and Member of Rajya Sabha, **Mrs. Rashmi Mittal,** Pro-Chancellor LPU, **Dr. Lovi Raj Gupta**, Pro Vice Chancellor, LPU and **Dr. Monica Gulati,** Registrar, LPU, for their vision 'Think Big', motivation and support.

I am equally thankful to the entire staff of School of Bioengineering and Biosciences, specifically the research advisory committee (RAC) members for the positive criticism, to make this work a wonderful one.

Special thanks to tertiary education trust fund, Nigeria with a grant number TETF/ES/UNIV/ALIERO/TSAS/2022, for the financial support to carry out the research, without which this journey would have been impossible. The efforts of **Dr. Mustapha Abubakar** Department of Botany, **KSUSTA,** Nigeria and **Dr. Mustapha Sale Na'Allah**, Department of Crop Science, KSUSTA, Nigeria in providing technical assistance is highly appreciated.

Words may not be sufficient in describing the moral and financial assistance by my beloved late father Mr. Haruna Abdullahi), and uncle, Professor A.I. Yakubu, **Mrs. Hauwau Muhammed and Mr. Auwalu Haruna' Ubansar Daraga,** from the beginning of this academic journey up till now. I pray to God almighty to reward them abundantly for all they have done to me, forgive their short comings and make the paradise be their final abode.

Before I conclude, I have to deliver my heartfelt gratitude to all friends and well-wishers, specifically Mrs. Sabila Adamu, Mrs. Asmau Umar, Ahmed Anas, Abdulsamad Anas, Ummu Salma Anas, Aisha, Fadila, Nasiba, Rabibiatu, Lasmar Garba, Anas B Dauda and Abdulahi Muhammad Dankurmi for their prayers and support throughout this great academic journey.

Anas Hamisu

ABSTRACT

In order to evaluate the effectiveness and efficiency of mutagens to determine their usefulness in producing favorable mutations and selecting mutants with desired traits, a mutation treatment must not result in chromosomal abnormalities, bio-physiological alterations, or genotoxic consequences that lower cell viability in order to be effective. The soybean (*Glycine max* (L.) Merrill), belongs to family Leguminosae, as “golden bean” and “miracle crop” of the twenty-first century. It comprises 20% high-quality oil, 40% protein, 36–40% starch, vitamins A, B, and D, 6–7% minerals (including calcium, iron, manganese, phosphorus, copper, and thiamine), 8–10% fiber, and phytochemicals such as flavonoids, saponins, alkaloids, steroids, and tannins. Soybean is utilized in numerous food products, such as tofu, soy sauce, plant-based milk, and meat alternatives, while its oil is commonly employed for culinary purposes. Soybean meal serves as an additive in animal feed. Additionally, it is utilized in industry for the manufacture of biofuels and functional meals. Considering the diverse functions of soybean in different areas of human perspective, crop productivity needs to be enhanced globally, and particularly in the developing nations. However, there was too much dependence on cowpea (*Vigna unguiculata*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), in the study area in Punja which has led to tremendous increase in market price and these crops require too much water for irrigation and thus, soybean might serve as alternative in terms of yield, oil, protein, fiber essential amino acid, and saturated fatty acid etc.

This research was conducted in the agricultural research farm of Lovely Professional University in Punjab, India, during the years 2022/2023 and 2023/2024. The experimental design utilized a randomized complete block design (RCBD) with three replications. The goal of the experiment was to find the mutant soybean variety that produced the most yield by testing its response to three different concentrations of three chemical mutagens: sodium azide (SA), ethyl methyl sulfonates (EMS), and methyl methane sulfonate (MMS). The concentrations were 0.2%, 0.4%, and 0.6%. The data were gathered and analyzed using SPSS software (version 22) and a test. Duncan's multiple range test (DMRT) was used to separate the means at the 5% level of

significance. Among the two varieties, the highest seed germination percentage (76.0% seedlings/plot and 87% seedling/plot) was recorded in SL958 (0.4% SA) in first- and second-year trial. while the lowest (30.33% seedlings/plot and 51.00% seedlings/plot) was observed in 0.6% MMS as compared to the control (SL744:53%, SL958:76% and SL744:64%, SL958:78% at 10 days after sowing, respectively). In the first-year trial, the average plant height was observed to be highest in SL958 (0.4% SA) for the two years trial (37.84 ± 1.32 cm and 36.76 ± 1.75 cm) and lowest (20.58 ± 0.30 cm and 18.44 ± 1.65 cm) in SL744 (0.6% SA), as compared to the controls (SL958: 26.09 ± 0.62 cm and SL744: 27.48 ± 0.74 cm). SL958 variety showed the highest plant height with SA treatments especially at 0.4% concentration, while MMS-treated plants showed stunted growth at 0.6% in both the two trials (2023/2024 and 2024/2025). Untreated plants took longest time to reach 50% flowering and podding (68 days for SL744, 63 days for SL958). Early flowering occurred in plants treated with 0.4% SA (39.67 days in SL958).

Second-year trials showed similar trends, with mutagen-treated plants flowering earlier than controls (this is because soybeans are photoperiod-sensitive, they flower based on day length, and mutations might make them less sensitive to day length, leading to earlier flowering. However, hormones like auxins and gibberellins affect flowering, and changes in hormone biosynthesis or signaling pathways caused by mutagens can accelerate early flowering). SA-treated plants (0.4%) podded earlier (69 days after sowing) and more abundantly than untreated controls (82 days after sowing), while SL744 podded in 77 days (compared to 86 days for the control). EMS and MMS delayed these processes, with the highest delays observed in the 0.6% MMS treatment. Mutagen treatment affected the pod and seed length and seed count. SL958 treated with 0.4% SA produced the longest pods (4.7 cm) and the highest number of seeds per pod (4.04 seeds). SL744 had shorter pods and fewer seeds per pod at higher concentrations of mutagens (0.6% MMS).

Pollen grains from mutagen treated plants showed significant morphological diversity, with 0.4% EMS and 0.4% SA treatments producing healthier, more regular grains compared to those received from 0.6% MMS treatment.

The average leaf count at 12 weeks after sowing was highest (234.33 ± 3.09 tetrafoliate leaves/plant) in SL958 (0.4% SA) while lowest (87 leaves/plant) in 0.6% MMS as compared to the control (SL744; 180.00 ± 1.63 and SL958; 160.73 ± 1.05). The highest total leaf area was recorded in the SL958 and SL744 M1plants which was 3625.8 ± 1.43 cm^2 and 2311.03 ± 3.65 cm^2, respectively. Seeds of the SL958 variety treated with 0.4% SA resulted in the development of tetrafoliate leaves with a broad leaf base and the maximum yield (277.55 ± 1.37 pods/plant) compared to the narrow pentafoliate leaves obtained through the treatment with EMS. However, in the SL744 variety, the same treatment led to tetrafoliate leaves with a comparatively lower yield of 206.54 ± 23.47 pods/plant as compared to the control (SL744 164.33 ± 8.58 and SL958 229.86 ± 0.96). Leaf variations, SA and EMS caused more favorable mutations (e.g., broad, tetrafoliate and pentafoliate leaves), while MMS led to negative leaf variations (narrow, trifoliate leaves). The highest protein content (47.04 ± 0.87% TSP) was recorded in the SL958 (0.4% SA) M_2 seeds followed by a content of 46.14 ± 0.64% TSP in the SL744 (0.4% SA) M_2 seeds, whereas the lowest content (38.13 ± 0.81% TSP) was found in SL958 (0.6% MMS). The highest protein percentage was recorded in T9 (0.4% SA) for both SL958 and SL744, while the lowest was observed in T_7 (0.6% MMS). Control treatments displayed lower protein content than those subjected to T_9 treatment, outperforming all other treatments across both years and varieties. Lipid percentage was significantly influenced by the mutagens, with the highest values seen in T9 (0.4% SA) in both varieties. The lowest lipid content was consistently recorded in T_7 (0.6% MMS) during the first-year trial 2023. In the second year, T_9 remained the top performer with a significant lipid increase compared to controls, while T_7 continued to show the lowest lipid content. The result showed that, T9 (0.4% SA) also exhibited the highest fiber content across both years and varieties, while T_7 (0.6% MMS) exhibited the lowest fiber percentage. SL958 generally had better fiber content than SL744. Phytochemical analysis, reveal that both soybean varieties showed the presence of key phytochemicals, such as alkaloids, the highest values (81.34) were treated with 0.4% AS, and the lowest values (21.38) were treated with 0.2% MMS, flavonoids, the highest values (227.17) were treated with 0.2% EMS, and the lowest values (106.36) were treated with 0.4% MMS, saponins, the highest values (86.58) were treated with 0.6% MMS, and the lowest values (35.59) were treated with 0.6% EMS and tannins, the highest values (94.39) were treated with 0.4% EMS, and the lowest values (68.44) were treated with 0.6% SA across all treatments including control SL744 (76.63) and

SL958 (84.56).

It was found that the 0.4% SA treatment in SL958 proved to be efficient in generating the highest leaf area (tetrafoliate and pentafoliate leaves), early flowering, podding and maturing, and the highest harvest index. It also improved biochemical traits like protein, lipid, and fiber, and it produced the highest yield of M1 and M2 plants (the first and second generations after mutation).

In contrast, MMS, particularly at 0.6%, had the most negative effects on plant growth, yield, and biochemical properties in both varieties (SL744 and SL958) of M_1 and M_2. This study demonstrates that targeted mutagenesis, especially with sodium azide, can be a powerful tool for enhancing soybean yield and quality, contributing significantly to agricultural productivity. Therefore, it could be recommended that chemical mutagens be used as a substitute for physical mutagens to improve soybean production in the area.

Future breeding programs should focus on stabilizing these mutants (M_3 and subsequent generations) to develop commercially viable, high-yield varieties with enhanced protein, lipid, and fiber content. The molecular basis of mutations induced by SA, EMS, and MMS should be explored. Identifying key genes responsible for early flowering, increased pod production, and biochemical enhancements can provide valuable insights for soybean improvement through gene editing or marker-assisted selection. Changes in hormonal pathways (auxins, gibberellins) cause plants to flower and pod early. More research into how mutagens affect these pathways could lead to the development of hormone-modifying treatments that optimize growth and yield in other crops. Continued research in this direction will contribute to global food security and agricultural innovation.

Preface

Soybean (*Glycine max*) is one of the most significant crops globally, revered for its exceptional nutritional, industrial, and agricultural value. As a leguminous plant, it serves dual purposes, as it is a vital source of high-quality protein and oil for human consumption while also enriching soil fertility through nitrogen fixation. Soybean's versatility extends beyond food products, finding applications in biofuels, pharmaceuticals, and eco-friendly materials, making it indispensable in modern agriculture and industry.

Despite its global prominence, soybean production faces challenges such as climate change, pest resistance, and limitations in genetic diversity. In response to scientific advancements (Geographic Information System, Genetically Modified, Phytohormones, Physical mutagens and chemical mutagens, has emerged as promising tools to enhance its Soybean growth, resilience, and nutritional profile. Mutagens, whether physical (e.g., radiation) or chemical (e.g., EMS, MMS, SA etc.), are instrumental in inducing genetic variation, offering opportunities for improving phenological, physiological, and biochemical traits in crops.

This research explores the multifaceted effects of mutagens on soybean, including their impact on phenology, meiotic processes, and yield parameters. By investigating how specific concentrations of mutagens influence growth, development, and nutrient enhancement, the study aims to unlock new strategies for optimizing soybean cultivation. The insights gained will contribute to addressing the ever-growing demand for sustainable and nutritionally enriched soybean varieties in the study area.

This preface underscores the importance of soybean research and sets the stage for a comprehensive study of its interaction with mutagens, promoting agricultural science innovation.

The present thesis entitled **"Influence of mutation on stability analysis and performance of soybean (*Glycine max* L.) varieties for yield and quality traits under Punjab condition"** encompasses the details of the studies undertaken and analyses of results obtained under 6 major chapters as described below:

Chapter 1 - Introduction: This chapter includes a brief introduction of the soybean plant, statement of the research problems, justification/research rationales, scope of the study, hypothesis, research aims and objectives.

Chapter 2 - Review of Literature: This chapter summarizes the different works undertaken on the soy bean origin and distribution, botanical description, the review on the physical and chemical mutagens, impact of mutagens on biological parameters, impact of mutagens on chromosomes, application of chemical mutagens and crop improvement.

Chapter 3 - Methodology: This chapter includes the, procedures for soybean seed treatments with mutagens, **experimental Design,** quantitative and qualitative parameters, pollen grain analysis and biochemical analysis.

Chapter 4. - Results and Discussion: This chapter includes the results and discussion related to the studies on influence of mutation on stability analysis and performance of soybean varieties for yield and quality traits under Punjab condition.

Chapter 5 - Summary Conclusions and Recommendation

The study that has been presented in this thesis and the findings are summarized in this chapter.

Chapter 6 - Bibliography: This chapter contains citations of references used in the present investigation.

Chapter 6 - Appendix: This chapter contains comprehensive list of publication, conferences and workshops attended.

Anas Hamisu

List of abbreviations

SPSS	Statistical package for social sciences
SD	Standard deviation
SEM	Standard error mean
Cm	Centimeter
cm^2	Centimeter square
CD	Critical difference
DF	Degree of freedom
CRD	Completely randomized design
G	Gram
IU	International unit
Kg	Kilogram
L	Liter
Ha	Hectare
H	Hour
DAS	Days after the sowing
FW	Fresh weight
DW	Dry weight
LA	Leaf area
M	Meter
Min	Minutes
Mg	Milligram
mL	Milli liter
mL/L	Mill liter per liter
NS	Non-significant
pH	Hydrogen iron concentration
RBD	Randomized block design
SEM	Scanning electron microscopy
UV	Ultra-violet
V/V	Volume by volume
w/v	Weight by volume
°C	Degree celsius
%	Percentage
SA	Sodium azide
EMS	Ethyl methane sulfonate
MMS	Methyl methane sulfonate
WAS	Week after sowing
SPAD	Soil Plant Analysis Development
TSP	Total soluble protein

Table of contents

List of Tables

List of Figures

CHAPTER 1

Introduction

1.0. INTRODUCTION

1.1. Background of the study

The term plant mutation refers to a rapid heritable alteration in the genetic material of a plant at both the gene and chromosomal levels. Scientists use mutations to study the features and functions of genes that control both qualitative and quantitative traits in plants. These traits include disease resistance, early maturation, and semi-dwarfism, as well as increased seed productivity, seed weight, biomass, and oil content. This makes it easier to improve the genes of crops that are important to the agriculture (Chopra 2005; Beyaz et al. 2017). Mutation breeding, also known as variant breeding, is a method for developing new crop varieties by producing genetic variability in plants using chemical or physical means (Beyaz et al. 2017).

The benefits of mutant breeding are as follows: (i) enhancing economic attributes and quality characteristics in crops in a short timeframe; (ii) conveying traits from a mutant variety to its progeny; (iii) the method is straightforward and cost- effective; (iv) dependable; and (v) safe and environmentally sustainable. The drawbacks are as follows: (i) it requires careful selection for the mutant that has the desired trait; (ii) random genetic recombination could lead to unexpected outcomes; and (iii) its effectiveness depends on the genes already in existence having the desired traits (Bado et al. 2015; Patil et al. 2020). Two types of mutagens are physical and chemical. Physical mutagens include (a) ionizing (particulate: X-rays and gamma rays; non-particulate: UV rays) and ionizing (UV rays); and (ii) chemical mutagens include (a) deamination agents like nitrous acid; (b) alkylating agents like EMS (ethyl methane sulfonate), MMS (methyl methane sulfonate), and mustard gas; (c) base analogues like 5- bromouracil and 2-aminopurine; (d) acridines like acriflavine and proflavin; and (e) other chemicals like hydroxylamine and sodium azide. Chemical mutagens are manageable, accessible, economical, efficient, and exhibit superior specificity compared to physical mutagens (Tamilzharasi et al. 2022).

However, mutagens are genotoxic which can lead to cell death in severe cases, chromosomal instability such as breakages, rearrangements, and translocations, and meiotic anomalies such as unoriented chromosomes, chromosome stickiness, and lagging chromosomes (Khan et al 2009).

The genetic makeup of economic crops has been improved by mutation breeding along with a significant increase in crop production. The improvement of economic traits and quality characteristics for the improvement of crops within a short period can be achieved with the help of induced mutagenesis. Induced morphological mutants are useful for the development of improved varieties when they are used in cross-breeding programs (Amoanimaa et al. 2022).

Despite their benefits and drawbacks, mutagens have been used to create several better cultivars of many crops, including a wheat mutant (Sharbati Sonora) of an amber-coloured grain variety with a higher protein and lysine content developed through gamma irradiation (Bakshi et al.2020); a rice mutant of the TEMB-1 variety with a semi-dwarf phenotype (90–95 cm) that matures early (130–135 days), lodging-resistant, aromatic, and has a high yield (4.5–5 tons per ha) (Lu et al.2008; Kurata et al. 2005); a barley mutant of the Diamant variety with large kernels that matures early and has high yields (Bouma et al. 1976); a soybean mutant of the TGX-1954 variety that matures early, drought tolerant, disease resistant, and has a higher yield (Jephter et al. 2017); a chickpea mutant of the BGM-413 variety that is salinity tolerant, tastes good, can be used in cooking, matures early, has a high yield, and has a high protein content (Kharkwal et al. 2005); a cotton mutant of a virescent variety with early maturation, disease resistance, drought tolerance, and a higher yield capacity (Song et al. 2015); a sugar cane mutant of the Co0238 variety that has a high fibre content (13.05%) (Ram et al. 2020); a tea mutant that is early sprouting, resistant to disease, and suitable for fine green tea developed through irradiation (Co60c) (Yang et al. 2003); and a vegetable mutant of the *Capsicum annum* variety with a high yield, increased vitamin content, and disease resistance (Devi et al. 2011; Devi et al. 2021). Thus, many features, including yield, lodging resistance, disease resistance, maturity, culm length, etc., have been developed through mutation breeding.

The soybean (*Glycine max* (L.) Merrill), family Leguminosae, widely is known as the "golden bean" and the "miracle crop" of the twenty-first century (Dilawari et al. 2022). It contains high-quality oil (20%), protein (40%), starch (36–40%), vitamins A, B, and D, 6–7% minerals (calcium, iron, manganese, phosphorus, copper, and thiamine), 8–10% fibre, and phytochemicals such as flavonoids, saponins, alkaloids, steroids, and tannins (Alghamdi et al. 2018).

In India, soybean is also used in various food products, including tofu, soya sauce, simulated milk, and meat products, and the oil is frequently used for cooking (Sedibe et al. 2023). Soybean

meal is used as a supplement in livestock feed. Apart from this, it is used in industries for the production of biofuels and functional foods (Nuthalapati et al. 2024). The industrial use of soybeans ranges from the production of yeasts and antibodies to the manufacture of soaps and disinfectants (Chandirakala et al. 2010). The top five soybean-producing nations worldwide are Brazil (120.7 MT), the United States (116.4 MT), Argentina (43.9 MT), China (20.3 MT), and India (13 MT). More than 90% of the world's production comes from these countries (Sedibe et al. 2023). India is the fifth largest soybean-producing country in the world. Madhya Pradesh, Rajasthan, and Maharashtra contribute 88% of the country's total soybean production (Chandirakala et al.2010). Soybean can play an important role in crop diversification in Punjab, provided it is able to compete with paddy, wheat, and cowpea in terms of economics. Yellow mosaic is a serious disease in the northwest's plains, causing yield losses up to 80 percent. Thus, the development of high-yielding and yellow-mosaic-resistant varieties is the main breeding objective of the soybean breeding programme at Punjab Agricultural University, Ludhiana, India. The two most recent varieties, SL958 and SL744, have been released. These have medium levels of maturity, are non-shattering, have a light-yellow grain with a grey hilum, and are resistant to the yellow mosaic virus (Gill al. 2015; Pandey et al. 2024).

Considering the diverse functions of soybean in different areas of human perspective, crop productivity needs to be enhanced globally, and particularly in the developing nations (Shea et al.2020). However, this study assesses the impact of three chemical mutagens, sodium azide, ethyl methyl sulphonates, and methyl methane sulphonate, on the two aforementioned soybean varieties through field trials, with the aim of unveiling their potential to generate genetic variability and screening the improved soybeans with a higher yield potential.

1.2. **Statement of the research problem**

In the present world there is too much dependence on cowpea (*Vigna unguiculata*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), which has led to tremendous increase in market price and soybean might serve as complement in terms of oil, protein, essential amino acid, and saturated fatty acid etc.

In the 1970s and 1980s, as reported by the International Food Policy Research Institute, Punjab was among the highest producers of soybean with a net domestic product (NDP) of 5.1–6.1 MT on average compared to the national average of 3.4 for India (Gulati 2002). Agriculture in Punjab is limited to wheat and rice, which has resulted in the excess mining of underground water resources for irrigation. Over the past 54 years, this has lowered the water table to the extent of 15 cm per year. Soybean production might serve as a supplement or as part of crop rotation, since it requires a lower amount of water.

Soybean research in terms of crop improvement is still at a low level in Nigeria compared to other grain legumes such as cowpea, groundnut, pigeon pea, and chickpeas and farmers still cultivate the traditional varieties (Adeniyan et al. 2007). In large parts of sub-Saharan Africa particularly in Northan part of Nigeria, small holder agricultural production has remained consistently low and food security is catastrophically poor. Mutation breeding is one of the plant breeding techniques used for creating genetic variability in yield contributing traits and to improve the yield of crops (Adeniyan et al. 2007).

There is need to carry out research to provide base line information on soybean varieties to the local communities. Therefore, this was undertaken to investigate the mutagenic effects and stability performance of soybean crop. There are still significant fluctuations in production that are mostly caused by inefficiencies at the farm level. However, the use of chemical mutagens is known to enhance the genetic variability of crop plants and induce mutations thereby facilitate the development of improved varieties at a swifter rate.

The study may provide information on the effect of chemical mutagens for induction of mutation. The treatments can possibly lead to an increase in beneficial genetic variability in soybeans which might be useful to plant breeders.

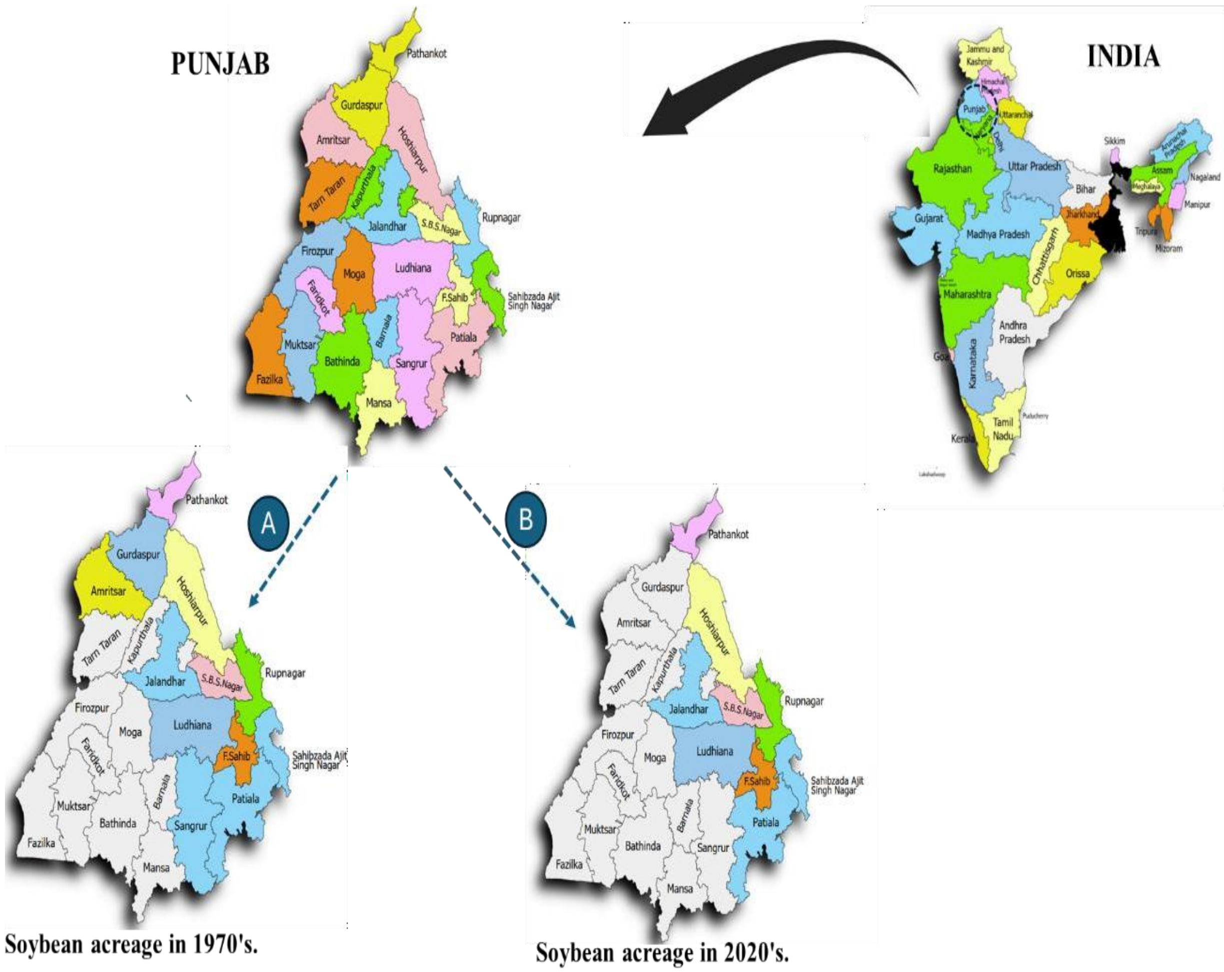


Fig 1.1. Map of soybean cultivation**. (A):** soybean acreage in 1970s; **(B):** soybean acreage in 2020s.

1.3. Soybean production in India year wise

This **fig.1.2.** provides agricultural data over 12 years (2011–2023), showing trends in area (in 1000 hectares), production (in 1000 tons), and yield (production per unit area). The area cultivated is generally increasing, starting at 10.109 (2011–2012) and peaking at 12.918 (2020–2021). The area declined slightly in 2021–2022 but rose again to 12.7 in 2022–2023. Production fluctuates significantly throughout the years. In 2012–2013, the production peaked at 12.186, accompanied by a moderate expansion of the area. Production hit a low of 6.929 in 2015–2016, despite a relatively larger area, likely due to the poor weather, pest outbreaks, or lower yield efficiency. By 2021–2022, production returned to 11.9 and 11.5 in 2022–2023. Yield was highest in 2011–2012 at 1.18 and declined thereafter, reaching a low of 0.6 in 2015–2016. Yield improves slightly in the following years but remains unstable, peaking again around 0.98 in 2016/2017 and 2018–2019. By 2022–2023, the yield is at 0.91, a gradual recovery but still below the initial levels.

This data implies fluctuations in agricultural efficiency, likely due to environmental factors, policy changes, or farming practices. Sustained recovery in yield is necessary for improved agricultural sustainability.

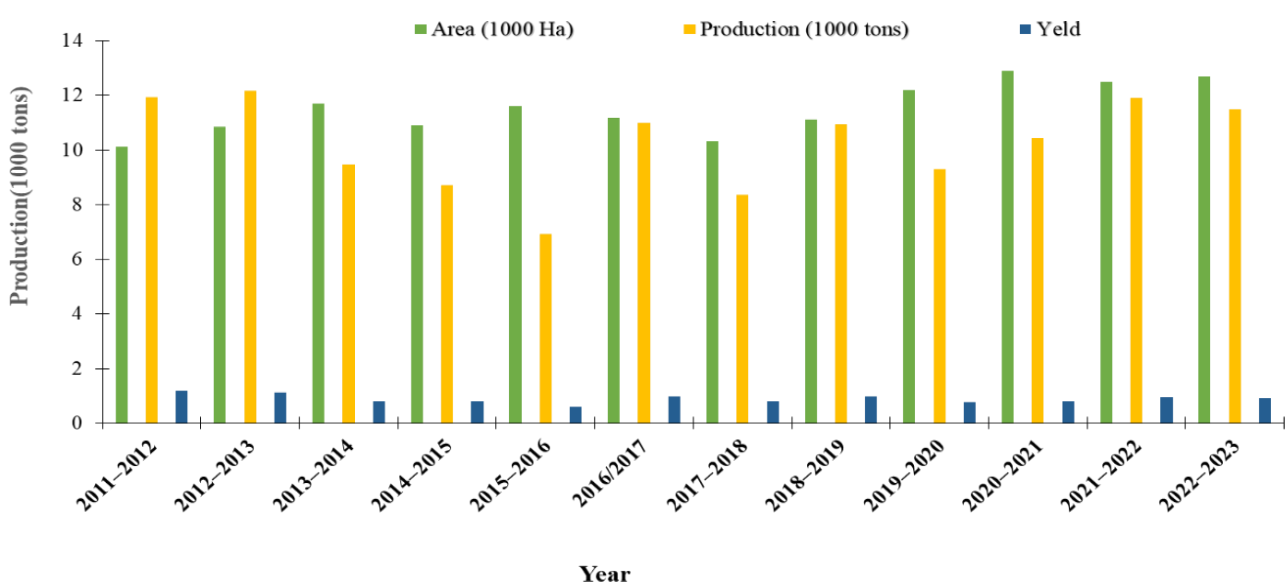


Fig 1.2. Soybean production year wise.

1.4. Justification/Research rationales

The selection of soybean among other crops is essential due to its significant oil-rich seeds and protein content for human and animal consumption. The significant crop, which faces a critical production constraint due to low quality seed, insect pest and seed shattering prior to harvest, has prompted enquiries regarding methods to keep seeds within the pods in the field before harvesting. Various mutagens have been employed for mutagenesis in soybean, and their effects have been documented (Khan and Tyagi 2013). Mutant soybean types must be produced to develop enhanced cultivars (Espina et al. 2018). Mutagenesis in this crop enhances soybean variability, thereby increasing the likelihood of develop new mutants that are beneficial for breeding programs focused on enhancing the agronomic performance of the seeds (Nobre et al. 2019). Chemical mutagens have been used to induce mutations, leading to mutant varieties exhibiting enhanced phenotypic differences, particularly mutants with superior agromorphological traits (Espina et al. 2018). Identifying, producing, and employing cultivars with pod shattering resistance, high yield, high protein content, and disease resistance, helps mitigate yield losses (Kataliko et al. 2019). The widespread use of soybeans and their five co-products (tofu, soymilk, soynut, miso, natto and soy sauce) has increased the necessity of generating new cultivars with optimal traits (Nobre et al. 2019). The study might provide information on the effects of chemical mutagens such as methyl methane sulphonate, ethyl methyl sulphonates, and sodium azide in soybean (SL958 and SL744) varieties. The treatments (0.2%, 0.4%, and 0.6%) may increase beneficial genetic variability in soybean plants, which could be useful to plant breeders. This method is meant to create genetic variability, high protein content, stop soybean pods from breaking and disease resistance, which will help farmers increase plant yield and productivity while also promoting and improving the marketing of quality indigenous products both in the India specifically Punjab State and abroad.

Therefore, this present study needs to be undertaken to investigate the mutagenic effects as a means of increasing the beneficial variability and nutrient content in soybean plants.

1.5. Scope of the Study

Field trials were used in the current study to assess how chemical mutagens affected different varieties of soybean (SL744 and SL958). The findings of this study could have a significant impact on society, especially Punjabi farmers, as they could reduce reliance on cowpea, rice, and wheat. To best of my knowledge, no research has been conducted on the influence of mutation on stability analysis and performance of soybean varieties for yield and quality traits under Punjab condition within the study area.

1.6. Research aims and objectives

The study aims to determine the potential of chemical mutagens (EMS, MMS and SA) in generating genetic variability that may be used in developing an improved soybean variety with high yield potential.

1.7. The specific objectives were:

1. To evaluate the phenological parameters of soybean under the effect of mutagens.
2. To evaluate the effect of different concentrations of mutagens on meiotic division in the pollen grain of soybean.
3. To determine the effect of different concentrations of SA, EMS and MMS on the quantitative and qualitative parameters of soybean at harvest.
4. To evaluate the concentration at which mutagens enhanced nutrient in soybean.

CHAPTER 2

Review of literature

2.0. REVIEW OF LITERATURE

2.1. Soybean origin and distribution

The soybean (*Glycine max* L. Merr), is a very nutritious and legume crop. In terms of hierarchy, soybeans are the most important source of both protein and oil. It contains the highest protein among food legumes, and comes second to groundnuts in terms of oil content (Alghamdi 2004; Fekadu et al. 2009; Mishra et al. 2024).

It is native to North and Central China; soybeans are frequently regarded as one of the earliest crops to be grown (Hymowitz 1970). The emperor 'Sheng Nung, of China first mentioned soybeans in a collection of writings called 'Pen Ts'ao Kong Mu', which detail the different flora of China and were written in the year 2838 B.C. Soybeans were first domesticated in the eastern half of China between the eleventh to seventeenth centuries B.C., according to historical and geographical evidence (Hymowitz, 1970). In 1765, soybeans were brought to the United States (Hymowitz and Harlan 1983), thereafter in 1893, they were brought to Canada, where they were first grown as hay crops in Ontario. In India soybean was introduced in 1977 by traders from Indonesia over the Himalayan routes and through Burma (now Myanmar). Consequently, soybeans have been historically cultivated on a limited scale in Himachal Pradesh, the Kumaon Hills of Uttar Pradesh (now Uttaranchal), eastern Bengal, the Khasi Hills, Manipur, the Naga Hills, and Madhya Pradesh, Punjab and Delhi (Singh 2006).

2.1.2. Botanical description of soybean (*Glycine max* (L.) Merrill)

The cultivated soybean, *Glycine max* (L.) Merr, is a diploidized tetraploid (2n = 40) belonging to the family Fabaceae, subfamily Papilionoideae, tribe Phaseoleae, and genus Glycine Wild (the ancient soybean). It is categorized as *Glycine max*. It is an upright bushy herbaceous annual that can attain a height of 1.5 meters. The primary leaves are unifoliate, opposite, and oval, while the secondary leaves are trifoliate and alternate. The nodulated root system comprises a taproot from which a lateral root system develops. Most cultivars possess tiny trichomes, however, glabrous types also exist. The papilionaceous flower comprises a tubular calyx with five sepals, a corolla of five petals (one banner, two wings, and two keels), one pistil, and nine merged stamens along with a single distinct posterior stamen. The stamens form a ring at the base of stigma and extend one day prior to pollination, during which the raised anthers form a ring around the stigma. The pod is either straight or slightly curved, measuring from two to seven centimeters in length, and comprises two halves of a single carpel connected by dorsal and ventral sutures. The seed's shape, typically oval, can differ among cultivars, ranging from almost spherical to elongated and flattened (Mishra et al. 2024).

2.1.3. Global production of soybean

The top five soybean-producing nations worldwide are Brazil (120.7 MT), the United States (116.4 MT), Argentina (43.9 MT), China (20.3 MT), and India (13 MT). More than 90% of the world's production comes from these countries (Sedibe et al. 2023). India is the fifth largest soybean-producing country in the world. Madhya Pradesh, Rajasthan, and Maharashtra contribute 88% of the country's total soybean production and 12% from others State (Chandirakala et al. 2010; Voora et al. 2024). However, the leading producers in Africa comprise Nigeria, Ethiopia, Sudan, Burkina Faso, Cameroon, Chad, Mali, and Rwanda. Nigeria holds the top position in Africa, contributing approximately 43% of the continent's overall production (FAOSTAT 2019). In Nigeria, soybean ranks as the second most significant grain legume following cowpea in terms of production, with an estimated yield of approximately 1.2 tons per hectare harvested in 2020, accounting for 43% of the total soybean production in Africa (FAOSTAT 2022; Eseigbe et al. 2024). The cultivation of soybeans in Nigeria is primarily limited to the Guinea and Sudan Savannah regions of the country. Kebbi, Sokoto, Zamfara, Katsina, Kano, and Jigawa are recognized as the leading states in soybean production. Significant production also occurs in Borno, Yobe, Kaduna, Niger, Benue, and Adamawa (Iwuchukwu et al. 2018). The production has recently extended to the middle belt to encompass other northern and southern regions of the country that were previously deemed unsuitable or marginal for cultivation (Remanandan and Asiegbu 1993; Iwuchukwu et al. 2018).

Soybean demonstrates adaptability to various environmental conditions, thriving particularly in deep, fertile, and well-drained loamy soils (Iwuchukwu et al. 2018). In Nigeria, these soils (deep, fertile, and well-drained loamy soils) are prevalent in the Northern Guinea Savannahs and the Sudan Savannah regions (Ogbonna 2011). The soybean is classified as a quantitative short-day plant, which results in a quicker flowering response under shorter day lengths (Garner and Allard 1920). Consequently, the influence of photoperiodism and temperature response plays a crucial role in identifying regions suitable for cultivar adaptation. Soybean cultivars are classified according to adaptation bands that extend in an east-west direction, influenced by latitude and day length. Thirteen maturity groups exist, ranging from MG 000 in the northern regions (45° latitude) to MG X close to the equator. In each maturity group, cultivars are categorized as early, medium, or late maturing (Garner and Allard 1920).

Table 2.1. Annual soybean production by different country (FAO STA 2023/2024).

Country	Global production (%)	Total production (2023/2024, metric tons)
Brazil	39	153 million
United State	29	113.34 million
Argentina	12	48.1 million
China	5	20.84 million
India	3	11.88 million
Paraguay	3	11 million
Canada	2	6.98 million
Russia	2	6.8 million
Ukraine	1	5.2 million
Bolivia	0.92	3.65 million
Nigeria	0.3	1.150,000 metric tons
Ethiopia	0.1	230,000 metric tons
Ghana	0.1	225,000 metric tons
Iran	0.1	165,000 metric tons

2.1.4. Climate and soil requirements in soybean production

The predominant cultivation of soybeans occurs during the main wet season (MWS), integrating effectively into an upland crop rotation alongside maize, pigeon peas, sesame, and groundnuts. Being a nitrogen-fixing legume, proper inoculation with rhizobia will result in the retention of some nitrogen in the soil for subsequent crops to utilize. The cultivation of this crop occurs in warm environments, specifically within tropical, subtropical, and temperate climates. Soybean exhibits resistance to low and extremely high temperatures; however, growth rates decline when temperatures exceed 35°C or fall below 18°C. In certain varieties, the onset of flowering can be postponed when temperatures fall below 24°C. The minimum temperatures are 10°C, while for crop production, they are around 15°C. A mere 25 to 30 percent of the flowers yield pods, with the ultimate count influenced by the plant's vigor throughout the flowering phase. Annual fluctuations in temperature can result in variations in flowering patterns. Soybean thrives in a warm and humid environment. The optimal temperature range for the majority of the varieties seems to be between 26 to 30 °C. Soil temperatures reaching 15.5°C or higher promote swift germination and robust seedling development. Reduced temperatures generally result in a postponement of the flowering process. The length of the day is a crucial determinant for the majority of soybean varieties, as they are classified as short-day plants. Soybean cultivation in northern India is typically initiated from the third week of June through the first fortnight of July. If temperature falls below 21°C or rise above 32°C, it may negatively impact flowering and pod setting. Extreme temperatures exceeding 40°C pose a significant threat to seed production. The ideal temperature range for soybean cultivation is between 20 and 30°C, while temperatures reaching 35°C and higher are deemed detrimental to production. The ideal soil temperature for germination and the initial growth of seedlings ranges from 25 to 30°C (Shea et al. 2020).

Soybeans have the potential to be cultivated year-round in tropical and subtropical regions, provided that water resources are accessible. A good soybean crop necessitates 400 to 500 mm of water throughout the season. The necessity for high moisture levels is paramount during germination, flowering, and the pod formation stage. Nonetheless, arid conditions are essential for maturation. Soybeans exhibit some tolerance to short periods of water logging; however, the deterioration of seeds poses a significant challenge during the rainy season. Soybean has the capacity to grow and produce yields with a minimum of 180mm of in-crop rainfall; however, one might anticipate a yield reduction of 40–60 percent in comparison to optimal conditions. The optimal range for rainfall is between 500-1000mm. Crop failure occurs if the rainfall received during the growing season is below 180 mm. Drought often leads to uneven crop establishment, which can result in crop mortality or significantly reduced yields. Extended dry spells exceeding 5 days are observed in sandy, gravelly, or shallow soils that possess a water storage capacity of less than 35 mm in the root zone, leading to frequent instances of crop water stress. In dense soils, the crop can endure a period of 15 days without precipitation. Implementing reduced tillage, preserving ground cover, or utilizing crop residues like rice straw or maize residue can significantly mitigate the effects of drought. This approach lowers soil temperature and surface evaporation, thereby enhancing the soil's capacity to retain moisture. The addition of crop residues to the soil, a practice referred to as mulching, can lead to an increase in soybean yield (Ulafić et al. 2020).

The soybean is classified as a short-day plant, meaning it flowers during periods of limited sunlight and responds to the decreasing length of days. Every variety possesses a specific day length that needs to be attained before flowering can commence. In the Northern Plain Zone of India, specifically in Punjab, Haryana, and Delhi, the optimal period for soybean planting is from mid-

June to the first week of July. When choosing varieties for cultivation, it is important to consider that day length differs by location and region, which will influence the maturity of the varieties (ICAR 2023). Soybean is fundamentally a short-day plant; however, its response to day length differs among varieties and is influenced by temperature. Developed varieties are specifically adapted to relatively narrow latitude ranges. The duration of daylight affects the developmental pace of crops. For short-day varieties, longer days can lead to delayed flowering and the growth of taller plants with additional nodes. Short days accelerate the flowering process, especially in late-maturing varieties. The total growing period spans from 100 to 130 days or even longer. Soybean is frequently cultivated as part of a rotation system along with cotton, maize, and sorghum. The spacing between rows ranges from 0.4 to 0.6 meters (Ulafić et al. 2020).

Optimal conditions for soybean cultivation are found in well-drained, fertile loam soils that maintain a pH range of 6.0 to 7.5. Sodic and saline soils impede the germination of seeds. Waterlogging poses a detrimental effect on the crop. An increase in 20% protein content and 16% decrease in oil content were observed as soil pH rose from 4.5 to 7.0. Soybeans exhibit tolerance to slightly acidic soil, with an optimal pH range of 5.5 to 6.5. However, they exhibit intolerance toward strongly acidic soils (below pH 4.5) due to the potential toxicity of aluminum (Al) and manganese (Mn) in these conditions. On the contrary, cultivating soybean in soils with a pH exceeding 8 is discouraged due to the potential for micronutrient deficiencies, including zinc (Zn) and iron (Fe) (Hoeft et al. 2000).

The soybean plant exhibits limited drought tolerance due to its relatively shallow root system, which restricts its ability to absorb water during arid conditions. Consequently, due to drought stress, soybeans grow sub optimally in sandy soils and those with limited water retention, such as gravelly or shallow soils. Insufficient rainfall on clay soils diminishes the likelihood of successful seed germination and plant establishment. It is advisable to cultivate soybeans only if the soil possesses adequate stored moisture. Soybeans exhibit vulnerability to waterlogging during the period from emergence to the four-leaf stage. After this stage, soybeans demonstrate a notable tolerance to waterlogging when compared to other non-rice crops, providing an additional incentive for their cultivation. Soybean appears to exhibit a greater tolerance for flood irrigation techniques compared to other crops (Vivian et al. 2013).

2.2. Agronomic practice in soybean farming

2.2.1 Crop rotation in soybean cultivation

The crop planted before the soybeans has an impact on possible yield. Planting soybeans consecutively can lead to an increase in diseases and pest issues over time. Furthermore, research indicates that growth-inhibiting allelopathic compounds are released from decomposing soybean residues in the soil, which adversely affects the cultivation and production of soybeans. Growing soybeans continuously will not result in an adequate yield. Rotating soybeans leads to higher yields than those obtained from continuous soybean cultivation (Shea et al. 2020).

2.2.2. Land preparation for soybean cultivation

Intensive summer plowing with a reversible moldboard plough exposes hibernating insects to severe temperatures and predatory birds while also promoting nutrient flow and soil water infiltration. Therefore, it is recommended to conduct a deep plowing every 3-4 years, supplemented by a standard plowing in the summer, and then follow up with two criss-cross harrowing or cultivations to break up soil clods, thereby creating an optimal seedbed for successful soybean cultivation. Additionally, performing sub-soiling every 4-5 years at 10-meter intervals alleviates sub-soil compaction and promotes rainwater infiltration, thereby supporting continuous crop development even under drought conditions (Vivian et al. 2013).

2.2.3. Sowing techniques in soybean cultivation

Due to enhanced mechanization and the accessibility of tractor-drawn seed drills, farmers have shifted from bullock-drawn sowing implements, such as dufan/tifan, to utilizing multi-crop seed drills that offer adjustable row spacing and seed rates as per the requirements of the crops. Normally, these seed drills are designed for flat sowing of 5–9 rows of soybean crops, with an adjustable inter-row interval of 14–18 inches. In light of the current climatic anomalies and unpredictable precipitation, the following strategies may be employed to alleviate the negative impacts of climate change. We can cultivate ridges and furrows using the Broad Bed Furrow (BBF). The BBF seed drills provide the capability to activate irrigation channels after every 4–5 rows. The furrow mechanism is installed at both ends of BBF seed drills. The process of planting ridges and furrows (FIRB, Furrow Irrigated Raised Bed) involves the following steps: The seed drill developed by the institution is applicable for sowing in each row or paired rows on ridges. The soybean planting period in India varies by zone, as illustrated in Table 2.2. below.

Table 2.2. Zone wise optimum sowing time seed rate and row spacing for soybean in India.

Zone	Sowing time	Seed rate (Kg/ha)	Spacing (cm)
North Eastern Hill	15^{th} June- 30^{th} June	55	45
North Plain	20^{th} June-5^{th} July	65	45
Eastern	15^{th} June -30^{th} June	55	45
Central	20^{th} June -5^{th} July	65	45
Southern	15^{th} June -30^{th} June	65	30

2.2.4. All India Coordinated Research Program on Soybean

The initiative operates in 20 states, with 34 coordinating centers (22 primary and 12 voluntary/need-based) designated for conducting location-specific research and assessment/validation of soybean technologies. To guarantee optimal functionality and suitability of the technologies, the states have been classified into six primary zones, as detailed below (ICAR 2023).

Northern Hill Zone: Himachal Pradesh and the Hill Region of Uttarakhand. The **Northern Plain Zone** includes Punjab, Haryana, Delhi, the North Eastern Plains of Uttar Pradesh, the Plains of Uttarakhand, and the Eastern region Bihar. **The Eastern Zone** comprises Chhattisgarh, Jharkhand, Bihar, Orissa, and West Bengal North. **Eastern Hill Zone** comprises Assam, Meghalaya, Manipur, Nagaland, and Sikkim. **The Central Zone** includes Madhya Pradesh, the Bundelkhand Region of Uttar Pradesh, Rajasthan, Gujarat, and the North-West Region of Maharashtra. **The southern zone** includes Karnataka, Tamil Nadu, Telangana, Andhra Pradesh, and the southern part of Maharashtra.

2.2.5. Seed germination

Farmers are recommended to assess the germination viability of the seeds they have acquired before sowing. A minimum germination rate of 70% is essential to achieve optimal plant population and, consequently, good production. This can be accomplished by sowing 400 seeds in a 4-by-4-meter plot while maintaining moisture. Emergence is recorded daily from days 5 to 8 until the count stabilizes. The germination test may alternatively be conducted by putting seeds between two sheets of newspaper and rolling them with a damp towel for 3-4 days (ICAR 2023).

Fig 2.1. Seed germination (A): 4 days after sowing, (B): Days after sowing (ICAR 2023).

2.2.6. Management of weeds in soybean cultivation

Early preparation of the seed bed is essential for effective weed control. Weed control can primarily be approached through chemical and cultural methods. Weeds engage in intense competition with soybeans for nutrients and other essential natural resources. Therefore, managing them is crucial at the right stage. This can be accomplished through a range of approaches, including mechanical, agronomical, and chemical methods for weed control. In the chemical approach, herbicides can be applied before germination or pre-emergent (planting) treatments, either before or after post-emergent treatments. Conversely, the cultural method involves manual weeding to clear the field of unwanted plants (Vivian et al. 2013). Uncontrolled weeds can lead to soybean yield reductions ranging from 20% to 70%. The losses in yield are influenced by the timing of weed emergence, the species of weeds involved, and the length of time those weeds remain in the field. The management of weeds is a crucial operation in vertisols (clay-rich soils that shrink and swell with changes in moisture content) and related soils due to the persistent rainfall and favorable conditions. (Vivian et al. 2013; ICAR 2023).

2.2.7. Harvesting and threshing

Harvesting soybeans typically involves uprooting the entire plant or using a sickle to cut the stalk. However, in cases where the crop is planted over extensive areas, a bush cutter connected to a gasoline engine and binder is employed for efficiency. Recently, a combined harvester has been utilized on large-scale farm households. Typically, harvested soybeans are dried in the sun after being tied into bundles (Shea et al. 2020). The optimal timing for harvesting is crucial for soybeans, as it can lead to yield loss from shattering and a decrease in seed viability due to exposure to field weathering. Rainfall during the maturity stage negatively impacts the quality of soybeans produced. Thus, it is recommended that farmers harvest their crops at the optimal time when 90% of the pods have changed color to yellow, as this serves as the appropriate indicator for harvesting. Harvesting at this stage does not negatively impact germination. The current moisture percentage of soybean seed is approximately 14-16%. To ensure the viability of seeds and prevent loss or mechanical damage, threshing should occur at a speed of 350 to 400 rpm. To ensure the integrity of the harvested soybeans, it is essential to store them in a secure location if threshing is delayed, thereby preventing potential damage from rain or shattering. The threshed soybean should be subjected to sun drying for 3–4 days to reduce the moisture content to 10%, which is crucial for preventing fungal infections during storage. The storage area must maintain a cool temperature, provide adequate aeration, and remain free from insects. It is advisable to maintain the soybean bags in an upright position whenever feasible. When stacking, it is advisable to limit the height to 4–5 bags, not exceeding 5 feet, while utilizing a platform to ensure the viability and germination of soybean seeds are preserved. When transferring the seed bags to the storage facility, they must be positioned with care on the designated platform. The seed bags must be kept away from direct contact with the floor or wall. Moisture seepage in the walls and floor could potentially lead to the transmission of diseases, so it's important to prevent such conditions for effective storage (Shea et al. 2020).

2.2.8. The economic importance of soybeans

Soybean is an economical and abundant source of protein, serving as a staple food in the diets of both animals and humans in various regions globally. Soybean is a primary source of vegetable oil and animal protein feed globally (Sugiyama et al. 2015). Its protein level is around 40–42% and it ranks second to groundnut in oil content (18–22%) (Robert 1986; Pagano and Miransari 2016). Products derived from soybeans encompass infant food, baked goods, confectionery, cooking oil, meat alternatives, processed meats, salad dressings, soy sauce, tofu, margarine, and miso (Ogedengbe and Bello 2018). Moreover, muscle tiredness and obesity can be mitigated by soy protein (Agyei et al., 2015), as soybeans lack carbohydrate content and serve as a good protein source, particularly for individuals with diabetes. Soybeans are utilized industrially in the production of candles, fertilizers, insecticides, pharmaceuticals, plywood, wallboard, linoleum, varnish, soaps, disinfectants, fire extinguisher fluid, and paints. The oil content contains triglycerides, which are seen as a potential source for biodiesel manufacturing (triglycerides react with an alcohol in the presence of a catalyst like sodium hydroxide or potassium hydroxide and fatty acids are converted into methyl or ethyl esters, which are the biodiesel molecules). Soybean is beneficial for aquaculture due to its nutritional benefits, sustainability, and cost-effectiveness. (Masuda and Goldsmith 2009). The influence of agriculture on soil structure and the alteration of soil species occupants has been enhanced (Pagano et al. 2011; Wall and Nielsen 2012). Soybean is a principal crop farmed worldwide that can influence various parts of the ecology. Microbes in the soil are among the most significant components of the ecosystem. Among the primary farmed crops (maize, rice, and wheat), soybean is the sole leguminous crop linked to arbuscular mycorrhizal fungi and rhizobia, presenting opportunities for further utilization. In 2011, Pagano and Covacevich talked about the benefits of arbuscular mycorrhizal fungi in agro-ecosystems. (Pagano and Covacevich are researchers known for their work in plant-soil interactions, particularly focusing on mycorrhizal fungi). They also said that people are becoming more aware of how intensive farming and the use of agrochemicals can hurt the diversity and activity of soil microbiota, as well as the quality of the soil, which changes the number and types of symbiotic fungi that live there. Mutualistic relationships, such as arbuscular mycorrhizal fungi, are important for soybean cultivation (Simard and Austin 2010; Pagano 2012). More and more, beneficial rhizospheric bacteria are being used as biofertilizers in farming. This means that we need to learn more about how different inocula affect the physiology and growth of soybeans. Dwivedi et al. (2015) asserted that soybean cultivation enhances the fixation of symbiotic nitrogen. The primary role of nodules on soybean roots is the fixation of atmospheric nitrogen via symbiotic nitrogen fixation; hence, they supply nitrogen for seed formation and plant growth. The chemical makeup of soybean is displayed in Table 2.3.

Table 2.3. Chemical composition of soybean per100g of dry matter (Lokuruka 2010**).**

Chemical contents	USDA nutrition data base
Water	8.45 g/100 g
Carbohydrates	30.16 g/100 g
Total lipids	19.94 g/100 g
Energy	1866 kcal/100 g
Protein	36.49 g/100 g
Fiber	16.2 g/100 g
Calcium	277 mg/100 g
Magnesium	280 mg/100 g
Phosphorus	704 mg/100 g
Sodium	2 mg/100 g
Iron	15.7 mg/100 g
Zinc	4.89 mg/100 g
Vitamin C, total ascorbic acid	6 (mg/100 g)
Thiamine	47 (mg/100 g)
Riboflavin	14 (mg/100 g)
Vitamin B6	0.87 (mg/100 g)
Niacin	0.87(mg/100 g)
Vitamin K	0.084 (μg/100 g)
Vitamin A	1.623 (μg/100 g)

.

2.3. Limitations/constraints in soybean cultivation

2.3.1. Common varieties of soybean

The Indian Council of Agricultural Research Indian Institute of Soybean Research (ICAR- IISR) has developed several enhanced breeding lines that have been utilized by various countries to produce and release improved production varieties (Dupare 2023). The ICAR-IISR has developed multiple soybean varieties, including NRC2, NRC128, and others. These varieties are designed to fulfill the requirements of farmers by exhibiting high yields, short to medium maturation periods, and increased oil content. In recent years, the institute has developed and released several specialty soybean varieties, such as NRC 127 and NRC 142, which lack both KTI and lipoxygenase-2 acid, and NRC 147, which is recognized for its high oleic acid content. The institute has registered vegetable-type soybean genotypes with ICAR-NBPGR in New Delhi. Various genotypes have been commercialized by prospective corporate clients for large-scale multiplication to address specific requirements.

2.3.2. Biotic factors affecting soybean cultivation

All diseases and pests that affect grain soybeans (Hartman et al. 2016) also affect vegetable soybeans, but their significance varies depending on the production regions globally. Most efforts to breed soybeans have focused on making them resistant to common pests and diseases. As a result, most modern cultivars have genes that make them resistant to diseases like soybean rust, Phytophthora root and stem rot, soybean cyst nematode, and soybean aphids. No comparable initiative has been undertaken to cultivate vegetable soybeans for disease and insect resistance, rendering the crop generally more susceptible to diseases and pests.

2.3.3. Damage caused by insects

According to Norsuwan et al. (2015), the main bugs that attack vegetable soybeans in Thailand are whiteflies (*Bemisia tabaci*), bean flies (*Melanagromyza sojae*, *M. phaseoli*, *Ophiomia centrosematis*, *Dolichostigma sp*.), beetroot armyworms (*Spodoptera litura*), stink bugs, leafhoppers, aphids, bean pod borers (*Etiella zinckenella*), leafhoppers, and beetroot armyworms in general. Putting out yellow sticky traps can cut down on bean fly (*Melanagromyza spp*.) and pod borer infestations in vegetable soybeans by 68.5% and 51.6%, respectively, compared to the control group that didn't do anything to control the insects.

2.3.4. Diseases caused by pathogens

Soybean crops are susceptible to various diseases, categorized as fungal, bacterial, viral, and nematode-related. Soybean rust, caused by *Phakopsora pachyrhizi*, is the primary foliar disease impacting soybeans worldwide. The cultivation of vegetable soybeans in China is mostly concentrated in the southeastern coastal region, where anthracnose is widespread due to the warm, humid climate (Zhu et al. 2022). Downy mildew, bacterial pustule, Fusarium pod rot, Cercospora blight, purple seed stain, and damping off were named by Lord et al. (Load et al. 2021) as major diseases that affect soybeans in the mid-Atlantic United States. The susceptibility of the Swarna Vasundhara and Karune varieties to yellow mosaic disease (YMD) considerably obstructs the dissemination of the crop over northern and central India. Major diseases impacting vegetable soybeans in Thailand comprise the soybean mosaic virus and purple seed stain. The latter does not affect yield but reduces germination and seed quality.

2.3.5. Abiotic factors affecting soybean cultivation

Inadequate rainfall or irrigation results in aborted blooms, diminutive pods, and withered beans in vegetable soybeans, significantly diminishing the output (Chen et al. 2021). In their study of vegetable soybeans under controlled environmental conditions, (Moloi and van der Merwe 2021) discovered that the tolerance responses of ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR), particularly during the flowering stage, operate synergistically to reduce hydrogen peroxide (H_2O_2) production and lipid peroxidation, thereby enabling H_2O_2 to participate in the signaling processes that induce drought tolerance. The elevation of total soluble sugars (TSS) during blooming and proline during pod filling was significant as a response to drought tolerance. AGS429 was the sole variety that markedly elevated H_2O_2 levels under drought stress.

2.3.6. Crop management practices

Despite advancements in varietal enhancement for improved yield, inadequate crop management methods have hindered the profitable and sustainable cultivation of vegetable soybeans. Inadequate nodulation can impede the nitrogen fixation capacity of plants, resulting in diminished yields. The depth of planting directly influences seedling emergence, resulting in suboptimal plant stands (Crawford et al. 2019). Close spacing between plants results in excessively high densities in the field, prompting competition for soil moisture, nutrients, and solar radiation (Petter et al. 2016), ultimately leading to suboptimal plant growth and reduced output. Soil potassium deficit can adversely affect crop growth and production levels (Lima et al. 2013). Salt stress negatively impacts the growth and yield of vegetable soybeans. It extends the time of bean filling, diminishes bean size, and eventually impacts production potential (Ghassemi et al. 2009). Waterlogging stress resulting from high soil moisture in the field is harmful to crops at any growth stage. The primary obstacles to improved crop production are escalating labor shortages and the absence of herbicide-tolerant varieties (Zhang et al. 2017). The extreme perishability of vegetable soybean pods has limited the harvesting period. This provides farmers with little time to harvest and sell the pods before they become yellow and unmarketable in fresh marketplaces (Nolen 2016). Labour expenses constitute around 62% of the overall crop production costs, predominantly attributed to manual harvesting (Garber et al. 2019). Mechanical harvesting can decrease total production costs by approximately 28%; however, the mechanical damage incurred during harvesting might diminish yield by up to 24% compared to hand harvesting (Garber et al., 2019). In Taiwan, a 190 HP FMC 7100 harvester operating at 3400 rpm maintained an actual loss of approximately 5%, with damaged pods at around 7%, and lowered the total cost of production by nearly 20% (Shanmugasundaram et al. 2001). Dhaliwal and William (2020) indicated that the economically best plant density for machine-harvested vegetable soybeans varied between 87,000 and 120,000 plants per hectare. In the United States, significant barriers to the commercial cultivation of vegetable soybeans include the efficacy of mechanical harvesting and the expense of human harvesting, which remains a prevalent method among small farmers (Lord et al. 2021; Tadesse et al. 2015). In Thailand, mechanical harvesting has gained popularity for vegetable soybeans in recent years because of its significant reduction in harvest costs.

2.3.7. Quality seeds production

High-quality seed is crucial for yielding a superior crop, influenced by various aspects including growth season, location, management inputs, diseases, insect pests, postharvest handling, and storage conditions. Locations with optimal temperature, low relative humidity, and arid conditions during seed maturity should be chosen for seed production. The quality of crops from the dry season surpasses that of the rainy season crops. In Japan, the vegetable soybean seed crop is cultivated in Hokkaido, where chilly, arid environments and moderate temperatures facilitate the production of high-quality seed. In Taiwan, seed production primarily occurs during the autumn season, when the climate is cool and arid. (Shanmugasundaram et al. 2001). In Thailand, the sowing of seed production takes place during the dry season, specifically from December to mid-January in the northern region. The primary distinction in cultural methods between seed crops and grain soybeans lies in pest and disease management. Robust integrated pest and disease management strategies must be implemented to safeguard the quality of the mature seed. The pesticide residue is not a significant concern if it does not impact the succeeding vegetable soybean crop. In regions with significant diurnal temperature fluctuations, many varieties will fracture upon maturity.

2.4. Impact of mutagens on biological parameters.

Numerous studies have examined the impacts of physical and chemical mutagens, as well as their combined treatments, on various biological parameters including germination, survival, injury, and sterility (Khan et al. 2005). Chaudhary (1983) observed a uniform decrease in germination across various wheat varieties when exposed to high doses of gamma rays. Khanna (1991) reported the influence of seed treatment using various concentrations of EMS on the germination and growth of chickpea seedlings. A proportional decline in germination percentage was observed with the increased concentrations of EMS. Khanna and Maherchandani (1981) reported a reduction in seed germination with an increase in the dose of gamma rays in chickpeas. Lal et al. (2009) investigated the mutagenicity of gamma rays, sodium azide, and their various combinations in the M1 generation of blackgrams, noting that higher concentrations of sodium azide led to a reduction in M1 germination rates. The survival of plants was influenced by varying doses of gamma rays and sodium azide. The combined treatments of gamma rays and sodium azide exhibited a more pronounced depressive effect on seedling growth.

2.5. Economic effects of mutation breeding.

Mutation breeding has significantly improved the yield per hectare and uplifted the socio-economic status of farmers. A mutation breeding program represents a valuable strategy that could allow mutant crops to better endure specific environmental stresses, such as water scarcity, extreme temperatures, and pH levels, or to reproduce at a faster rate compared to wild-type crops. Modern plant breeders and farmers have the opportunity to utilize a vast array of natural biodiversity, which can be significantly expanded through the use of mutation induction techniques. This advancement leads to a substantial economic influence on agriculture and food production, currently estimated in billions of dollars and millions of cultivated hectares (Kharkwal and Shu 2009). Over the last seventy years, more than 2250 varieties have been introduced globally, originating either as direct mutants or from their descendants (Ahloowalia et al. 2004). The induction of mutations through radiation has emerged as the predominant technique for the direct development of mutant varieties. The primary approach in mutation-based breeding focuses on improving well-adapted plant varieties by modifying one or two key traits that restrict their productivity or improved their quality value. Furthermore, the economic impact of the chosen mutant varieties of rice, barley, cotton, groundnut, pulses, sunflower, rapeseed, and Japanese pear, along with various mutation- derived varieties, has led to a synergistic effect on enhancing both the yield and quality of the crops, optimizing agronomic inputs, facilitating crop rotation, and boosting consumer acceptance. Unlike the currently protected plant varieties or germplasm, which face growing limitations on their utilization, the induced mutants have been freely available for plant breeding purposes. A variety of mutants have significantly influenced the enhancement of yield and quality in numerous seed-propagated crops across borders. Ahloowalia et al. (2004) indicate that induced mutations are set to play an increasingly significant role in developing crop varieties with traits like modified oil, protein, and starch quality, improved uptake of specific metals, deeper rooting systems, and enhanced resistance to drought, diseases, and salinity, all of which are essential for environmentally sustainable agriculture.

2.6. Impact of chemical mutagens on plant growth

Sodium azide is a strong mutagen, and the growth of plant tissues is significantly inhibited with increasing concentration and duration of treatment. Mutational effects of this mutagen have been shown to be very effective in tomatoes, changing things like germination rate, root length, seedling height, seedling survival, number of branches per plant, and yield per plant (Adamu et al. 2007).

The impact of varying concentrations of NaN_3 treatments on root length was significantly noted across different crops. The shortest root length (9.10 cm) was recorded on day 14 in the barley group that was treated to 2.5 mM NaN_3 for 3 hours. This was different from the groups that were exposed to NaN_3 for 3 hours and were tested on day 7. This treatment had a statistically significant impact on leaf length, particularly noticeable on day 14 (Ilbas et al. 2005). The NaN_3 treatment changed the length of the coleoptile in groups that were exposed to it for 3 or 4 hours. In barley, the coleoptiles were shortest in groups that were exposed to 3 mM NaN_3 for 3 hours (2.54 cm) and 2 mM NaN_3 for 4 hours (1.59 cm) (Ilbas et al. 2005). The decline in seedling survival is ascribed to cytogenetic damage and physiological disruptions (Sato and Gaul 1967). The higher sensitivity at higher mutagenic levels is due to a number of factors, such as changes in the metabolic activity of cells, the inhibitory effects of mutagens, and the upset of the balance between growth regulator promoters and inhibitors (Krishna et al. 1984).

2.7. Effect of chemical mutagens on chromosome

Cytological analysis of mitotic or meiotic behavior is regarded as one of the most reliable indicators for assessing mutagen potency. Therefore, a fundamental component of most mutation research involves the studies of meiotic anomalies and their genetic implications. It also provides a crucial indicator for assessing the sensitivity of plants to different types of mutagens. The mechanism of action and the nature of mutations created by sodium azide are becoming understood and it has been accelerated by the discovery of a mutagenic metabolite formed by sodium azide. It has been shown that sodium azide damages chromosomes during mitosis in barley (Nillan et al. 1975), beans (Kihlman 1959). Sodium azide generated chromosomal aberration frequencies that were comparable to or slightly higher than those of the untreated controls. The primary abnormalities caused by sodium azide are translocations, lagging chromosomes, chromosomal bridges, and sticky chromosomes. Chromosome stickiness happens when chromosomes aren't folded properly into their individual chromatids. This causes chromatin fibers to mix and sub chromatid bridges to connect the chromosomes. Additionally, the occurrence of lagging chromosomes escalates with higher doses of sodium azide (Ramel et al. 1976). The assembly and migration of spindle fibers during cell division is an ATP-dependent process. Having less ATP production and availability may make it harder for spindle fibers to organize in sodium azide-treated root tip cells. This can change how the chromosomes are arranged at the metaphase plate and how they move to their respective poles during anaphase (Ramel et al. 1976). The abnormalities in spindle formation and chromosome segregation during mitosis will lead to chromosomal anomalies, such as lagging chromosomes, sticky chromosomes, and bridge formation. It has been shown that sodium azide lowers the rate of germination and increases chromosomal abnormalities in the mitotic cells of plant root tips. These effects depend on the dose. Mutagens produce structural alterations in chromosomes and generate mutations, which may be responsible for the disruption of pairing among homologous chromosomes. Khan et al. (2009), showed that sodium azide damages chromosomes, which causes bridges formation during mitotic division. This, in turn, makes phenotypic aberration. It also has a significant role in genetic sterility, as demonstrated in rice without alterations in vigor (Mensah et al. 2005). Sodium azide and colchicine are both polyploidizing and mutagenesis chemicals, utilized over an extended period to generate polyploid plants. The carcinogenic impacts of sodium azide on plant shape, chlorophyll content, sterility, and yield were previously validated by Ahoowalia (1967) and Castro et al. (2003).

2.8. Application of chemical mutagens and crop improvement

The presence of genetic variability is necessary for the crop improvement. The variability available to the breeders comes from spontaneous or artificially induced mutations. Plant breeding involves procedures that increase genetic variation, select desirable genotypes, evaluate selected genotypes, and finally multiply, and release new cultivars (Al-Qurainy & Khan 2009). Mutational breeding generates a knowledge base that guides future users of mutation technology for crop improvement. In mutation breeding, the enhancement of the genetic variation is made through the influence of different mutagens. Despite the advantages and limitations of this method, it has been applied in the development of numerous improved cultivars and in different crops, such as wheat, rice, barley, soybean, lupines, vegetables, ornamentals, etc. Several traits have been subjected to mutation breeding such as yield, lodging resistance, disease resistance, maturity, culm length, etc. The artificial mutation is a practical mean to achieve genetic improvement in crop species and it is done with physical and/or chemical mutagens that enlarge the mutation frequency, when compared to the spontaneous occurrence (Mullangie et al. 2024). However, for extensive use of these mutants in plant breeding, high production efficiency is essential. This means that the utility of any mutagen depends not only on its effectiveness (mutation factor/dose) but also on its efficiency. The effectiveness of a mutagen has no practical implications since radiations and chemical mutagens are relatively inexpensive (Al-Qurainy & Khan 2009). On the other hand, lower levels of mutagens efficiency can limit their uses. Mutagenic efficiency is the production of desirable changes that are free from associations with undesirable genetic alterations. This is generally measured by the proportion of the mutation frequency in relation to damages associated to mutagenic treatments such as height reduction, chromosome breakages, sterility, lethality, etc. The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in different selected plants by mutagens (Khan et al. 2009). The ability of these mutagens to enter the cell of living organisms to interact with the DNA produces the general toxic effects associated with their mutagenic properties. Thus, their effects are mainly due to the direct interaction between the mutagen and the DNA molecules. Sodium azide mutagenesis can not only generate diverse resistance but also provide an efficient method for breeding disease resistant varieties (Al-Qurainy & Khan 2009).

Maize embryonic calli derived from immature embryos of inbred line 18-599 were treated with gamma (γ) ray and sodium azide (NaN_3), and selected on high osmotic medium containing 1.0% NaCl for drought tolerant mutant (He et al. 2009). A combination of 20 Gy of γ ray and 1 mmol/L of NaN_3 was identified to be most effective for the mutation. The drought tolerance of a mutated line 18-599M was significantly higher than its parental 18-599. Genetic polymorphism between 18-599M and 18-599 was amplified by 7 of 700 pairs of SSR primers. Three insertions, three deletions and seventeen base substitutions were found during exon 1 of glutathione S-transferase gene by sequence alignment. Two of the base substitutions changed its coded amino acids, which were probably responsible for the induced drought tolerance. Silicon deficient mutant of rice was obtained with treatment of sodium azide and produced mutant was best as forage crops because it is easily digestible by animals (Nakata et al. 2008).

The CAS-7 mutant of sunflower possesses a multiple phenotype having a reduced triacylglycerol seed content, a modified intraplastidial fatty acid synthesis, together with a seedling blocked growth and poor green colour and reduced chloroplast development (Venegas-Caleron et al. 2008).

The plant growth (plant height, leaf area per plant, fresh and dry weight of root, stem and leaf) of *Eruca sativa* has been improved in green house experiment at 3 mM concentration of sodium azide (Al-Qurainy, 2009). At this concentration of sodium azide, all mutant had higher fresh and dry weight compared to control plants. Similarly, the % seed germination of *Eruca sativa* at various concentration of sodium azide varied and % germination decreased as the concentration of sodium azide increased and also with increased treatment duration (Khan et al. 2009). Thus, its mutagenicity proves a breeding tool for crop improvement. The numerous mutants produced under the treatment of sodium azide and great influence was found on seed shape and seed size of tested common bean mutants (Jeng et al. 2010). The NaN_3 induced mutation also occurs in the levels of total phenolics, anthocyanins and proanthocyanidines and various kinds of antioxidant activities. NaN_3 induced mutations also affects the profile of manomeric anthocyanidines, with two (cyaniding and pelargonidin) or three (delphinine, cyaniding and pelargonidin) monomeric anthocyanins were detectable in the seed coat depending on the tested mutants. Majority of mutants tend to have greater antioxidant activities (DPPH, radical scavenging, FRAP, LPI ability and ABTS- radical scavenging activity) than the wild type (Jeng et al. 2010). The enhanced antioxidative abilities are related to the higher levels of total phenolics, anthocyanins and proanthocyanidins accumulated in the seed coat.

2.9. Sodium azide (SA)

It is acknowledged as a chemical mutagen and has been employed to enhance agronomic traits in crop plants. Plants modified through sodium azide treatment demonstrate superior survival rates in challenging environmental conditions, as well as increased yields, greater resilience to stress, extended shelf life, and lower agricultural inputs when compared to traditional varieties (Ikhajiagbe et al. 2013). The simultaneous application of sodium azide and cytokinin has been noted to markedly affect vegetative growth parameters, influencing factors such as leaf number, chlorophyll content, nitrate reductase activity, stomatal density, and stem diameter (Dewi et al.

2016). The effectiveness of sodium azide in promoting genetic variability and improving agronomic traits depends on the concentration used and the particular crop species being examined. Sodium azide has been shown to influence seed germination, shoot length, and root length while also inducing a high frequency of chlorophyll-deficient mutations (Khan and Tyagi 2009). The decrease in seed germination observed in mutagenic treatments can be attributed to delays or inhibitions in the physiological and biological processes essential for germination. These processes encompass the inhibition of mitotic activity (Sato and Gaul 1967), hormonal imbalances (Ananthaswamy et al. 1971), and alterations in enzyme activity (Chrispeds and Varner 1967). The observed stimulatory effect could potentially be attributed to the actions of growth hormones. The function of mutagens could involve enhancing enzymatic activation and stimulating the young embryo, leading to an elevated rate of cell division. This impact extends beyond germination, influencing both vegetative growth and flowering as well. The most significant reduction in the duration until 50% flowering occurred as a result of varying impacts of mutagens, which disrupted seed metabolism and the initiation of DNA synthesis, as noted by Mathew et al. (2015). Findings from (Anantha swamy et al. 1971) regarding Pigeon pea were consistent. The existing literature regarding germination inhibition as a result of mutagen doses indicates that potential factors may include the inhibition of auxin synthesis, a decline in assimilation mechanisms, and disturbances in enzyme formation.

2.10. Ethyl methyl sulfonate (EMS)

It is a chemical mutagen commonly used to create genetic variability in crop species. A recent study conducted by Chen et al. (2020) explored the effects of EMS-induced mutagenesis on agronomic, yield, and quality traits in two significant peanut genotypes, Huayu 22 and Yueyou 45.

Different concentrations of EMS were utilized for varying durations, leading to the emergence of mutants that displayed noticeable changes in phenotypic traits, including plant height, branching structure, leaf shape, yield metrics, and quality features. Among the observed mutants, significant variants were identified, including those exhibiting dwarfism, unique leaf pigmentation and shape, increased oil and/or protein content, altered seed dimensions, and changed seed coat coloration. The findings revealed that the mutations were heritable in subsequent generations (M_3), thus confirming these mutants as significant genetic assets for peanut improvement efforts. The application of EMS-induced mutagenesis has proven effective in generating diversity in agronomic traits among different legume varieties, as highlighted in research conducted by Opoku et al. (2020). The effectiveness of EMS mutagenesis is rooted in its capacity to swiftly induce changes in genetic material, resulting in a range of both favorable and unfavorable phenotypic outcomes. As a result, EMS-induced mutagenesis presents a valuable strategy for increasing genetic diversity and possibly improving agronomic characteristics in cultivated crops. Ethyl methane sulfonate is a chemical mutagen that is currently utilized in plant breeding to enhance genetic variability in genes of agronomic significance in species beneficial for agriculture. It mainly leads to single base point mutations through the induction of guanine alkylation, which results in GC to AT transitions. The impact varies among clones of a single genotype as well as among different genotypes within the same category (Gutiérrez et al.2024)

2.11. Methyl methane sulfonate (MMS)

It often known as methyl methane sulfonate (MMS), is a chemical mutagen used to induce genetic variability in plants. The mechanism of action entails the induction of point mutations by alkylating specific nucleotides within the DNA sequence. MMS-mediated alkylation predominantly affects guanine residues, leading to single nucleotide changes (Chen et al. 2023). These modifications include alterations in the bases guanine (G), adenine (A), thymine (T), and cytosine, along with the phosphonate backbone. Although there are few direct investigations concentrating on methane sulfonate, findings from research on similar mutagens such as ethyl methyl sulfonate (EMS) indicate that MMS is probably to have similar impacts on agronomic traits in plants.

Hypothesis

2.12. Hypothesis

The soybean (*Glycine max* (L.) Merrill), family Leguminosae, widely is known as the "golden bean" and the "miracle crop" of the twenty-first century (Dilawari et al. 2022). It contains high-quality oil (20%), protein (40%), starch (36–40%), vitamins A, B, and D, 6–7% minerals (calcium, iron, manganese, phosphorus, copper, and thiamine), 8–10% fibre, and phytochemicals such as flavonoids, saponins, alkaloids, steroids, and tannins (Alghamdi et al. 2018).In India, soybean is also used in various food products, including tofu, soya sauce, simulated milk, and meat products, and the oil is frequently used for cooking (Sedibe et al. 2023). Soybean meal is used as a supplement in livestock feed. Apart from this, it is used in industries for the production of biofuels and functional foods (Nuthalapati et al. 2024). Therefore, this study was undertaken to investigate the mutagenic effects and stability performance of soybean crop. However, the use of chemical mutagens is known to enhance the genetic variability of crop plants and induced mutations thereby facilitate the development of improved varieties at a swifter rate.

Objective 1: To study the phenological parameters of soybean under the effect of mutagens.

Alternate Hypotheses (H_a): The application of chemical mutagens significantly alters the phenological parameters of soybean, such as germination, flowering, and maturation, compared to untreated controls. **Null Hypotheses (H_0)**: The application of mutagens has no significant effect on the phenological parameters of soybean compared to untreated controls. We reject the null hypothesis and accept the alternate hypothesis.

Objective 2: To study the effect of different concentrations of mutagens on meiotic division in the pollen grain of soybean. **Alternate Hypotheses (H_a)**: Different concentrations of mutagens significantly affect meiotic division in soybean pollen grains, causing chromosomal abnormalities and altered pollen viability. **Null Hypotheses (H_0)**: Different concentrations of mutagens have no significant effect on meiotic division in soybean pollen grains. We reject the null hypothesis and accept the alternate hypothesis.

Objective 3: To study the effect of different concentrations of SA, EMS, and MMS on the quantitative and qualitative parameters of soybean at harvest. **Alternate Hypotheses (H_a)**: The application of SA, EMS, and MMS at different concentrations significantly influences the quantitative (yield, size, weight) and qualitative (protein, oil, fiber) parameters of soybean at harvest. We reject the null hypothesis and accept the alternate hypothesis. **Null Hypotheses (H_0)**: The application of SA, EMS, and MMS at different concentrations does not significantly affect the quantitative or qualitative parameters of soybean at harvest. We reject the null hypothesis and accept the alternate hypothesis. We reject the null hypothesis and accept the alternate hypothesis.

Objective 4: To study the concentration at which mutagens enhance nutrients in soybean. **Alternate Hypotheses (H_a)**: Specific concentrations of mutagens significantly enhance the nutrient content of soybean compared to untreated or excessively treated samples. We reject the null hypothesis and accept the alternate hypothesis. **Null Hypotheses (H_0)**: Mutagens at any concentration have no significant effect on the nutrient content of soybean. We reject the null hypothesis and accept the alternate hypothesis.

Chapter 3

Materials and Methods

3.0. Study area

The research was carried out at Lovely Professional University agricultural research farm (31.3192◦ N latitude and 75.5723◦ E longitude) during the year 2023/ 2024 and 2024/2025 seasons. The area lies within the sub-tropical belt in Northwest India. Semi-arid deserts of Rajasthan lie towards the southern border. It is characterized by three major seasons: summer (April to June); rainy (July to September); and winter (October to March). The climate ranges from sub- humid (northeast) to semi-arid (southwest), with harsh winters and summers. The area is characterised by sandy loam soil. Temperature ranges from 4 ◦C to 47 ◦C. Average annual rainfall is 650 mm

3.1. Seed Procurement and Germination Test

Soybean seeds were obtained from Punjab University of Agriculture, Ludhiana, India (30.9041◦ N latitude and 75.8066◦ E longitude). Two soybean varieties (SL958 and SL744) were selected based on their characteristics, including their high yield, medium level of maturity, and soil preference. Prior to experiment, the germination test was conducted by broadcasting a hundred seeds of SL744 and SL 958 in two earthen pots filled with fertile organic soil. Following the placement of seeds, and lightly covered with a layer of soil. Irrigation was maintained to moist the soil when required, and the germination percentage was closely observed over five days, with results indicating up to 95% germination. The cleared and manually ploughed site for seed production was laid out according to the experimental plot design (RCBD) measuring 8 × 60 m^2, and seeds were sown accordingly.

Table 3.1: Varietal description of SL744 and SL958 soybean varieties.

Characteristics	Description	
	SL744	**SL958**
Plant Height	3 to 5 feet tall	3 to 5 feet tall
Number of primary branches	1-3	1-3
Number of secondary branches	4.72	4.72
Number of leaves/plants	75-250 leaves	75-250 leaves
Number of pods per plant	200-250 pods	200-250 pods
Grain color	Light yellow grain with grey hilum	Light yellow grain with black hilum
Protein and oil content	41.7% protein content, 20.2%	42.3% protein and 21.0% oil
Resistance	Resistance to soybean mosaic and yellow mosaic virus	Resistance to yellow mosaic virus and soybean mosaic virus
Maturity time	139 days	142 days
Seed yield	About 7.3 quintals per acre on Average	About 7.3 quintals per acre on Average
Soil type	Fertile, non-saline/alkaline and well drained loamy soil	Fertile, non-saline/alkaline and well drained loamy soil

3.2. Soil analysis

Soil testing was conducted at the experimental site before sowing to determine the soil status, adopting the method established by Walkley and Black (1934). A random sampling method was employed, involving the collection of ten soil samples from ten distinct locations at a depth of 0–15 cm within the field. Twenty grams (20g) of soil was measured from each sample and transferred into a silver plate to create a composite sample of total 200g approximately. From the composite samples, a representative sample was made and put through a 2 mm sieve. It was then tested using standard methods for total organic carbon (TOG), pH, and nutrient availability (NPK) (Table 3).

Table 3.2: Methods of testing the properties of the experimental soil.

S/No	Soil properties tested	Method deployed
1	pH	Glass electrode method pH Meter (Motsara and Roy 2008)
2	Organic carbon (%)	Rapid Titration Method (Walkley and Black 1934 as modified by Nelson and Sommers 1982)
3	Nitrogen (kg/ha)	Kjeldahl method as (Bremner and Mulvancy 1982)
4	Phosphorus (kg/ha)	Olsen and Sommers (1982)
5	Potassium (kg/ha)	Flame photometry (Helmke and Sparks 1996)

3.3. Seed treatments with mutagens

Chemicals were obtained from Loba chemie Pvt. Ltd., Mumbai, India. The seeds were soaked in distilled water for 6 h and later on treated with ethyl methane sulphonate (EMS), methyl methane sulfonate (MMS), and sodium azide (SA) for 6 h. They were immersed at different concentrations (0.0%, 0.2%, 0.4%, 0.6%) of three chemicals, respectively. The 0.0% concentration was the control. Fifty mL phosphate buffer solution was added to each treatment so as to maintain the osmotic content of the cell at pH of 3. Another set of seeds were soaked in distilled water and phosphate buffer solution as a control. The procedure was periodically repeated at room temperature (25–30 ◦C). After the end of the treatments, seeds were extensively rinsed with running tap water approximately 8–10 times. Subsequently, both treated and untreated control seeds were sown in the field using a randomized complete block design with three replications to induce mutation generation (Jabeen et al. 2004).

Table 3.3: Detail of mutagenic treatments on the soybean seed varieties.

Mutagens used	Concentration (%)	Duration of presoaking (h)	Duration of treatments (h)
Untreated control	0.00	6:00	6:00
Ethyl methane sulphonate (EMS)	0.2	6:00	6:00
‘’ ‘’	0.4	6:00	6:00
‘’ ‘’	0.6	6:00	6:00
Methyl methane sulphonate (MMS)	0.2	6:00	6:00
‘’ ‘’	0.4	6:00	6:00
‘’ ‘’	0.6	6:00	6:00
Sodium azide (SA)	0.2	6:00	6:00
‘’ ‘’	0.4	6:00	6:00
‘’ ‘’	0.6	6:00	6:00

3.4. Preparation of chemical formulations

Ultimately, after evaluating the efficacy of mutagens, a specific chemical formulation was developed based on the effective doses of all the chosen active chemical mutagens (EMS.MMS & SA). The formulation obtained from these chemicals can therefore be utilized as an alternative method for generating beneficial mutations, as shown in the table 3.4. below.

Table 3.4: Detail of mutagenic formulation treatments on the soybean seed varieties.

Treatments	Details	Concentration (%)
T1	Untreated control	0.00
T2	EMS	0.2ml in 99.8ml water (0.2%)
T3	‘’ ‘’	0.4ml in 99.6ml water (0.4%)
T4	‘’ ‘’	0.6ml in 99.4ml water (0.6%)
T5	MMS	0.2ml in 99.8ml water (0.2%)
T6	‘’ ‘’	0.4ml in 99.6ml water (0.4%)
T7	‘’ ‘’	0.6ml in 99.4ml water (0.6%)
T8	SA	0.2g in 99.8ml water (0.2%)
T9	‘’ ‘’	0.4g in 99.6ml water (0.4%)
T10	‘’ ‘’	0.6g in 99.4ml water (0.6%)

3.5. Experimental Design

The land area measuring 620 m^2 was divided into 60 plots (3 columns of 20 plots each), each measuring 1.5 m^2, with a spacing of 0.75 m between plots and 1 m gap (as irrigation channel) between replications (Fig 3.1). Each plot consisted of four ridges that were spaced 0.37 m apart, with the two innermost ridges serving as net plots. The alley of rows was utilized for both destructive and non-destructive sampling, and the border rows were used for trash sampling. The experimental design included a randomized complete block design (RCBD) with three replications. Prior to planting, the soil was plowed, harrowed, and ridged to eliminate debris, weeds, and clods, ensuring adequate aeration and moisture for crop development. Seeds were planted with a 75-cm spacing on each ridge immediately after the rainfall during the third week of

May 2023/2024 and 2024/2025. Three seeds were manually planted in each hole and subsequently trimmed to two plants per stand at two weeks after sowing (WAS). The crops were harvested manually with a hoe and sickle. Matured pods were harvested and sun-dried to decrease moisture content, followed by manual threshing to separate the seeds from the chaff.

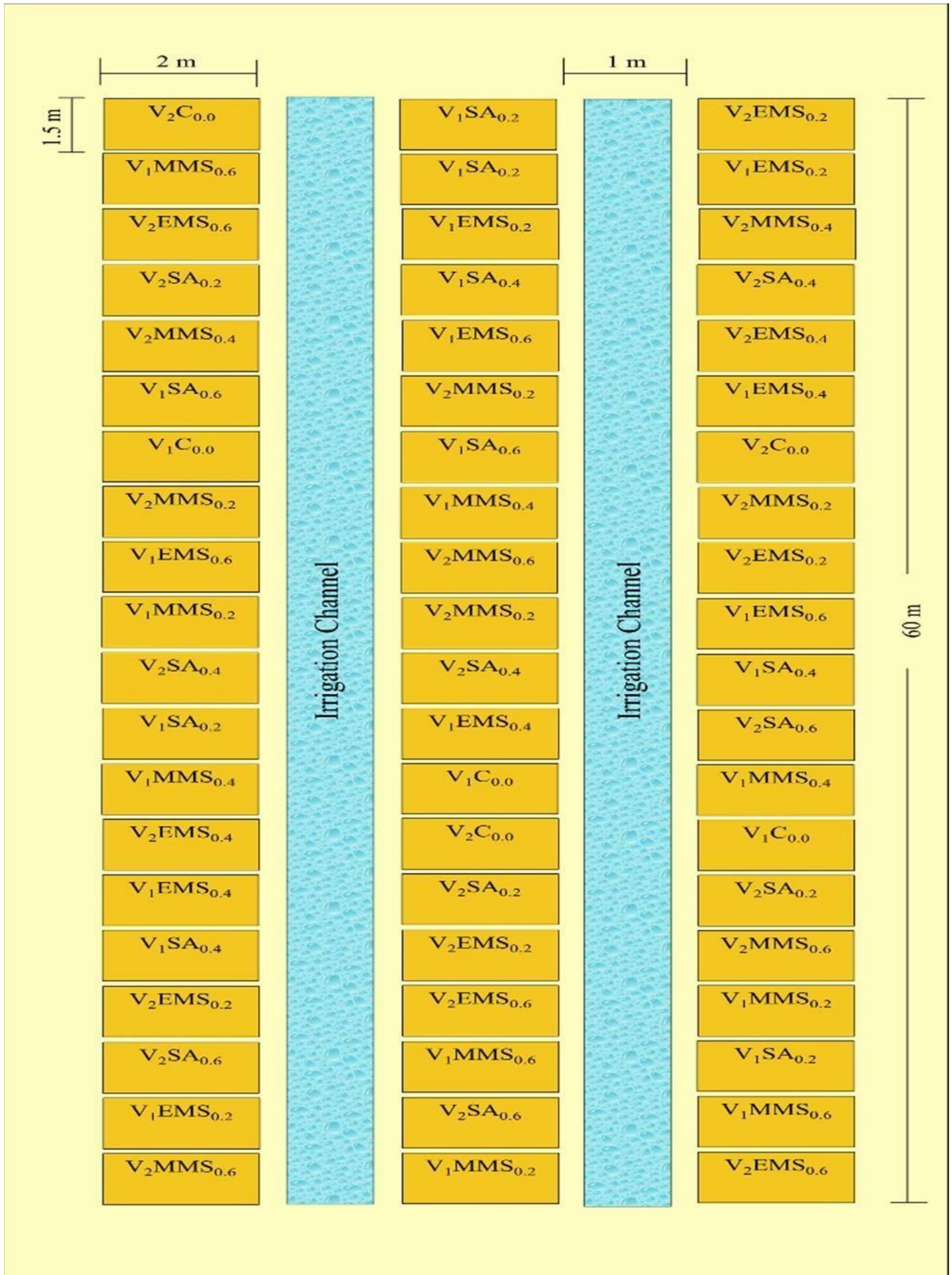


Fig 3.1. Field layout (8 × 60 m) and experimental design using RBD. V1: variety 1 (SL744), V2: variety 2 (SL958), C: control. Chemicalxx (xx = 0.2, 0.4, 0.6%), EMS: ethyl methane sulfonate, MMS: methyl methane sulfonate, SA: sodium azide.

Fig 3.2. A view of the field preparations at the LPU agricultural research farm. **(A)**: Harrowing, **(B)**: Seeds Sowing, **(C)**: Irrigation.

Fig 3.3. A field view after sowing. **(A)**: Seedling stage, **(B)**: Vegetative stage, **(C)**: Estimation of chlorophyll content.

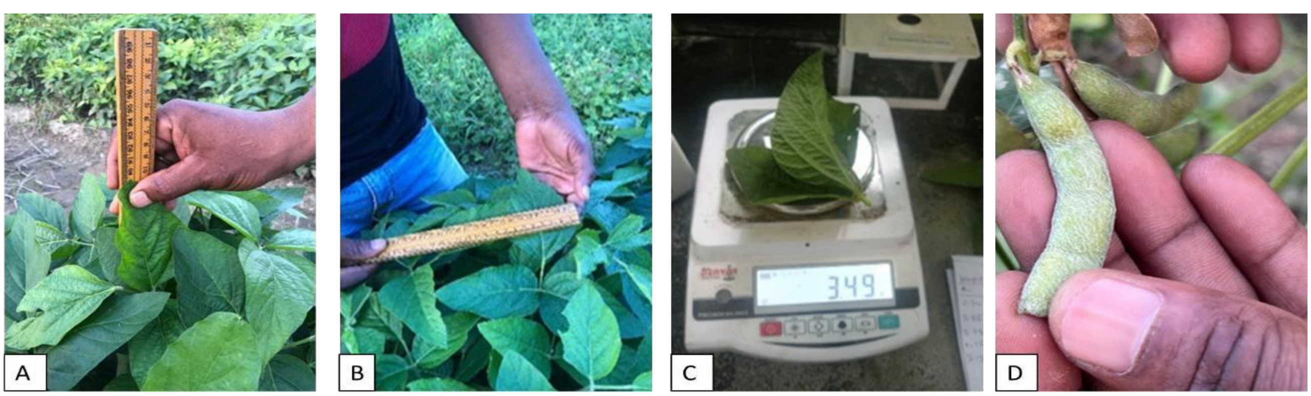


Fig 3.4: Data collection on plant growth parameters. **(A)**: Plant height, **(B)**: Leaf area, **(C)**: Fresh weight, **(D)**: Pod.

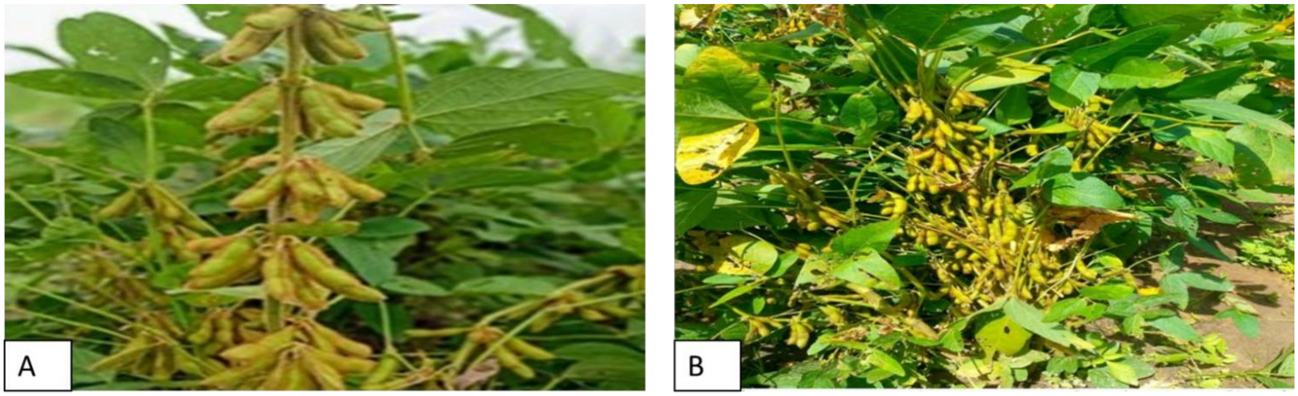


Fig 3.5. Soybean varieties. **(A)**: SL744 variety, **(B)**: SL958 variety.

Fig 3.6. Final growth stage of soybean. **(A)**: Maturing stage, **(B)**: Harvesting stage, **(C)**: Threshing stage.

3.6. Assessment of quantitative and qualitative parameters

Using the methods described by Anas et al. (2024), many aspects of plant growth were recorded, including seed germination, plant height, leaf count, leaf area, leaf variation, days to 50% flowering, days to 50% podding, days to maturing, pod length, seeds per pod, pod weight, 100-seed weight, seed size, and seed coloration, biological yield, grain yield and harvest index. These traits were recorded for the control plant as well as M_1 and M_2 plants of two soybean varieties (SL744 and SL958) at different stages of development after planting.

3.6.1. Germination count

Germination counts were recorded starting on the fourth day under field conditions. The data were presented in terms of percentage of the control group. Germination rate = (Number of germinated treatment seeds / Total number of seeds sown) × 100

3.6.2. Plant height

The plant's height was measured from the stem at soil level to the terminal node at three distinct stages of development (week 4, week 8, and week 12) using a tape measure in centimeters (cm). Five plants were randomly chosen from each plot, and the averages were recorded.

3.6.3. Number of leaves

The leaf count per plant was recorded at intervals of 6, 8, and 10 weeks after sowing. Five plants were randomly chosen from each plot, and the averages were recorded.

3.6.4. Leaf area measurement

The leaf area (LA, cm^2): It was calculated using the formula F (L x W), where F represents a constant factor (0.75), L denotes leaf length, and W signifies leaf breadth.

3.6.5. Fresh weight (FW) and dry weight (DW, g) of leaves were recorded from each treatment plot using a digital weighing system (M-METLAR digital balance).

3.6.6. Chlorophyll Content ($nmol/cm^2$): The chlorophyll content was measured using a Minolta chlorophyll meter (SPAD 502, Illinois, U.S.A.).

3.6.7. Days to 50% Podding: This refers to the time interval between planting and the point at which 50% of the plants produced podding was recorded.

3.6.8. Days to 50% flowering: The interval from sowing to the date when 50% of the plants had flower was recorded.

3.6.9. Days to Maturity: The duration from sowing to the point at which the plant reaches physiological maturity (95% browning) was recorded.

3.6.10. The number of pods per plant: It was determined by counting the pods on each of the five selected plants at maturity and calculating the average.

3.6.11. The pod length (cm): It was measured using a tape measure with five randomly selected plants, and the average was recorded.

3.6.12. The number of seeds per pod: It was determined by counting the seeds in five randomly selected pods from each of the five chosen plants and the average was recorded.

3.6.13. The total weight of 100 pods: The randomly selected pods from each five plants was measured in grams (g) using an electronic scale (M-METLAR digital balance).

3.6.14. The weight of 100 seed: The randomly picked seeds from each five plant was measured in grams (g) using an electronic scale (M-METLAR digital balance) and values were recorded

3.6.15. The seed yield per plant: The yield was determined by recording the weight of cleaned seeds from five randomly selected plants, with the average recorded in grams.

3.6.16. Biological yield per plant: The individual sun-dried plants were weighed before threshing. The combined weight of the five plants was measured, and the average was recorded in grams.

3.6.17. Harvest index (HI): The harvest index was calculated using the formula: harvest index = (grain yield of plant / biological yield of plant).

3.7. Biochemical Analysis

3.7.1. Seed processing

The soybean seed (SL958 and SL744) varieties were pounded to a coarse powder using an electric blender. The mass of the powder was determined using an analytical weighing balance. The powder was packed in a sealed bag, labeled, and stored in a dry cool place for further experiment.

3.7.2. Determination of Protein Content (%)

Micro Kjeldahl method was used to analyse the protein content of the control, $M_1$and M_2 seeds of each variety (SL744 and SL958), which involved three steps: digestion, distillation, and titration (AOAC 1995).

3.7.3. Apparatus: Among other items, there are beakers, funnels, desiccators, Kjeltec equipment, retort stands, burettes, fume cupboards, conical flasks, and filter paper.

3.7.4. Reagent**:** 50g of concentrated tetraoxosulphate (VI) acid (H_2SO_4), 0.1% N of H_2SO_4 in 2.5 mL/L/DW, 10g of 40% NaOH solution (40 mL each), nitric acid (HNO_3), 10 mL (10x20=200 mL) of H_2SO_4, 1g of selenium power, and a 4% boric acid indicator were heated to 420 °C for 140 minutes.

3.7.5. Procedure: This analysis consists of three steps: titration, distillation, and digestion (AOAC 1995).

3.7.6. Digestion: 20 ml of HNO_3 and selenium power were added to the sample mixtures in a micro-Kjeldahl flask containing 2 g of the dried sample. The mixture was then heated continuously in a fumed cupboard using a digestion block (heater) until the nitrogen in the sample was reduced to ammonium sulphate (AOAC 1995).

3.7.7. Distillation: A microKjeldahl flask was filled with 10 cm3 of the sample aliquot, 40 cm^3 of the distilled water, and 20 cm^3 of 40% NaOH after the digest had been diluted to 50 cm^3 with distilled water. In order to create a green distillate, it was put into a flask with 10 cm3 of boric acid and a few drops of methyl orange indicator.

3.7.8. Titration: The distillate in the flask was titrated with 0.01N H_2SO_4, resulting in a colour change from green to purple at the endpoint. The titer data were recorded, and the average titer value was calculated. This was used to determine the percentage of nitrogen.

The % nitrogen (N) and crude protein content were calculated as mentioned below:

Calculation:

% Nitrogen (N) = (TV × NA × 0.014 × DF)/ (volume of aliquot × sample weight) × 100

% Crude protein (g) = C.F × % N

where,

TV = Titre value

NA = Normality of acid Sample weight = 2 g.

DF = Dilution factor Vol. of aliquot = 10 mL

Conversion factor C.F = 6.25.

3.7.9. Determination of Lipid Content (%)

Two grams of the powdered seed sample (control, $M_1$and M_2 seeds) of each variety (SL744 and SL958) were placed in separate labelled thimbles, and their mouths were covered with cotton wool. N-hexane (200 mL) was then added into the 250 mL extractor flask. The covered porous thimbles were placed in a condenser, and the Soxhlet apparatus was assembled. Extraction was allowed for about 5–6 h. Thereafter, the extracted sample in the thimbles was transferred into a dried crucible. The weight of the crucible and extracted sample was taken as W_1. The crucible containing the sample was oven-dried at 105–110 ◦C for 1 h and later cooled in a desiccator, and the weight was taken as W_2 (AOAC 1995). The % weight of lipid was calculated as mentioned below:

Calculation:

W_0 = Weight of empty crucible

W_1 = Weight of crucible + grounded extracted sample (oil) before

W_2 = Weight of empty crucible + weight of extracted sample (oil) after drying

% weight of lipid = $(W_2 - W_1)/ (W_1 - W_0) \times 100$

3.7.10. Estimation of Fiber Content (%)

For estimation of seed fibre content, two grams of the powdered seed sample (control, $M_1$and M_2

seeds) of each variety (SL744 and SL958) was introduced into separate beakers followed by addition of distilled water (100 mL) and 10% H_2SO_4 (20 mL). The beaker was kept on a hot plate for 30 min. Thereafter, the suspension was filtered and then heated again using 1.25% NaOH for 30 min, filtered, and rinsed with hot water. It was allowed to drain, and the residue was scraped into a pre-weighed crucible (W_1). It was then put into a muffle furnace to dry for 2 h at 600 ◦C, kept in a desiccator to cool, and weighed as W_2 (AOAC 1995). The percentage fibre content was then calculated as shown below:

Calculation:

W_0 = Weight of empty crucible W_1 = Weight of crucible + residue

W_2 = Weight of empty crucible + weight of residue after drying

% Crude fibre = $(W_1 - W_2)/ (W_1 - W_0) \times 100$

3.8. Qualitative phytochemical analysis

Soybean seeds were subjected to qualitative phytochemical analysis to determine some phytochemical classes, utilizing the methodologies outlined by (Trease and Evans 2009; Riaz et al. 2018; Usman et al. 2020). The following tests were conducted from this stock solution: Alkaloids, Flavonoids, Glycosides, Saponins, and Tannins.

3.8.1. Test for Alkaloids (Meyer's test)

3ml of the seed extract were mixed with 1m of 1% hydrochloric acid in a test tube. A few drops of Meyer's reagent (potassium mercuric iodide solution) were then added. The results indicate the presence of alkaloids by producing a whitish-yellow turbidity or precipitate.

3.8.2. Test for Saponins (Foam Test)

To generate a steady, enduring froth, 0.5 ml was vigorously agitated with 2 ml of distilled water in a test tube. Saponins were present by a foamy lather form lasted longer than 10 minutes.

3.8.3. Test for Flavonoids (Alkaline reagent test)

A few drops of sodium hydroxide (NaOH) solution were added to the extracts in a test tube. The presence of flavonoids was indicated by a golden hue that became colorless with the addition of dilute acid.

3.8.4. Test for Tannins (Ferric test)

The colour produced was not observed after applying a 5% ferric chloride (FeCl3) solution to 2-3 ml of the extract. Condensed tannins have a dark green hue, but hydrolyzed tannins display a blue-black coloring.

3.9. Quantitative phytochemical analysis

3.9.1. Preparation of seed processes

The dried seed materials were homogenized to a fine coarse powder using an electric blender and then stored in an airtight container until further use. Methanol solvent was used for extraction. 10 grams of coarse powders of seed was soaked in methanol in 100ml container and allowed to stand for 30 min on a water bath with occasional shaking. These flasks were then kept on a rotary shaker at 200 rpm for 24 hours. Finally, sample extract of methanol was prepared using a Soxhlet apparatus and was filtered through Whatman No. 1 filter paper that had been cleaned, and a rotary evaporator was used to dry them out under vacuum at 40°C. Thus, the obtained dried extracts were stored at 4°C in labeled and sterile bottles (Madhu et al.2016).

.**3.9.2.** Quantitative estimation of alkaloids

To 1 mL of the methanolic extract, 5 ml of pH 4.7 phosphate buffer was added, and 5 ml of BCG (Bromocresol Green) solution and shake a mixture with 4 ml chloroform. The chloroform-colored layer formed was carefully collected in a 10 ml volumetric flask. The absorbance of the complex chloroform layer was measured at 470 nm against a blank prepared as above but without extract. Atropine is used as a standard material, and the assay is compared with atropine equivalents (Madhu et al.2016).

3.9.3. Quantitative estimation of flavonoid

Total flavonoid content was determined by the aluminum chloride method using catechin as a standard. 1 ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume); after 5 min, 0.3 ml of 5% sodium nitrate and 0.3 ml of 10% aluminum chloride were added. After 6 min of incubation at room temperature, 2 ml of 1 ml sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm and compared to a blank. The results were given as mg catechin/g dried extract (Madhu et al.2016).

3.9.4. Quantitative estimation of saponins

The test extract was dissolved in 80% methanol, 2 ml of vanillin in methanol was added, mixed well, and the 2 ml of 72% sulfuric acid solution was added, mixed well, and heated in a water bath at 60°C for 10 min. Absorbance was measured at 544 nm against the reagent blank. Diosgenin is used as a standard material, and the assay is compared with Diosgenin equivalents (Madhu et al.2016).

3.9.5. Quantitative estimation of tannins

1 mL of the methanolic extract was added to 20 mL of 70% methanol, then shake for 30 minutes. The extract was then filtered. 1 ml of extract was placed into a test tube, then 5 ml of vanillin reagent (4% vanillin in methanol) and 5 ml of HCl reagent (8% in methanol) were added and mixed thoroughly. The mixture was incubated for 20 minutes at room temperature. The absorbance was read at 500 nm using a spectrophotometer (Madhu et al.2016).

3.10. Meiotic analysis

For the meiotic studies, five randomly chosen M_2 (mutation 2) plants' young flower buds were fixed in freshly made Carnoy's fixative (a 3:1 mix of absolute alcohol and acetic acid) for 24 hours. The buds were then rinsed and stored in 70% alcohol. This study used a scanning electron microscope (SEM) at the Central Instrumentation Facilities (CIF) of Lovely Professional University, India to look at pollen grains and squashed anthers in 2% aceto-orcein (Naaz et al., 2024).

3.11. Statistical Analysis

Experimental data were obtained from five randomly selected plants in each experimental plot (Figure 3.1). A two-way ANOVA was conducted using SPSS software (version 22) to analyze the data, with means separated by Duncan's multiple range test (DMRT) was conducted at a 5% significance level.

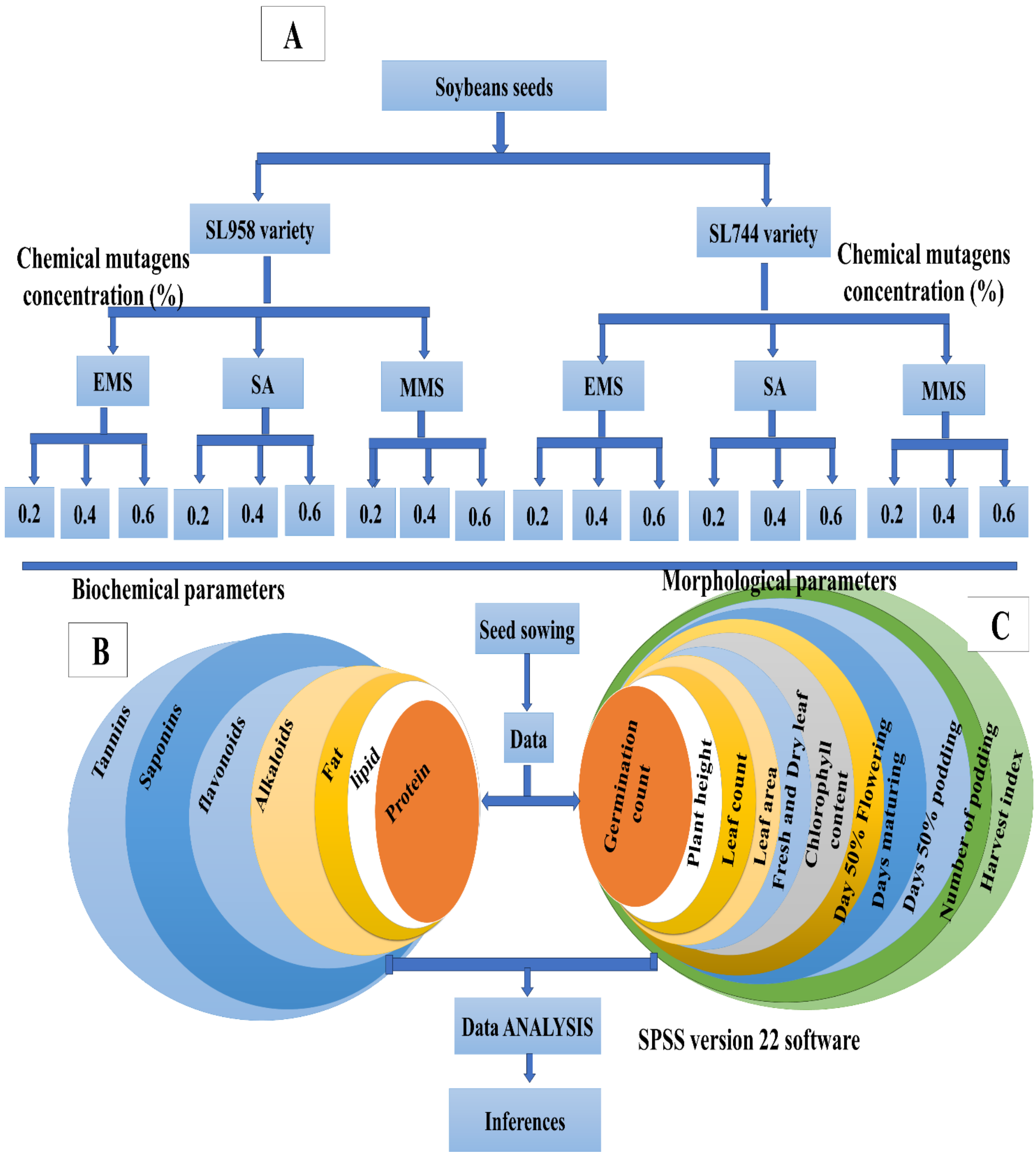


Fig 3.7. Work plan**.** A: Seed treatments, B: Biochemical parameters, C: Morphological parameters.

CHAPTER 4

Results

CHAPTER FOUR

4.0. Results

4.1. Soil properties

The analysis of the soil properties indicates that the pH level is within the normal range (6.70), with low organic carbon content (0.36%). The concentrations of nutrients are as follows: nitrogen at 245 kg/ha, phosphorus at 17.5 kg/ha, and potassium at 173 kg/ha, all of which are categorized as medium levels, as detailed in table 4.1.

Table 4.1. Physico-chemical properties of the experimental soil.

S/No	Soil properties	Standards	Result	Status
1	pH	6.0 –7.5	6.70	Normal
2	Organic carbon (%)	0.3 –0.5	0.36	Low
3	Nitrogen (kg/ha)	245–480	245	Medium
4	Phosphorus (kg/ha)	16.5–19.6	17.5	Medium
5	Potassium (kg/ha)	153 –458	173	Medium

4.2. Effect of chemical mutagens on seed germination percentage (%)

To calculate the germination percentage, 96 seeds were sown in each plot. After seed germination, the seedlings were counted, and seed germination percentages were recorded for control and test plots (as shown in Fig 4.1 A&B. & Fig 4.2 A&B.), which were compared statistically. The seed germination percentages were used to plot the graphs (*x* axis: chemical treatments; *y* axis: seed germination percentage) using MS Excel version 13. The graphs (Fig 4.1 A&B & Fig 4.2 A&B) illustrate the M_1 and M_2 seed germination rates for two varieties (SL 958 and SL 744) under various treatments (0.2%, 0.4%, and 0.6%) at 4, 8, and 10 days. The treatments include various concentrations of EMS, MMS, and SA, along with a control for two consecutive seasons, 2023/2024 and 2024/2025, respectively.

The highest seedlings (65 seedlings) were recorded in SL744, treated with 0.4% SA 10 days after sowing, and the lowest seedlings (42 seedlings) those treated with 0.6% MMS. However, the highest seedlings (76 seedlings) were recorded in SL958, treated with 0.4% SA at 10 days after sowing and the lowest seedling (30.33 seedlings) treated with 0.6% MMS. The result was significantly different from the untreated control (SL744: 53, SL958: 76 seedlings) during the first-year experiments. Moreover, in the second-year trial, EMS exhibited the lowest germination at lower (0.2%) concentrations (65 seedlings), which was recorded but showed higher inhibition at higher (0.6%) rates (only 53 seedlings at day 10). Moreover, MMS exhibited a negative impact on germination when compared to the control; even at the highest concentration of 0.6% MMS, only 48 seedlings germinated by day 10. Generally, MMS suppressed germination at all concentrations in both varieties. While SA showed a positive impact on germination, particularly at the 0.4% concentration (reaching 70 seedlings by day 10), it appears to yield the best results overall in both varieties. However, SL 958 variety untreated control showed a steady and significant increase in germination from 28 seedlings on day 4 to 78 seedlings on day 10. Similarly, the 0.2% EMS treatment was initially lower than the control at day 4 (22 vs. 28), but it overtook the control by day 8 (52 vs. 50). By day 10, it remained slightly below the control (73 vs. 78). At 0.4% EMS the germination rate outperformed the control at day 4 (32 vs. 28) and day 8 (56 vs. 50), but it fell short of the control at day 10 (65 vs. 78). At all-time points, the germination consistently fell short of both the control and lower EMS concentrations, with only 57 seedlings recorded by the 10th day. All concentrations of MMS (0.2%, 0.4%, and 0.6%) exhibited decreased germination in comparison to the control and EMS treatments. At 0.2% MMS, there was a slight increase in germination up to day 10, but it remained low overall (49 seedlings). At 0.4% and 0.6% MMS, germination was slightly higher than 0.2% MMS at day 10 (50 and 51 seedlings), but overall germination was still reduced. At 0.2% SA, the initial germination was higher than the control at day 4 (32 vs. 28), but the final germination at day 10 (61 seedlings) was lower. However, at 0.4% SA, it significantly outperformed all other treatments, with germination reaching 87 seedlings at day 10, which was significantly higher than the control's 78 seedlings, and this was observed across all varieties. Meanwhile, at 0.6% SA, early germination was comparable to 0.4% SA, but growth was slower by day 10, reaching 62 seedlings.

In cultivars SL958 and SL744, the M_2 generation had a much greater seed germination percentage of 86.7% compared to 72% in M_1 generation.

4.3. Specific Treatment Effects

4.3.1. EMS (Ethyl Methane Sulfonate): The 0.4% EMS concentration had the best overall germination performance for both SL 958 and SL 744, with higher germination rates at days 8 and 10 compared to the control and other EMS concentrations. The 0.6% EMS concentration clearly inhibited germination, as seen by the reduced seed germination at all time points in both varieties.

4.3.2. MMS (methyl methane sulfonate): MMS consistently stopped germination in both types, with lower rates than the control, EMS, and SA treatments. Although, the difference between concentrations of MMS (0.2%, 0.4%, and 0.6%) were minor, but it indicated that even low doses of MMS are quite inhibitory.

4.3.3. SA (Sodium azide): SL 958 showed the most dramatic positive response to 0.4% SA, with germination rates surpassing the control (87 seeds by day 10). For SL 744, the 0.4%

SA concentration also yielded the best results, with 70 germinated seeds by day 10. The 0.6% SA concentration, though lower than the 0.4% SA treatment, still promoted better germination.

4.4. Variety-Specific Responses

SL 958 appeared to be more sensitive to EMS, with noticeable inhibitory effects at higher concentrations (0.6% EMS). However, it responded positively to SA, especially at 0.4% concentration, with significantly higher germination rates. On the other hand, the SL744 variety showed more resistance to EMS across concentrations, with the 0.4% EMS treatment performing similarly to or better than the control. SA at 0.4% also showed the best germination in this variety, as shown below.

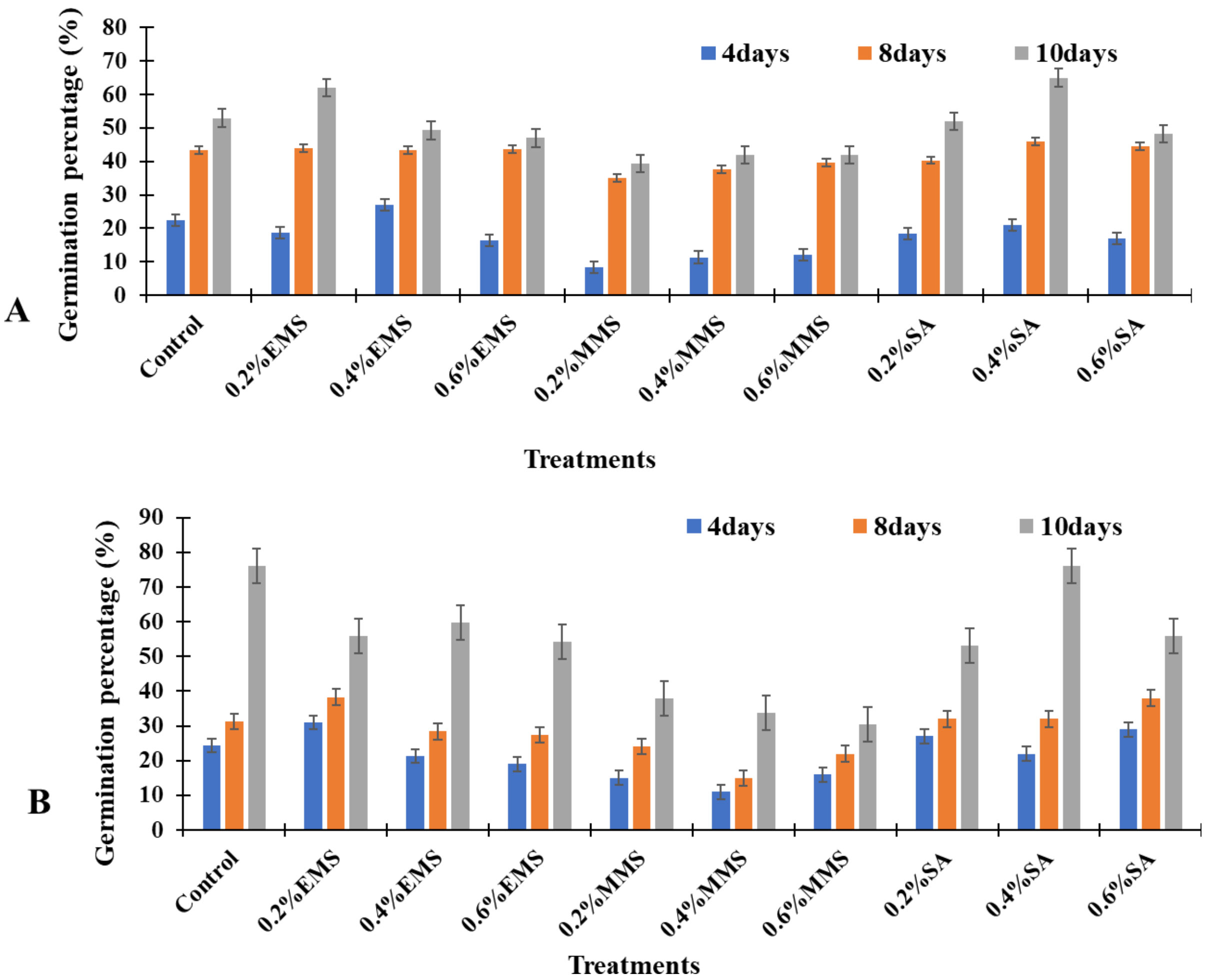


Figure 4.1. M_1 generation: Effect of chemical mutagens on seed germination percentage (%). **(A)** Effect on SL744 soybean varieties. **(B)** Effect on SL958 soybean varieties. DAS = Days after sowing.

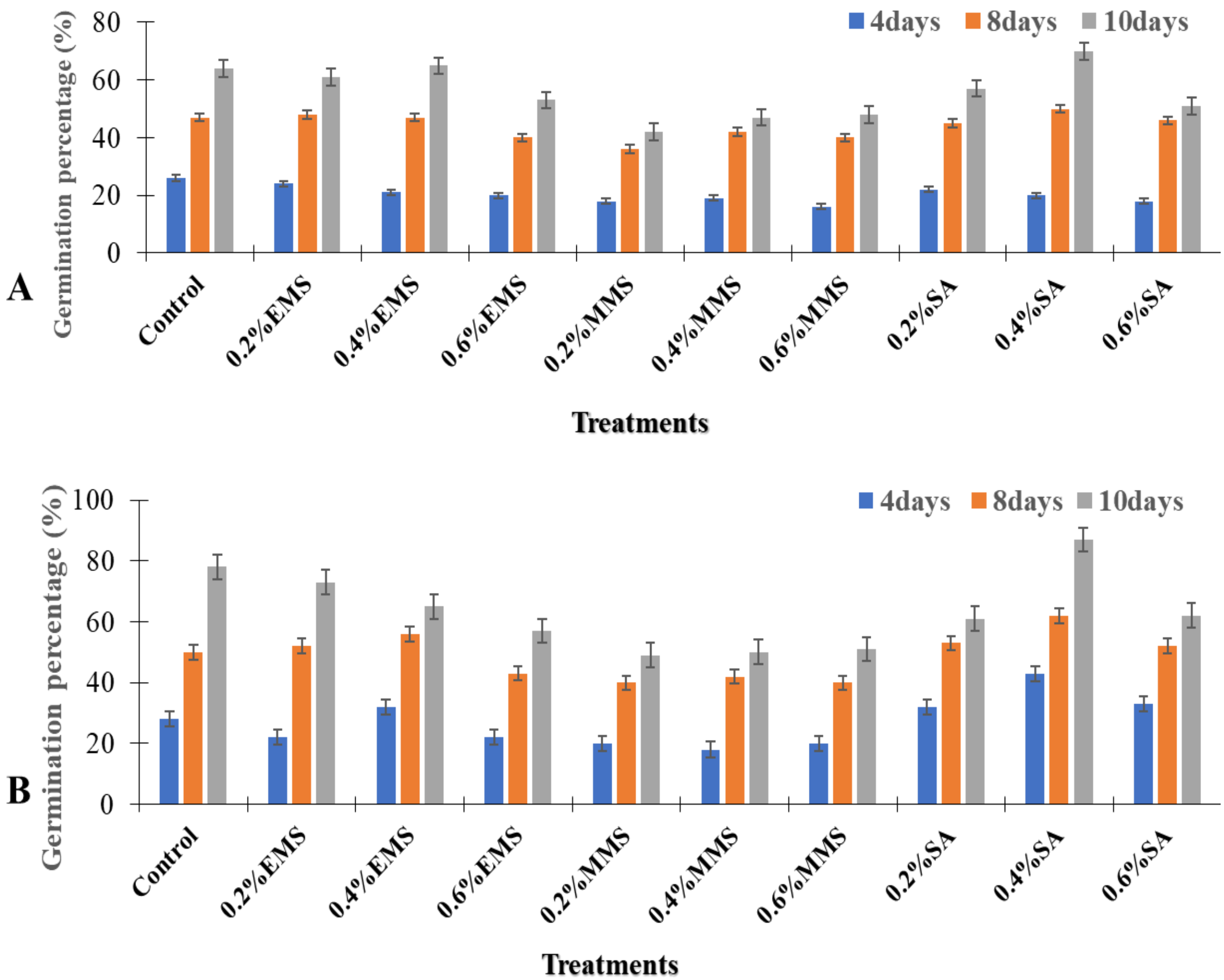


Figure 4.2. M_2 generation: Effect of chemical mutagens on seed germination percentage (%). **(A)** Effect on SL744 soybean varieties. **(B)** Effect on SL958 soybean varieties. DAS = Days after sowing.

4.5. Effects of chemical mutagens on quantitative and qualitative growth parameters in the field experiment

4.5.1. Effect of chemical mutagens on plant height (cm) in M_1 and M_2 plant

The impact of chemical mutagens on plant height during the two consecutive cropping periods (2023/2024 and 2024/2025) is presented in Table 4.2, with data collected at all intervals in the field experiments. In the majority of the data recording periods during the two cropping seasons, the results showed significant differences from each other and from the untreated control plots, M_1 and M_2, with a statistical significance of 5%. At 12 WAS, the T_9-treated (0.4% SA) plots exhibited the highest plant height (37.84± 1.32 cm) for the SL958 variety, followed by T_2 (35.06±1.42 cm) with 0.2% EMS. Meanwhile, the lowest height (21.90 0.63 cm) was recorded for T_7 (0.6% MMS). Moreover, for the SL744 variety at 12 WAS, the T_2 (0.2% EMS)-treated plots exhibited the highest plant height (24.80 ± 1.07 cm), and the lowest height was recorded for T_{10} (20.58 ± 0.30 cm) with 0.6% SA. These results, however, differed significantly from those of the untreated control plants (SL958: 26.09 ± 0.62 cm and SL744: 27.48 ± 0.74 cm) during the first-year study. In the second-year experiment, T_9 records the highest plant height (36.76 ±1.75 cm) for the SL 958 treated with 0.4% SA. Meanwhile, the T_7 had the least plant height (19.66 ± 0.56 cm) at 12 WAS. Moreover, for the SL744 variety at 12 WAS, the T_2 (0.2% EMS) treated plot exhibited the highest plant height (28.74±1.04), and the lowest height was recorded for T_7 (18.44 ±1.65 cm) treated with 0.6% MMS. The results, however, differed positively from the untreated control plant (SL958: 26.7±0.99 cm, SL744: 28.74±1.04 cm).

Table 4.2. Effect of chemical mutagens on plant height (cm) at different weeks after sowing.

	Data taking periods											
	2023/2024						2024/2025					
	SL744W4	**SL958W8**	**SL744W8**	**SL958W8**	**SL744W12**	**SL958W12**	**SL744W4**	**SL958W8**	**SL744W8**	**SL958W8**	**SL744W12**	**SL958W12**
T1(Control)	13.29±0.68^{cd}	9.29±1.01^{a}	26.13±0.84^{a}	21.99±1.42^{d}	27.48±0.74^{a}	26.09±0.62^{e}	14.786±0.91^{bcd}	16.62±0.30^{bc}	27.08±1.25^{a}	25.72±0.69^{c}	28.74±1.04^{a}	26.7±0.99^{b}
T2	14.13±0.96^{a}	15.19±0.89^{a}	25.37±0.52^{a}	29.33±0.63^{b}	24.80±1.07^{b}	35.06±1.42^{b}	17.16 ±0.59^{a}	17.946±0.61^{b}	25.48±1.19^{a}	33.44±1.47^{a}	27.42±1.47^{ab}	34.16±1.38^{a}
T3	12.10±0.80^{bc}	11.87±1.12^{bc}	21.29±0.57^{bc}	22.06±0.23^{d}	21.82±1.24^{cd}	24.27±0.73^{ef}	12.98±0.53^{cd}	20.14±0.43^{a}	22.08±0.58^{bc}	25.506±0.58^{c}	25.8±1.87^{c}	27.2±1.03^{b}
T4	10.13±0.54^{d}	9.75±0.82^{d}	20.87±0.78^{c}	17.43±1.09^{e}	24.17±0.87^{b}	22.44±0.51^{f}	11.764±0.47^{bc}	16.504±0.50^{bc}	22.26±1.03^{bc}	18.8±0.51^{g}	24.4±1.67^{c}	19.94±0.84^{de}
T5	14.26±1.15^{a}	14.65±0.81^{a}	22.60±0.93^{b}	26.34±0.90^{c}	23.02±0.82^{bc}	28.40±0.95^{cd}	14.04±0.87^{cd}	16.26±0.58^{c}	23.64±0.86^{b}	22.94±0.51^{d}	24.48±0.86^{c}	24.96±0.90^{e}
T6	9.99 ±0.21^{d}	9.99±0.21^{cd}	20.97±0.67^{c}	21.22±0.86^{d}	22.21±1.03^{cd}	25.53±1.71^{e}	12.984±0.58^{ef}	11.64±0.87^{e}	24.38±1.57^{b}	20.58±0.89^{f}	25.7±1.54^{c}	22.82±1.05^{c}
T7	9.39±0.82^{d}	9.73±0.64^{d}	17.99±0.74^{d}	15.97±1.00^{e}	21.77±0.17^{cd}	21.90±0.63^{f}	11.086±0.73^{ef}	9.92±0.93^{f}	16.72±1.73^{e}	17.664±0.84^{h}	18.44±1.65^{e}	19.66±0.56^{cd}
T8	13.96±0.64^{a}	13.49±0.90^{ab}	17.95±0.68^{d}	25.37±1.07^{c}	21.31±0.63^{cd}	30.54±2.37^{c}	10.92±0.95^{f}	13.02±1.02^{e}	22.02±0.84^{cd}	21.92±0.52^{e}	25.4±0.75^{c}	24.5±1.89^{c}
T9	14.19±0.94^{a}	14.25±0.93^{a}	19.19±0.74^{d}	36.35±1.5^{a}	23.18±0.61^{bc}	37.84±1.32^{a}	15.44±0.61^{ab}	14.06±0.67^{d}	24.34±2.57^{a}	27.784±0.54^{b}	27.66±1.22^{bc}	36.76±1.75^{a}
T10	11.09±0.83^{d}	10.12±0.99^{cd}	17.97±0.69^{d}	28.50±0.91^{b}	20.58±0.30^{d}	28.71±0.92^{cd}	12.766±0.97^{de}	11.72±0.79^{e}	20.74±1.05^{d}	22.66±2.16d^{e}	21.98±0.76^{d}	24.16±1.84^{c}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.2. Effect of chemical mutagens on number of leaves per plant in M_1 and M_2 plant

Data on leaf count were also collected from the same experimental plots to compare the effect of the three different chemical mutagens on the two soybean varieties (SL744 and SL958), as shown in Table 4.3. The results showed that at four weeks after sowing (4 WAS), there were significant differences in the number of leaves among the treatments. This indicated that the chemical mutagens had a strong effect on the leaf count. However, at eight and twelve weeks after sowing (8 and 12 WAS), there was a highly significant difference in the number of leaves among the treatments, indicating that they had a positive impact on the leaf count. At 4 WAS, the T_7 treatment (0.6% MMS) caused a significant reduction in the number of leaves (17.47 0.70 leaves/plant) in SL958, which was significantly different from the other treatments. At 8 and 12 WAS, the same treatment (0.6% MMS) exhibited the most significant reduction in the number of leaves (81.58 ± 4.03 leaves/plant and 87.00 ± 0.82 leaves/plant, respectively). These results differed significantly from those of all the treatments, including the untreated control plants (156.77 ± 1.05 leaves/plant and 160.73 ± 1.05 leaves/plant). At 12 WAS, the T_9 treatment (0.4% SA) recorded the highest leaf count (234.33 ± 3.09 leaves/plant) in SL958. The T_9 treatment (0.4% SA) also caused the most leaves to grow on SL744 (199.33 ± 2.62 leaves/plant), which was significantly higher than the untreated control plants (180.00 ± 1.63 and 160.73 ± 1.05) during the first year of the study. In the second-year experiment, T_9 records the highest number of leaves per plant (240.8±2.05 leaves/plant) for the SL 958 treated with 0.4% SA. Meanwhile, the T7 has the least plant height (118±8.51 leaves/plant): 12 WAS. Moreover, for the SL744 variety at 12 WAS, the T_9 (0.4% SA) treated plot exhibited the highest number of leaves/plant (233.2±3.56 leaves/plant), and the lowest number of leaves/plants was recorded for T_7 (119.8±3.49 leaves/plant) treated with 0.6% MMS.

The results, however, differed positively from the untreated control plant (SL958: 172.6±9.02 leaves/plant; SL744: 164.2±5.22 leaves/plant).

Table 4.3. Effect of chemical mutagens on number of leaves.

	Data taking periods											
	2023/2024						2024/2025					
	SL744W4	SL958W8	SL744W8	SL958W8	SL744W12	SL958W12	SL744W4	SL958W8	SL744W8	SL958W8	SL744W12	SL958W12
T1(Control)	25.40b±0^{c}	26.47±0.98^{d}	147.33±1.25^{b}	156.77±1.05^{bc}	180.00±1.63^{c}	160.73±1.05^{d}	26.6±2.07^{c}	30.2±1.48^{b}	148.6±6.88^{b}	158±8.51^{c}	164.2±5.22^{b}	172.6±9.02^{c}
T2	23.13±0.84^{cd}	34.44±0.97^{a}	133.00±2.16^{b}	217.80±5.37^{a}	179.33±2.49^{c}	229.00±5.35^{ab}	24.6±1.82^{d}	28±0.71^{c}	135.2±4.15^{c}	216.8±2.28^{b}	148±4.53^{bc}	226.6±513^{b}
T3	22.73±0.38^{cd}	24.78±1.03^{de}	126.00±1.63^{b}	155.87±5.56^{bc}	168.33±4.99^{c}	165.13±2.11^{d}	24±2.55^{d}	26.6±1.67^{d}	123.2±7.60^{g}	163.2±3.03^{c}	132.4±5.86^{bc}	172.6±5.32^{c}
T4	22.40±0.43^{cd}	24.18±0.95^{f}	77.87±1.33^{b}	152.20±5.37^{c}	135.53±5.72^{g}	164.80±2.47^{d}	24.2±2.77^{d}	22±1.58^{e}	110.8±2.59^{h}	140.4±2.07^{d}	127.8±5.81^{c}	153.2±5.36^{cd}
T5	31.27±1.16^{a}	28.51±1.25^{c}	178.80±3.96^{b}	218.00±1.63a	190.33±2.49^{b}	227.33±2.05^{b}	32±3.98^{b}	21.8±0.84^{e}	129.8±4.60^{e}	122.2±3.77^{f}	138.8±4.09^{bc}	132.6±4.83^{d}
T6	28.33±1.25^{ab}	22.20±0.59^{f}	134.00±0.82^{b}	215.67±3.68^{a}	179.80±3.84^{c}	220.07±2.37^{c}	30.8±2.68^{b}	22.2±1.30^{e}	125.8±2.68^{g}	129±4.64^{e}	135.2±2.17^{bc}	138.4±2.70^{e}
T7	19.20±1.07^{d}	17.47±0.70^{g}	120.33±1.25^{b}	81.58±4.03^{d}	156.53±3.13^{e}	87.00±0.82^{f}	16.4±2.07^{c}	17.6±1.14^{f}	108.8±6.83^{i}	109±9.70^{g}	119.8±3.49^{a}	118±8.51^{e}
T8	25.67±4.78^{bc}	25.17±0.94^{de}	120.67±1.70^{b}	102.60±6.25^{d}	142.07±2.25^{fg}	146.00±2.16^{e}	28.6±4.56^{c}	27.8±0.84^{c}	132.6±2.07^{d}	120.2±2.39^{f}	137±6.04^{bc}	133.6±5.08^{f}
T9	30.53±1.84^{a}	32.80±0.75^{ab}	200.00±2.62^{a}	193.87±2.81^{bc}	199.33±2.62^{a}	234.33±3.09^{a}	43±2.92^{a}	36±3.08^{a}	223.8±3.70^{a}	231.4±2.07^{a}	233.2±3.56^{a}	240.8±2.05^{e}
T10	28.20±0.86^{bc}	31.27±0.99^{b}	129.07±11.44^{b}	181.00±1.63^{b}	147.67 ±.05^{fg}	220.33±1.25^{c}	29±5.83^{c}	27.6±0.89^{cd}	123.8±3.70^{g}	107±3.81^{g}	132.8±4.49^{bc}	120.8±8.17^{a}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.3. Effect of chemical mutagens on leaf area (cm^2) in M_1 and M_2 plant

Table 4.4. shows the results of the effect of the chemical mutagens on the leaf area of the control and M1 plants. The results revealed that at 4 WAS, the T_9 plots (0.4% SA) recorded the highest leaf area (62.5 ± 0.82 cm^2) in SL958, while the T_7 plots (0.6% MMS) recorded the lowest (22.2 ± 0.00 cm^2). Similarly, at 4 WAS, the T_9 plots (0.4% SA) recorded the highest leaf area (61.06 ± 0.47 cm^2) in SL744, while the T_4 plots (0.6% EMS) recorded the lowest (22.72 0.00 cm^2). However, at 8 WAS, the response in T_2 (0.2% EMS) plots differed significantly ($p < 5\%$) from the untreated plots in having the highest leaf the area (2628.4 ± 1.16 cm^2) in SL958, while the T_8 (0.2% SA) plots had the lowest (148.9 ± 0.50 cm^2). In SL744, the T_5 (0.2% MMS) plots recorded the highest leaf area (2042.6 ± 1.20), whereas the T_4 plots (0.6% EMS) recorded the lowest (496.0 ± 1.08 cm^2) leaf area. At 12 WAS, the results followed a similar trend. The T_9 plot (0.4% SA) exhibited the highest leaf area (3625.8 ± 1.43 cm^2) in SL958, while the T_7 (0.6% MMS) plot recorded the lowest area (267.9 ± 1.09 cm^2). In SL744, the T_9 (0.4% SA) plots recorded the highest leaf area (2311.03 ± 3.65 cm^2), whereas the T_{10} plots (0.6% SA) recorded the lowest (731.54 ± 1.11 cm^2) leaf area compared to that of the untreated control (2190.6 ± 2.05 and 2256.6 ± 1.72 cm^2), during the first-year study. In the second-year experiment, T_9 records the highest number of leaf area per plant (3743.0±2.01 cm^2) for the SL 958 treated with 0.4% SA. Meanwhile, the T_7 having the least number of leaf area per plant (1131.7±0.72 cm^2), at 12WAS. Moreover, for the SL744 variety at 12 WAS, the T_9 (0.4% SA) treated plot exhibited the highest number of leaf area per plant (2423.5±2.91 cm^2), and the lowest number of leaf area per plant was recorded for T_7 (1175.8±2.95 cm^2) treated with 0.2% EMS. These results, however, differed significantly from untreated control plant (SL958: 2423.2±3.09 cm^2; SL744: 2213.1±2.34 cm^2).

Table 4.4. Effect of chemical mutagens on leaf area (cm^2).

	Data taking periods											
	2023/2024						2024/2025					
	SL744W4	SL958W8	SL744W8	SL958W8	SL744W12	SL958W12	SL744W4	SL958W8	SL744W8	SL958W8	SL744W12	SL958W12
T1(Control)	38.1± 0.41b	35.2 ±0.47^{c}	2111.2± 1.25^{a}	2226.1±1.07^{b}	2190.6±2.05ab	2256.6±1.72^{bc}	36.73±4.69^{c}	40.73±0.42^{d}	2116.3±4.69^{a}	2316±5.26^{c}	2213.1±2.34^{c}	2423.2±3.09^{b}
T2	59.80±0.47^{ab}	45.8 ±0.47^{b}	1605.3±1.92^{bc}	2628.4 ±1.16^{a}	1738.8±1.25b	2790.6±1.55^{ab}	57.23±0.85^{a}	49.27±0.90^{b}	1706.3±1.73^{d}	2733±1.50^{b}	1816.9±1.63^{f}	2817.6±1.84^{b}
T3	38.62± 0.47^{ab}	41.3 ±0.47^{ab}	1411.2 ±2.18^{c}	2069.1±1.28^{cd}	1527.4±1.03b	2557.9±1.51^{b}	36.23±1.04^{c}	43.37±1.36^{c}	1412.8±0.36^{e}	2267±2.51^{d}	1630.2±2.65^{j}	2115.0±2.54^{b}
T4	22.72±0.00^{b}	32.1±0.47^{cd}	496.0±1.08^{ef}	1578.9± 0.82^{c}	12854.6±1.5b	1865.9±0.86^{c}	20.90±0.44^{f}	37.30±0.56^{e}	631.0±5.14^{j}	1783±1.32^{e}	1175.8±2.95^{b}	1917.4±0.20^{b}
T5	52.22±0.47ab	57.2 ±0.00^{ab}	2042.6±1.20^{ab}	1018.6±0.81^{e}	2221.1±1.27ab	2440.8±0.86^{c}	48.70±1.23^{b}	43.47±0.71^{c}	2055.1±2.61^{b}	1786±3.56^{e}	2236.6±3.65^{d}	2541.8±0.25^{a}
T6	32.06±0.00^{ab}	34.9 ±0.82^{cd}	1357.4±1.95^{cd}	239.2 ±1.04^{de}	1947.2±1.58b	1091.1±0.75^{d}	31.58±2.41^{cd}	38.47±0.76^{e}	1356.9±3.61^{g}	1217±1.37^{g}	2163.0±0.75^{h}	1212.7±0.35^{b}
T7	28.33±0.82^{b}	22.2 ±0.00^{e}	1070.9±1.34^{cd}	236.3±1.70^{bc}	939.1±0.82c	267.9±1.09^{d}	26.03±1.20^{e}	20.01±0.60^{f}	1081.5±1.43^{h}	737±1.77^{i}	1233.7±1.75^{gh}	1131.7±0.72^{b}
T8	25.17±0.00^{b}	32.8 ±0.82^{cd}	520.0±0.16^{e}	148.9 ± 0.50^{f}	1633.8 ±2.03b	1946.1±1.25^{cd}	26.10±0.75e	41.60±0.98^{cd}	837.9±6.26^{i}	1483±1.30^{f}	1723.4±2.21^{g}	2123.8±0.40^{b}
T9	61.06±0.47a	62.5±0.82^{a}	1199.7±1.02^{cd}	2383.7±2.56^{ab}	2311.03±3.65a	3625.8±1.43^{a}	55.60±1.39^{a}	60.73±3.83^{a}	1195.9±1.50^{h}	2833±1.27^{a}	2423.5±2.91^{a}	3743.0±2.01^{b}
T10	37.50±0.40^{b}	33.4±0.67^{d}	1987.4± 3.47^{b}	998.2 ±1.54^{ef}	731.54±1.11c	2703.4±1.16^{c}	32.90±1.75^{d}	38.53±1.66^{e}	1869.2±6.72^{c}	1123±1.70^{h}	1962.8±1.88^{e}	1912.6±0.53^{b}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.4. Effect of chemical mutagens on the fresh weight (g) of soybean leaf

The effect of chemical mutagens on the fresh weight of soybean leaves is presented in **Table 4.5.** There was no statistical difference between the treatments and the untreated control group (P<5%) in either of the trial periods (2023/2024 or 2024/2025). In the first trial, T_5 had the lowest fresh weight value (2.63±0.54 g) of all the varieties. In the second trial, T_4 and T_7 had the lowest fresh weight values (2.67±0.58 g and 2.67±0.58 g, respectively), and the highest (3.67±0.58 g) were observed in T_9 and T_3 treated with 0.4% SA and 0.4% EMS. The results, however, were similar positively from the untreated control plant (SL958: 3.00±0.00 g, SL744: 3.00±0.00 g).

Table 4. 5. Effect of chemical mutagens on weight (g) of fresh leaf on M_1 and M_2 plant.

	Data taking periods			
	2023/2024		2024/2025	
Treatments	SL744	SL958	SL744	SL958
T1(Control)	$3.00±0.00^{a}$	$3.00±0.00^{ab}$	$3.00±0.00^{abc}$	$3.00±0.00^{de}$
T2	$3.00±0.00^{ab}$	$3.00±0.00^{bc}$	$3.00±0.00^{a}$	$3.00±0.00^{b}$
T3	$3.02±0.41^{b}$	$3.33±0.58^{ab}$	$3.33±0.58^{cd}$	$3.67±0.58^{b}$
T4	$3.00±0.00^{abc}$	$3.00±0.00^{ab}$	$3.00±0.00^{cd}$	$2.67±0.58^{bc}$
T5	$3.10±0.25^{ab}$	$2.63±0.54^{cd}$	$3.00±0.00^{cd}$	$2.67±0.58^{e}$
T6	$3.02±0.41^{ab}$	$3.00±0.00^{c}$	$3.33±0.58^{cd}$	$3.00±0.00^{b}$
T7	$3.01±0.25^{bc}$	$3.00±0.00^{bc}$	$3.00±0.00^{d}$	$2.67±0.58^{e}$
T8	$3.00±0.00^{d}$	$3.00±0.00^{b}$	$3.00±0.00^{ab}$	$3.33±0.58^{b}$
T9	$3.00±0.00^{de}$	$3.00±0.00^{ab}$	$3.33±0.58^{bc}$	$3.67±0.58^{a}$
T10	$3.00±0.00^{e}$	$3.00±0.00^{a}$	$3.00±0.00^{cd}$	$3.00±0.00^{cd}$

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.1.1. The impact of chemical mutagens on the dry weight (g) of soybean leaves

The dry weight of the soybean leaves was also affected by chemical mutagens. The results indicated that the T_9 (0.4% SA) plots had higher dry weight values throughout all data record periods, ranging from 1.284 to 0.21 g for the 1^{st} and 2^{nd}-year experiments. The least dry weight values were recorded in T_7 (0.558±0.05g) for the SL958. The weights of SL744 T_2 and T_8 were similar, though. They were 0.92 ± 0.0g and 0.928 ± 0.02g after being treated with 0.2%EMS and 0.4%SA, and they were 0.67±0.04g, 0.648±0.05g, and 0.638±0.04g after being treated with 0.2%MMS, 0.4%MMS, and 0.6%MMS. In the 2nd year trial, similar results were recorded for the SL958 variety, with T_7 and T_5 having the least dry weight values (0.612±0.05 and 0.621±0.06 g). As for the SL744 variety, the plot treated with T_8 (0.2% SA) had the highest weight (28.74±1.04). The plot treated with T_7 (1.21±0.02g) had the lowest height, and the plot treated with T_{10} (0.612±0.04g) had the lowest value. The results, however, differed positively from the untreated control plant (SL958: 0.936±0.02g; SL744: 0.823±0.03).

Table 4.6. Effect of chemical mutagens on dry weight (g) of soybean leaf.

	Data taking periods			
	2023/2024		2024/2025	
Treatments	SL744	SL958	SL744	SL958
T1(Control)	0.73 ± 0.03^{bc}	0.826 ± 0.02^{c}	0.823 ± 0.03^{bc}	0.936 ± 0.02^{c}
T2	0.92 ± 0.0^{ab}	0.934 ± 0.02^{ab}	0.945 ± 0.0^{b}	0.946 ± 0.02^{ab}
T3	0.738 ± 0.03^{bc}	0.926 ± 0.03^{b}	0.818 ± 0.03^{bc}	0.925 ± 0.03^{b}
T4	0.718 ± 0.02^{c}	0.868 ± 0.04^{bc}	0.738 ± 0.02^{c}	0.732 ± 0.04^{bc}
T5	0.67 ± 0.04^{cd}	0.750 ± 0.06^{d}	0.734 ± 0.04^{c}	0.621 ± 0.06^{d}
T6	0.648 ± 0.05^{d}	0.932 ± 0.03^{ab}	0.723 ± 0.05^{d}	0.823 ± 0.03^{ab}
T7	0.638 ± 0.04^{d}	0.558 ± 0.05^{e}	0.721 ± 0.04^{d}	0.612 ± 0.05^{e}
T8	0.928 ± 0.02^{a}	0.924 ± 0.02^{ab}	1.21 ± 0.02^{a}	0.934 ± 0.02^{ab}
T9	0.876 ± 0.05^{b}	1.284 ± 0.19^{a}	0.756 ± 0.05^{cd}	1.421 ± 0.19^{a}
T10	0.714 ± 0.04^{bc}	0.760 ± 0.05^{cd}	0.612 ± 0.04^{e}	0.751 ± 0.05^{cd}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.5. Effect of Chemical Mutagens on Leaf Variation

Plant morphology is considered to be an important tool for the screening of desirable mutants. In our study, chemical mutagens caused the appearance of leaf abnormalities, including unifoliate, bifoliate, tetrafoliate, and pentafoliate characteristics (Fig 4.3. I, Fig 4.3.II). Morphological changes, such as variations in leaf shape (broad and narrow leaves), were also observed in this study. Broad leaves were observed in the SL958 variety for 0.2% EMS (A: tetrafoliate), 0.2% MMS (G: tetrafoliate), 0.2% SA (D: pentafoliate), and 0.4% SA (H: tetrafoliate), while in the SL744 variety, broad leaves were observed for 0.2% EMS (J: tetrafoliate), 0.4% EMS (K: tetrafoliate), 0.6% MMS (O: trifoliate), 0.4% MMS (N: trifoliate), and 0.4% SA (Q: tetrafoliate). Narrow leaves with pointed tips along with thinner stems were observed in SL958 for 0.4% EMS (B: pentafoliate), 0.6% EMS (C: trifoliate), 0.4% MMS (E: trifoliate), 0.6% MMS F: trifoliate), and 0.6% SA (I: trifoliate), in comparison to the untreated control plants. Mean- while, in SL744, narrow leaves were observed for 0.6% EMS (L: tetrafoliate), 0.2% MMS (M: tetrafoliate), 0.2% SA (P: trifoliate), and 0.6% SA (R: tetrafoliate). It was interesting to note that in the pentafoliate leaves, the leaflets were much narrower and the area was much more reduced than in their tetrafoliate counterparts, which had a broad leaf base during the first-year trial.

However, during the second-year trial (Figs. 4.4. I and 4.4.II), morphological changes, such as variations in leaf shape (broad and narrow leaves), were also observed in this study. Broad leaves were observed in the SL958 variety for 0.2% EMS (A: tetrafoliate), 0.2% MMS (D: tetrafoliate), 0.2% SA (G: tetrafoliate), and 0.4% MMS (E: tetrafoliate); however, 0.4% EMS (B: pentafoliate), 0.4% SA (H: pentafoliate), while in the SL744 variety, broad leaves were observed for 0.2% EMS (J: tetrafoliate), 0.4% EMS (K: tetrafoliate), 0.6% EMS (L: tetrafoliate), and 0.4% MMS (M: tetrafoliate). 0.4% MMS (N: trifoliate), 0.6% MMS (O: trifoliate), 0.2% SA (P: trifoliate), 0.6% SA (R: trifoliate) and 0.4% SA (Q: tetrafoliate).

In SL958, plants treated with 0.6% MMS (F: trifoliate), 0.6% MMS (O: trifoliate), and 0.4% MMS (N: trifoliate) had narrow leaves with pointed tips and thinner stems than plants that were not treated (C: trifoliate, C_2: trifoliate). It was interesting to note that in the pentafoliate leaves, the leaflets were broad, and the area was much greater than in their tetrafoliate counterparts, which had a broad leaf base.

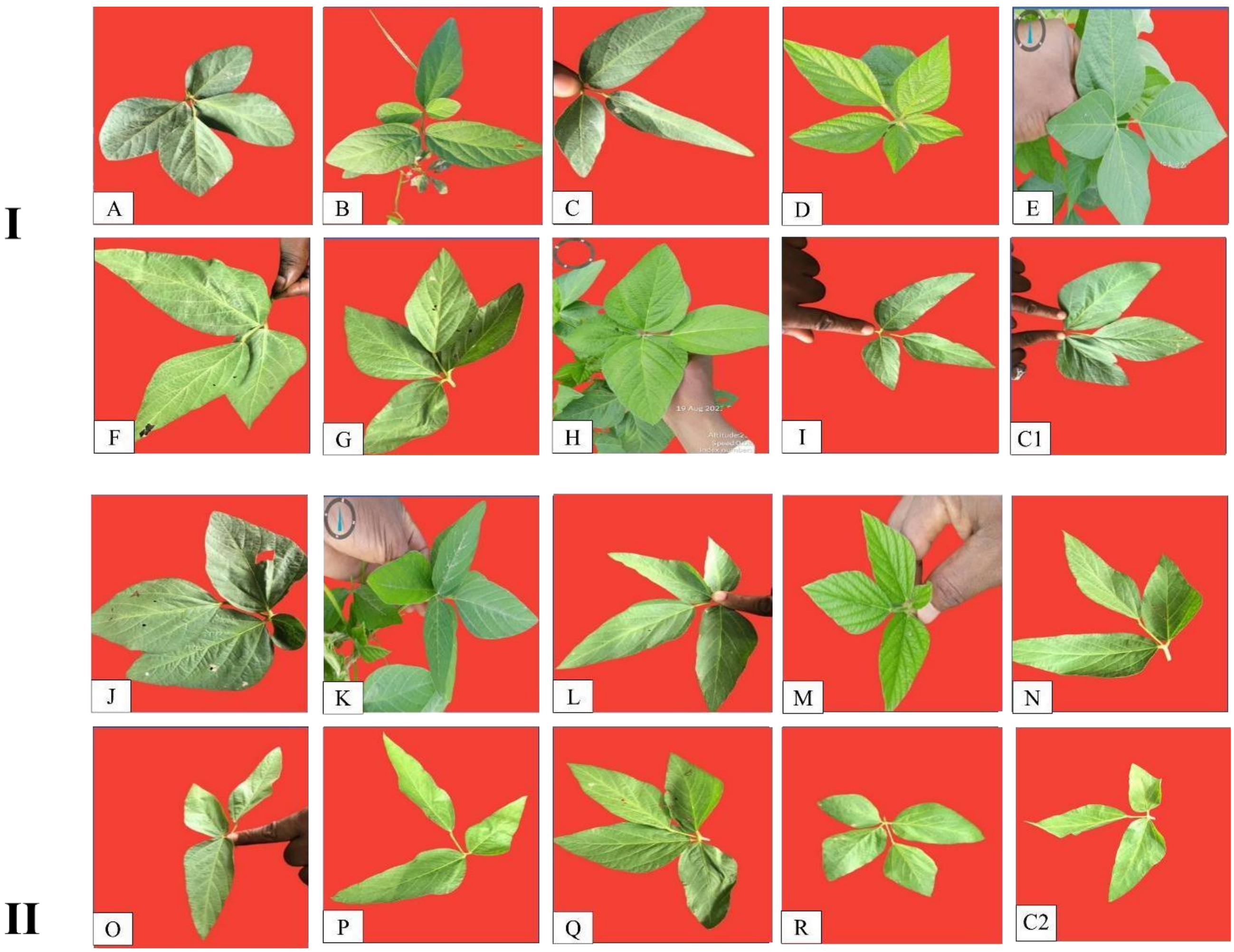


Figure 4.3. M_1 generation (I) Effect of different concentrations of three mutagens on the morphology of soybean (*Glycine max* L.) variety SL958 at 12 WAS. Mutagen I: Ethyl methane sulfonate (**A**) 0.2%, (**B**) 0.4%, (**C**) 0.6%. Mutagen II: Methyl methane sulfonate (**D**) 0.2%, (**E**) 0.4%, (**F**) 0.6%. Mutagen III: Sodium azide (**G**) 0.2%, (**H**) 0.4%, (**I**) 0.6%. (**C_1**) Control: SL958 (without mutagen treatment). (**II**) Effect of different concentrations of three mutagens

on the morphology of SL744 soybean (*Glycine max* L.) variety at 12 WAS. Mutagen I: Ethyl methane sulfonate (**J**) 0.2%, (**K**) 0.4%, (**L**) 0.6%. Mutagen II: Methyl methane sulfonate (**M**) 0.2%, (**N**) 0.4%, (**O**) 0.6%. Mutagen III: Sodium azide (**P**) 0.2%, (**Q**) 0.4%, (**R**) 0.6%. (**C_2**) Control: SL744 (without mutagen treatment).

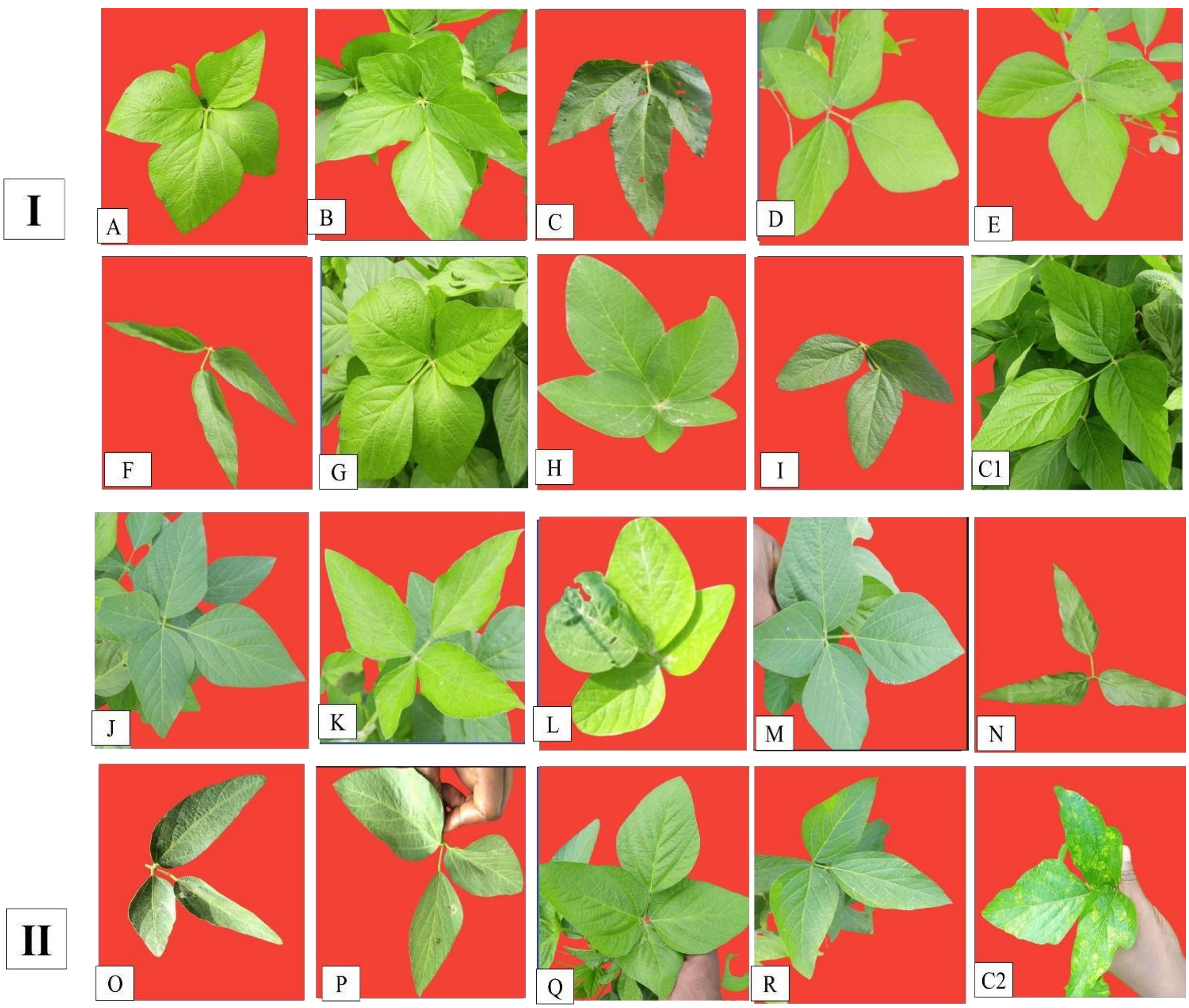


Figure 4.4. M_2 generation (**I**) Effect of different concentrations of three mutagens on the morphology of soybean (*Glycine max* L.) variety SL958 at 12 WAS. Mutagen I: Ethyl methane sulfonate (**A**) 0.2%, (**B**) 0.4%, (**C**) 0.6%. Mutagen II: Methyl methane sulfonate (**D**) 0.2%, (**E**) 0.4%, (**F**) 0.6%. Mutagen III: Sodium azide (**G**) 0.2%, (**H**) 0.4%, (**I**) 0.6%. (**C_1**) Control: SL958

(without mutagen treatment). (**II**) Effect of different concentrations of three mutagens on the morphology of SL744 soybean (*Glycine max* L.) variety at 12 WAS. Mutagen I: Ethyl methane sulfonate (**J**) 0.2%, (**K**) 0.4%, (**L**) 0.6%. Mutagen II: Methyl methane sulfonate (**M**) 0.2%, (**N**) 0.4%, (**O**) 0.6%. Mutagen III: Sodium azide (**P**) 0.2%, (**Q**) 0.4%, (**R**) 0.6%. (**C2**) Control: SL744 (without mutagen treatment).

4.5.6. Effect of chemical mutagens on days to 50% flowering

The time (days) to reach 50% flowering for the untreated control, M_1 and M_2 plants is displayed in Table 4.7. In SL744 and SL958, the untreated control plants took the most number of days to reach 50% flowering (68.00 and 63.33 days, respectively), while the T_9 (0.4% SA)-treated plants in SL958 took the least amount of time (39.67 and 0.94 days). However, the T_7-treated (0.6% MMS) M_1 plants took the greatest number of days (50.33 1.25 days) for the SL958 variety. Meanwhile, for SL744, T_8 (0.2% SA) took the greatest number of days to reach 50% flowering (51.33

± 0.47), while the T_9 (0.4% SA)-treated plants for SL744 took the least amount of time (46.67 ± 1.70 days) during the first-year study. In the second-year experiment, T_3 records the highest number of days to 50% flowering (67±3.06 days) for the SL 958 treated with 0.4% EMS. Meanwhile, the T_9 has the least number of days to reach flowering (50±2.89 days). On the other hand, for the SL744 variety, it took the T_1 plot (the untreated control) the longest (77±2.52 days) to reach flowering, while it took the T_2 plot (52±1.15 days) the shortest to reach 50% flowering.

The results, however, differed significantly from the untreated control plant (SL958: 65±2.52 days, SL744: 77±2.52 days).

Table 4.7. Effect of chemical mutagens on days to 50% flowering in M_1 and M_2.

	Data taking periods			
	2023/2024		2024/2025	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	68.00 ± 0.82^{a}	63.33 ± 1.25^{a}	77 ± 2.52^{a}	65 ± 2.52^{ab}
T2	47.67 ± 1.25^{de}	43.00 ± 0.82^{de}	52 ± 1.15^{de}	57 ± 3.51^{c}
T3	51.00 ± 1.63^{bc}	46.00 ± 0.82^{c}	62 ± 2.52^{bc}	67 ± 3.06^{a}
T4	51.00 ± 0.8^{bc}	45.00 ± 0.82^{cd}	55 ± 4.51^{d}	52 ± 1.15^{ef}
T5	47.00 ± 0.82^{e}	48.33 ± 1.70^{b}	52 ± 4.58^{de}	55 ± 2.52^{de}
T6	48.33 ± 1.70^{cde}	49.00 ± 0.82^{b}	57 ± 5.69^{c}	53 ± 3.00^{e}
T7	48.67 ± 1.25^{bcde}	50.33 ± 1.25^{b}	61 ± 6.66^{c}	56 ± 4.73^{d}
T8	51.33 ± 0.47^{b}	41.33 ± 0.47^{ef}	60 ± 2.52^{cd}	57 ± 2.08^{cd}
T9	46.67 ± 1.70^{e}	39.67 ± 0.94^{f}	63 ± 6.24^{bc}	50 ± 2.89^{g}
T10	50.00 ± 0.82^{bcd}	42.67 ± 0.47^{e}	67 ± 9.45^{b}	61 ± 1.53^{b}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.7. Effect of chemical mutagens on days to 50% podding in M_1 and M_2 plant

The time (days) to reach 50% podding in the untreated control and M1 plants is displayed in Table.4.12. It shows that the T_9 (0.4% SA)-treated M_1 plants of the SL958 variety exhibited early podding (69.00 ± 0.82 days) as compared to the untreated control plants (82.67 ± 1.25 days). Similar results were observed for the T_9 M_1 plants of the SL744 variety, for which podding occurred at 77.00 2.87 days, compared to the delayed podding of the untreated control plants (86.33± 0.94 days), during the first-year 2023 field trial. In the second-year 2024 experiment, T_1 records the highest number of days to podding (90.67±5.03 days to podding) for the SL 958 untreated control. Meanwhile, the T_9 has the least number of days to podding (63.67±4.04 days to podding). It took 93.00±4.58 days for the T_7 (0.6% MMS) treated plot to produce podding, which was the longest time. It took 77.33±5.03 days for the T_9 treated with 0.4% SA to produce podding, which was the shortest time. The results, however, differed positively from the untreated control plant (SL958: 90.67±5.03 days to podding; SL744: 86.00±7.21 days to podding).

Table 4.8. Effect of chemical mutagens on number of days to 50% podding.

	Data taking periods			
	2023/2024		2024/2025	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	86.33±0.94[a]	82.67±1.25[a]	86.00±7.21[bc]	90.67±5.03[a]
T2	80.33±0.94[e]	70.67±1.25[cd]	82.00±2.65[cd]	78.00±7.00[c]
T3	81.00±0.47[cd]	74.67±1.25b	85.00±4.36[c]	76.33±6.03[d]
T4	79.67±1.41[a]	74.33±1.25b	90.33±6.51[ab]	78.00±6.24[c]
T5	85.00±0.47[a]	80.00±0.82[a]	90.67±4.04[ab]	90.00±6.24[ab]
T6	85.33±3.56[bc]	80.67±1.25[a]	86.00±6.00[bc]	86.00±6.56[bc]
T7	84.33±0.47[a]	72.33±1.70[c]	93.00±4.58[a]	87.00±4.00b
T8	85.33±1.41[cd]	73.67±2.16[d]	81.67±3.06[d]	72.00±3.00[e]
T9	77.00±2.87[cd]	69.00±0.82[a]	77.33±5.03[e]	63.67±4.04[f]
T10	84.33±0.47[bc]	73.67±1.70[c]	89.33±3.51[b]	77.33±8.02[cd]

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.8. Effect of chemical mutagens on days to maturity

The results in Figure 4.4a show that treatment T6 had the highest values (141 days to maturing), followed by T_1, T_4, T_5, T_7, and T10 that were treated with untreated control, 0.6% EMS, 0.2% MMS, 0.6% MMS, and 0.6% SA. These treatments had similar values (140 days to maturing), as did treatments T_2, T_8, and T_9 that were treated with 0.2% EMS, 0.2% SA, and 0.4% SA. These treatments had the lowest values (138 days to maturing) and were treated with 0.4% EMS in SL744. However, in SL958, treatments T_1, T_5, and T_7, which were treated with untreated control, 0.2% MMS, and 0.6% MMS, recorded the highest values (143 days to maturing), while treatments T_3 and T_8, which were treated with 0.4% EMS, 0.2% SA, and 0.4% SA, respectively, recorded the lowest values (140 days to maturing). However, during the 2024/2025 field experiment. The highest values (142 days to maturing) treated with 0.6 MMS were observed in T_7, and the lowest values (137 days to maturing) were recorded in T_8 treated with 0.2% SA in SL744. Furthermore, in SL958, the highest value (142 days to maturing) is in T_5, T_7, and T_{10} treated with (0.2% MMS, 0.6% MMS, and 0.6% SA, respectively), and the lowest values (139 days to maturing) are in T_2 and T_8 treated with (0.2% EMS and 0.2% SA) in the SL958 variety.

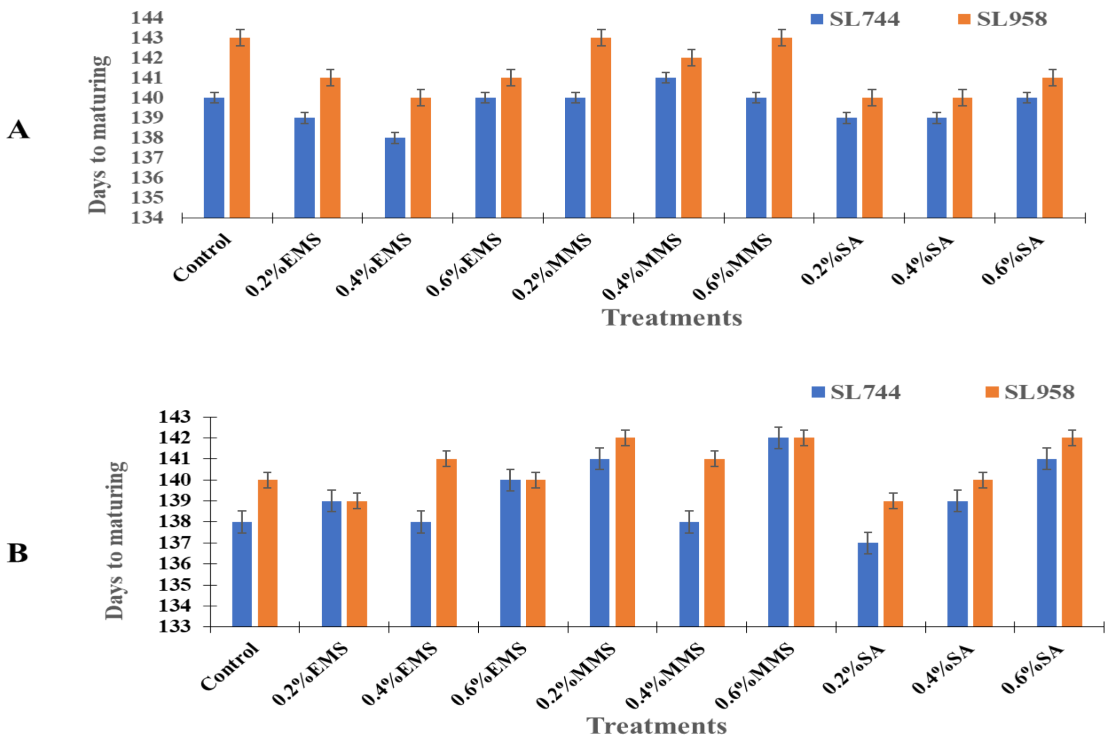


Figure 4.5. Effect of chemical mutagens on days to maturing. **(A)** Effect on days to maturing in 2023/2024 field trial (**B**) Effect on days to maturing in 2024/2025 field trial.

4.5.9. Effect of chemical mutagens on number of podding on M_1 and M_2 plant

The table 4.9. represent the effect of chemical mutagens on number of podding of M_1 and M_2 plant. The results indicated that, there's significant differences in terms of yield among both of the varieties, the highest yield (277.55± 1.37 pods/plant) was recorded for the M_1 plants of the SL958 variety treated with 0.4% SA (T_9), while the lowest (217.55± 2.20 pods/plant) was recorded for the T_4 plants treated with 0.4% EMS compared to the untreated control (229.86± 0.96 pods/plant). However, in the SL744 variety, the highest yield (273.00 ±4.55 pods/plant) was recorded for T_4 (0.4% EMS), while the lowest (112.2 pods/plant) was observed in the SL744 variety treated with 0.2% EMS (T_2). The results indicate that 0.4% SA effectively enhanced the yield of the SL958 variety, during the first-year study. In the second-year experiment, T_9 records the highest number of pods per plant (368.6±54.72 pod/plant) for the SL 958 treated with 0.4% SA. Meanwhile, the T_7 having the least number of pods per plant (188.4±14.22 pod/plant) 12WAS.Moreover, for the SL744 variety at 12 WAS, the T_9 (0.4% SA) treated plot exhibited the number of pos per plant (257.2±27.59 pods/plant), and the lowest height was recorded for T_7 (162.8±18.01 pods/plant) treated with 0.6%MMS. The results, however, differed positively from untreated control plant (SL958: 26.7±0.99 cm SL744: 28.74±1.04 cm).

Table 4.9. Effect of chemical mutagens on number of pods on M_1 and M_2 plant.

	Data taking periods			
	2023/2024		2024/2025	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	164.33±8.58d	$229.86±0.96^{e}$	$234.8±24.47^{c}$	$259.6±23.17^{b}$
T2	$112.22±5.92^{e}$	$228.44±1.23^{e}$	$219.2±21.21^{bc}$	$251.4±11.63^{b}$
T3	$264.11±3.09^{ab}$	$261.00±0.82^{b}$	$255±45.50^{a}$	$290.6±25.71^{b}$
T4	$273.00±4.55^{a}$	$217.55±2.20^{g}$	$194.4±27.36^{cd}$	$194±5.20^{c}$
T5	$260.44±30.99^{ab}$	$228.00±2.67^{d}$	$204.4±14.21^{cd}$	$198.8±23.64^{c}$
T6	232.22±1.10ab	$222.78±0.82^{e}$	$192.4±28.78^{de}$	$197.4±20.55^{c}$
T7	$179.33±33.07^{cd}$	$270.11±1.10^{f}$	$162.8±18.01^{e}$	$188.4±14.22^{c}$
T8	$146.66±50.87^{bc}$	$269.78±0.95^{b}$	$255.8±19.45^{bc}$	$276.6±53.27^{b}$
T9	$206.54±23.47^{de}$	$277.55±1.37^{b}$	$257.2±27.59^{a}$	$368.6±54.72^{a}$
T10	$216.67±4.34^{bc}$	$276.69±1.23^{a}$	$212.2±14.62^{c}$	$276.2±46.43^{b}$

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.10. Effect of chemical mutagens on pod length (cm)

The results indicate that, some of the pods that were treated with 0.2% EMS, 0.4% EMS, and 0.4% SA had the shortest lengths (3.1 ± 0.21, 3.1 ± 0.14, and 3.1 ± 0.64 cm), while the pod that was treated with 0.4% SA had the longest length (4.7 ± 0.14 cm) in SL958. These values were very different from one another. However, SL744, which had a 0.40 % SA concentration, had the longest length (3.8 ± 0.14 cm) in T_9, while T_7 had the shortest pods (3.1 ± 0.21 cm). In the second-year experiment, T_9 records the highest pod length (4.30 ± 0.20 cm) for the SL 958 treated with 0.4% SA. Meanwhile, the T_7, having the least pod length (3.27 ± 0.21 cm), was treated with 0.6% MMS. Moreover, for the SL744 variety, the T_3 (0.4% EMS) treated plot exhibited the highest pod length (3.93 ± 0.44 cm), and the lowest pod length was recorded for T_7 (3.23 ± 0.15 cm) treated with 0.6% MMS. The results, however, differed positively from the untreated control plant (SL958: 3.40 ± 0.20 cm, SL744: 3.27 ± 0.21 cm).

Table 4.10. Effect of chemical mutagens on pod length (cm).

	Data taking periods			
	2023/2024		2024/2025	
Treatments	SL744	SL958	SL744	SL958
T1(Control)	$3.1{\pm}0.28^{d}$	$3.2{\pm}0.14^{cd}$	$3.27{\pm}0.21^{c}$	$3.40{\pm}0.20^{bc}$
T2	$3.2{\pm}0.21^{cd}$	$3.1{\pm}0.21^{e}$	$3.30{\pm}0.17^{bc}$	$3.23{\pm}0.15^{c}$
T3	$3.4{\pm}0.57^{b}$	$4.0{\pm}0.21^{ab}$	$3.93{\pm}0.44^{ab}$	$3.53{\pm}0.51^{bc}$
T4	$3.2{\pm}0.28^{c}$	$3.1{\pm}0.14^{de}$	$3.30{\pm}0.26^{bc}$	$3.07{\pm}0.15^{c}$
T5	$3.5{\pm}0.07^{ab}$	$3.6{\pm}0.21^{b}$	$3.37{\pm}0.15^{abc}$	$3.33{\pm}0.25^{bc}$
T6	$3.2{\pm}0.21^{de}$	$3.5{\pm}0.28^{bc}$	$3.47{\pm}0.25^{abc}$	$3.33{\pm}0.21^{bc}$
T7	$3.1{\pm}0.21^{d}$	$3.2{\pm}0.21^{cd}$	$3.23{\pm}0.15^{c}$	$3.27{\pm}0.21^{bc}$
T8	$3.2{\pm}0.49^{cd}$	$3.1{\pm}0.64^{d}$	$3.90{\pm}0.36^{ab}$	$3.80{\pm}0.52^{b}$
T9	$3.8{\pm}0.14^{a}$	$4.7{\pm}0.14^{a}$	$3.91{\pm}0.47^{a}$	$4.30{\pm}0.20^{a}$
T10	$3.4{\pm}0.49^{bc}$	$3.4{\pm}0.21^{c}$	$3.57{\pm}0.47^{abc}$	$3.23{\pm}0.51^{c}$

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.11. Effect of chemical mutagens on number of seeds per pod

The number of seeds per pod was highest in SL958 with a value of 4.04 ± 0.19 seed per pod, treated with 0.4% SA in T_8. While the lowest values were recorded in T_7 (2.36 ± 0.24 seed per pod) treated with 0.6% MMS. These values were significantly different from each other. The most seeds were found in SL744 T_2, which had 3.38 ± 0.3 seeds per pod, while the fewest seeds were found in T_{10}, which had 2.2 ± 0.23 seeds per pod. This was very different from the untreated control, which had 2.44 ± 0.24 seeds per pod, and SL744 had 2.38 ± 0.24 seeds per pod. In the second-year experiment, T_9 records the highest number of seeds per pod (4.09 ± 0.12 seed per pod) for the SL 958 treated with 0.4% SA. Meanwhile, the T_{10} has the least number of seeds per pod (2.51 ± 0.9 seed per pod). Moreover, for the SL744 variety, the T_8 (0.2% SA) treated plot exhibited the highest value (3.74 ± 0.21 seed per pod), and the lowest value was recorded for T4 (2.7 ± 0.31 seed per pod) treated with 0.4% EMS. The results, however, differed positively from the untreated control plant (SL958: 2.09±0.14 seed per pod, SL744: 2.8 ± 0.21 seed per pod).

Table 4.11. Effect of chemical mutagens on number of seed per pod.

	Data taking periods			
	2023/2024		2024/2025	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	2.38 ± 0.24^{de}	2.44 ± 0.24^{ab}	2.8 ± 0.21^{d}	2.09 ± 0.14^{e}
T2	3.38 ± 0.3^{a}	3.69 ± 0.20^{ab}	3.3 ± 0.2^{ab}	3.82 ± 0.22^{ab}
T3	2.7 ± 0.22^{bc}	3.56 ± 0.30^{b}	2.9 ± 0.22^{b}	3.72 ± 0.34^{b}
T4	2.34 ± 0.32^{e}	3.22 ± 0.19^{b}	2.7 ± 0.31^{de}	3.32 ± 0.13^{ab}
T5	2.44 ± 0.21^{cd}	2.46 ± 0.23^{b}	2.84 ± 0.24^{c}	2.83 ± 0.28^{cd}
T6	2.42 ± 0.29^{d}	3.46 ± 0.30^{ab}	2.91 ± 0.26^{b}	3.21 ± 0.31^{bc}
T7	2.36 ± 0.30^{de}	2.36 ± 0.24^{b}	2.84 ± 0.32^{c}	2.71 ± 0.27^{de}
T8	3.36 ± 0.23^{a}	3.56 ± 0.27^{ab}	3.74 ± 0.21^{a}	2.93 ± 0.47^{c}
T9	2.94 ± 0.23^{b}	4.04 ± 0.19^{a}	3.21 ± 0.26^{ab}	4.09 ± 0.12^{a}
T10	2.2 ± 0.23^{c}	2.76 ± 0.29^{ab}	2.9 ± 0.26^{b}	2.51 ± 0.9^{d}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA.

4.5.12. Effect of chemical mutagens on 100 pods weight (g)

The statistical analysis reveals significant differences among varieties in Table 4.12 when it comes to the weight of 100 pods. The least 100 pod weight was recorded in T_{10}, SL958 (0.6% SA), with a value of 24.55 ± 2.12g, and the highest weight was recorded in T_9 & T_3 (30.80 ± 2.83g & 30.10 ± 2.12g) with 0.4% SA & 0.4% EMS. However, for the SL744 variety, T_9 recorded the highest value (32.10 ± 1.41 g), treated with 0.4% SA, and the least was observed in T_4 and T_7 with values of 25.02 ± 1.41 g and 25.03 ± 2.83 g, treated with 0.6% EMS and 0.6% MMS, respectively. These values were significantly different from one another and from the untreated control (SL958: 30.00 ± 2.12 g & SL744: 31.22 ± 1.41 g). Furthermore, SL958 and SL744 significantly differed from the control during the first-year study. In the second-year experiment, T_9 records the highest pod weight (33.67±3.06 g) for the SL 958 treated with 0.4% SA. Meanwhile, the T_7 has the least pod weight (26.00 ± 2.65g). Moreover, for the SL744 variety, the T_1, T_3, and T_9 (untreated control, 0.4% EMS, and 0.4% SA, respectively) plots exhibited the highest pod weight (31.33 ± 1.53 g, 30.00 ± 1.00 g, and 31.00 ± 2.00 g), and the lowest value was recorded for T_4 and T_7 (27.33 ± 2.08 g and 27.00 ± 2.00 g) treated with 0.6% EMS and 0.6% MMS. The results, however, differed positively from the untreated control plant (SL958: 30.33 ± 1.53 g, SL744: 31.33 ± 1.53 g).

Table 4.12. Effect of chemical mutagens on 100 pod weight (g).

	Data taking periods			
	2023/2024		**2024/2025**	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	31.22±1.41^{ab}	30.00±2.12b	31.33±1.53a	30.33±1.53^{ab}
T2	29.00±0.71^{c}	26.22±1.41^{e}	30.00±1.00^{abc}	31.67±2.52^{a}
T3	31.33±0.71^{ab}	30.10±2.12^{ab}	31.33±1.53^{a}	31.67±2.08^{a}
T4	25.02±1.41^{de}	29.22±2.12^{d}	27.00±2.00^{ab}	28.67±1.53^{ab}
T5	30.04±0.71^{b}	28.17±1.41^{de}	30.33±1.53^{ab}	26.67±1.53^{b}
T6	26.22±1.41^{d}	28.33±4.24^{de}	28.00±2.00^{bc}	28.00±3.00^{ab}
T7	25.03±2.83^{e}	29.45±0.71^{c}	27.33±2.08^{bc}	26.00±2.65^{b}
T8	28.08±1.41^{cd}	29.03±2.12^{cd}	29.67±1.53^{abc}	30.67±2.08^{ab}
T9	32.10±1.41^{a}	30.80±2.83^{a}	31.00±2.00^{a}	33.67±3.06^{a}
T10	30.02±0.71^{bc}	24.55±2.12^{ef}	30.00±1.00^{abc}	28.33±4.04^{ab}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.5.13. Effect of chemical mutagens on 100 seeds weight (g)

The highest value for 100 seed weight in T_9 was recorded in SL958 (SA Concentration 0.40%) with 10.8 g and the lowest value in T_{10} was recorded as 8.7 g in SL958 treated with 0.60% SA Concentration. However, in the SL744 variety, the highest 100 seed weight (10.5 g) was recorded for T_9 treated with 0.4% SA, while the lowest (8.1 g) was observed in the SL744 variety treated with 0.2% MMS (T_7) during the first-year study. In the second-year experiment, T9 achieved the highest 100 seed weight (10.2 g) for the SL 958 variety, which was treated with 0.4% SA. Meanwhile, the T_7 has the least 100 seed weights (8.2 g). Moreover, for the SL744 variety, the T_9 (0.4% SA) treated plot exhibited the highest 100 seed weight (9.8 g), and the lowest 100 seed weight was recorded for T_7 (7.8 g) treated with 0.6% MMS. The results, however, differed positively from the untreated control plant (SL958: 9.5 g, SL744: 8.9 g). These highest and lowest values were statistically significantly different from each other and from all the values of other varieties

Table 4.13. Effect of chemical mutagens on 100 seed weight (g).

Data taking periods				
	2023/2024		2024/2025	
Treatments	SL744	SL958	SL744	SL958
T1(Control)	9.2	9.0	8.9	9.5
T2	9.1	9.6	9.0	8.9
T3	9.0	9.6	8.7	9.2
T4	8.2	9.5	8.1	9.1
T5	8.3	9.1	8.7	9.6
T6	8.4	8.8	8.1	8.3
T7	8.1	8.0	7.8	8.2
T8	9.0	9.4	9.1	9.0
T9	10.5	10.8	9.8	10.2
T10	9.6	8.7	9.0	9.1

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = standard deviation ±, WAS = weeks after sowing, T = treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.6. Effect of chemical mutagens on the pollen grain of soybean flower

The pollen grains of a soybean flower (***Glycine max*** **L.**) are typically spherical or elliptical in shape and covered with a smooth or slightly textured exine (outer layer) (Mendieta & Granados, 2015). These grains are relatively small, with sizes ranging from approximately 25 to 35 micrometers in diameter, which were transported from the male anther to the female stigma, either within the same flower (self-pollination) or between different flowers (cross-pollination) (Razzaq et al., 2019). In our studies, the scan electron microscope (SEM) images depict a wide variety of soybean pollen grains, ranging from smooth to highly wrinkled surfaces and from small spherical shapes to larger, more irregular grains. The surface roughness, shape, and size of the pollen may indicate the effect of different chemical mutagens, environmental conditions, dehydration, or developmental stages, as shown below.

- **(A) :** An irregular and compressed structure, the grains appear to be clustered, with folds or wrinkles on the surface indicating potential dehydration or structural damage.
- **(B) :** The grains are oval-shaped, loosely packed, moderate in size, but comparatively smaller than those in A. The surface shows some distortion, with irregular textures that may indicate a treatment process affecting pollen structure.
- **(C) :** Rounded, discrete grains; scattered, small, uniform particles visible in isolation; smooth and round; less damage compared to A and B.
- **(D) :** Spherical or oval grains grouped together, slightly larger than C but still smaller than B. Wrinkled appearance, indicative of possible dehydration.
- **(E) :** Elongated and clustered pollen grains, relatively larger with noticeable folds. Very rough surface, with distinct ridges.

(F) : The grains are irregularly clustered, displaying deep indentations and larger pollen grains. The surface looks deeply wrinkled, with intricate textures, possibly due to environmental factors or treatment.

(G) : The grains are spherical and closely packed, while the pollen is medium-sized and rounded**.** The surface appears smoother than in other samples, though some surface depressions are visible.

(H) : The pollen grains are roughly circular, have irregular shapes, and are small, yet more spherical and distinct than F and G. Compared to F and G, it has a finer surface texture and less distortion**.**

(I) : The grains are elongated and exhibit some irregularity; their size is comparable to that of H. The surface appears mildly wrinkled, possibly indicating a mid-stage pollen condition.

(C1): The pollen grains are small, rounded, and arranged in clusters; their size is significantly smaller than those of other pollen**.** The surface is relatively smooth, showing few depressions or irregularities.

(J) : Mostly circular but showing signs of compression or flattening, medium-sized grains. The surface **is** moderately rough, with a coarse texture.

(K) : Elongated grains, slightly fused at the ends. Size **is** slightly larger than J. The surface appears wrinkled.

(L) : Small, isolated, spherical pollen grains**.** The surface is smooth, indicating a relatively healthy or undamaged state.

(M) : Small, rounded, spherical grains are clustered together. The surface is smooth.

(N) : The pollen grains are elongated and have a tubular shape, making them larger than those in other samples**.** The surface is rough and crumpled, possibly indicating external damage or stress.

(O) : Circular and elongated grains; larger, irregularly sized grains. The surface is wrinkled, indicating possible aging or damage.

(P) : Rounded pollen grains, fused in clusters, medium to large. The surface exhibits roughness and deep wrinkles, which may be a result of environmental conditions.

(Q) : The pollen grains are elongated, closely packed, and of moderate size. The surface has a medium level of roughness.

(R) : These are irregularly shaped, elongated pollen grains, similar in size to O and P. The surface is wrinkled and rough, indicative of stress or processing.

(C2): The pollen grains are rounded and clustered, with a size smaller than R**.** The surface is moderately smooth, indicating healthier or less affected pollen grains.

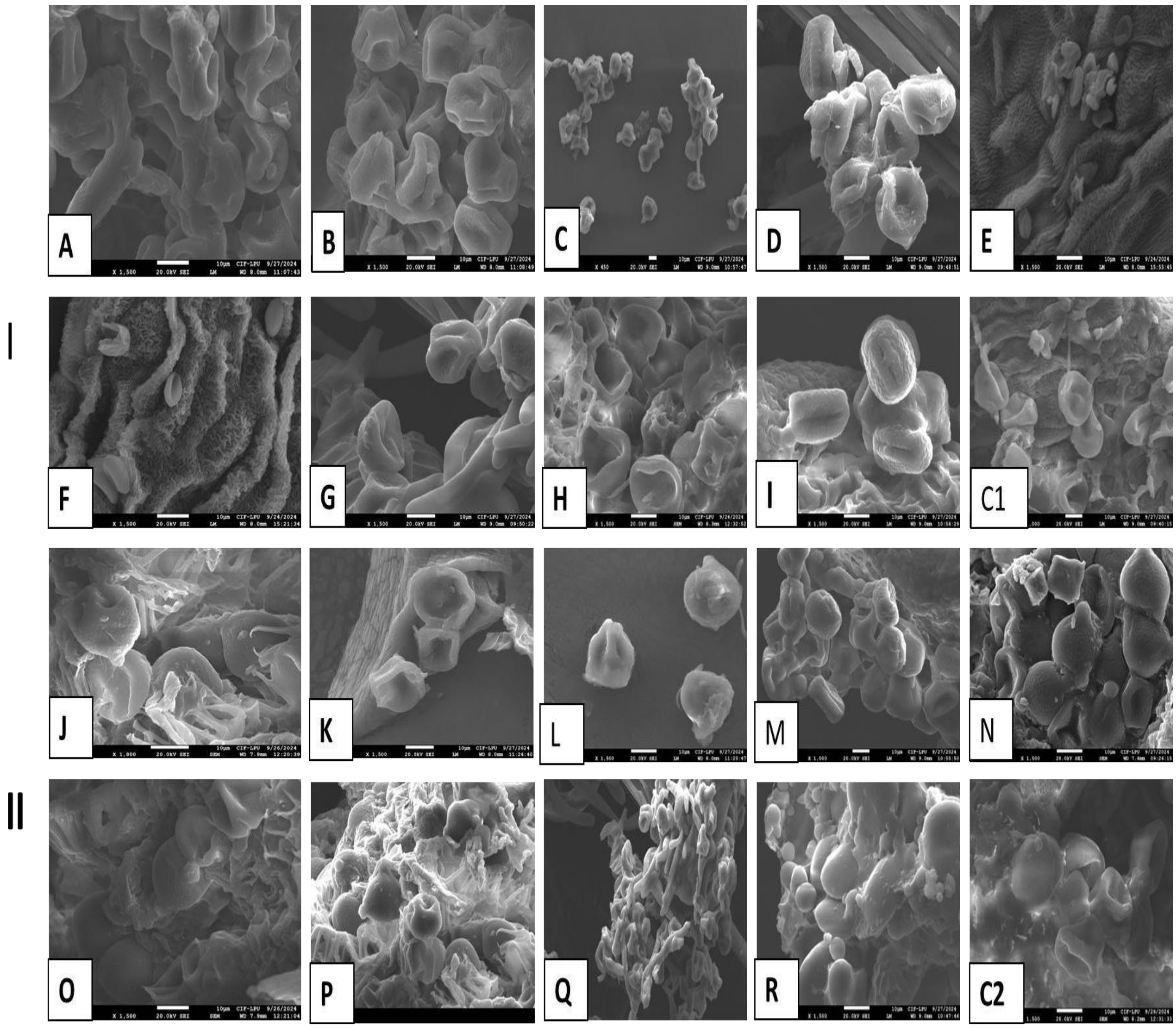


Fig 4.6. (I). Effect of different concentrations of three mutagens on the pollen grain of two soybean (*Glycine max* L.) varieties. Variety I: SL958-Mutagen I: Ethyl methane sulfonate (**A**) 0.2%, (**B**) 0.4%, (**C**) 0.6%, Mutagen II: Methyl methane sulfonate (**D**) 0.2%, (**E**) 0.4%, (**F**) 0.6%, Mutagen III: Sodium azide (**G**) 0.2%, (**H**) 0.4%, (**I**) 0.6%, (**C1**) SL958-Control. **(II)**. Effect of different concentrations of three mutagens on the pollen grain of two soybean (*Glycine max* L.) varieties 12WAS. Variety II: SL744 - Mutagen I: Ethyl methane sulfonate (**J**) 0.2%, (**K**) 0.4%, (**L**) 0.6%; Mutagen II: Methyl methane sulfonate (**M**) 0.2%, (**N**) 0.4%, (**O**) 0.6%; Mutagen III: Sodium azide (**P**) 0.2%, (**Q**) 0.4%, (**R**) 0.6%, **(C2)** SL744-Control.

4.7. Impact of chemical mutagens on Energy Dispersive X-ray Spectroscopy (EDS) in the pollen grain of soybean flowers.

The EDS analysis provides valuable insights into the elemental composition of soybean varieties. The flower sample plays a crucial role in assessing the suitability of these varieties for enhanced yield production.

4.7.1. The elemental composition of soybean (SL958) variety treated with 0.4% SA.

The results show that carbon (C) was observed to be 60.06%, and it also forms the backbone of organic molecules like carbohydrates, proteins, lipids, and nucleic acids. Soybean plants, like all plants, fix carbon through photosynthesis, storing it in structures like cellulose and starch. While nitrogen (N) was recorded at 5.51%, it plays a crucial role in the production of amino acids (proteins), nucleotides (DNA/RNA), and chlorophyll. Soybeans, being legumes, are known for their symbiotic relationship with nitrogen-fixing bacteria (Rhizobium), which explains the significant nitrogen content. Mutagens may not have significantly affected nitrogen fixation, which is crucial for soybeans' symbiosis with nitrogen-fixing bacteria. Furthermore, oxygen (O) was observed (30.18%). It plays a crucial role in respiration and metabolic processes. Magnesium (Mg) (0.27%) is essential in biological systems, often associated with enzyme function and photosynthesis in plants. Silicon (Si) (0.06%) provides structural support, enhances stress resistance, and helps in cell wall strengthening. The absence of phosphorus (P) (0.0%) in this data is unusual, given its essential role in ATP, DNA, and membrane phospholipids. This could be that the phosphorus content is below detectable levels in this specific analysis; furthermore, mutagen-induced disruption in phosphorus uptake, metabolism, or the plant’s ability to synthesize nucleotides and ATP efficiently—sulfur (S) (0.24%)—is crucial for synthesizing sulfur-containing amino acids (cysteine, methionine) and some vitamins (biotin, thiamine). It also plays a role in forming certain plant defense compounds, potassium (K) (1.56%); it regulates water balance, stomatal function, and enzyme activation in plants. It plays a crucial role in maintaining turgor pressure and photosynthesis efficiency, while Calcium (Ca) (0.83%) plays a crucial role in cell wall structure and stability, particularly in the process of pectin cross-linking. It also acts as a secondary messenger in signal transduction pathways. However, some of the trace elements (Manganese (Mn) - 0.01%; Copper (Cu) - 0.66%; Zinc (Zn) - 0.49%; Molybdenum (Mo) - 0%; Chlorine (Cl) - 0.12%) were also detected. The high carbon, nitrogen, and oxygen content reflects the effect of chemical mutagens and their role in organic molecule synthesis and photosynthesis. Elements such as Cu, Zn, and Mn bolster the functions of enzymes.

Table 4. The elemental composition of soybean variety (SL958) treated with 0.4% SA.

Elements	**Percentage (%)**
B	0.0
C	60.06
N	5.51
O	30.18
Mg	0.27
Si	0.06
P	0.0
S	0.24
Cl	0.12
K	1.56
Ca	0.83
Mn	0.01
Cu	0.66
Zn	0.49
Mo	0.0

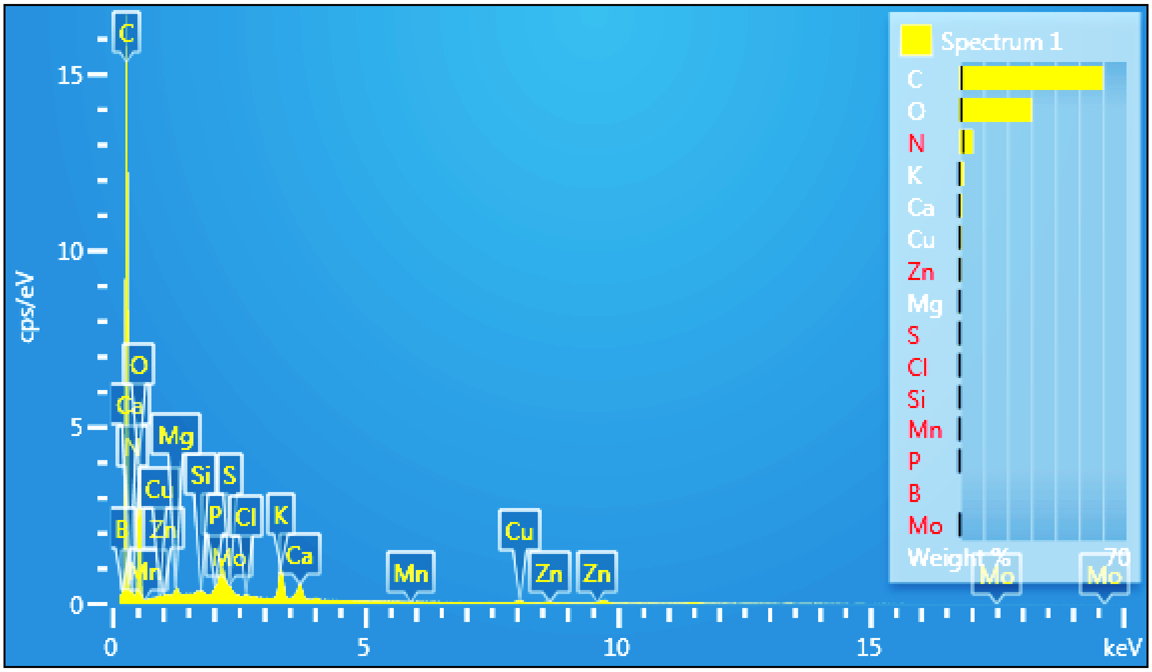


Fig 4.7. Effect of chemical mutagen on elemental composition of variety (SL958) treated with 0.4%SA.

4.7.2. The elemental composition of soybean variety (SL744) treated with 0.4% SA.

The results show that Carbon (C) is 57.24%, Nitrogen (N) is 2.01%, Oxygen (O) is 31.91%, Magnesium (Mg) is 0.31%, Silicon (Si) is 0.4%, Phosphorus (P) is 0.0%, Sulfur (S) is 0.0%, Chlorine (Cl) is 0.18%, Potassium (K) is 4.49%, Calcium (Ca) is 0.7%, Manganese (Mn) is 0.0%, Copper (Cu) is 1.69%, Zinc (Zn) is 1.07%, and Molybdenum (Mo) is 0.0%. Chemical mutagens can change the elements in soybean plants. This shows that while core metabolic processes like photosynthesis, carbon fixation, and protein synthesis are mostly still working, there may be problems with phosphorus uptake and molybdenum-dependent nitrogen fixation. Elevated copper and zinc levels suggest an increased need for stress responses, likely due to oxidative damage caused by the mutagens. These changes could lead to long-term developmental effects, even if immediate metabolic functions remain stable.

Table 4.15. The elemental composition of soybean variety (SL744) treated with 0.4% SA.

Elements	Percentage (%)
B	0.0
C	57.24
N	2.01
O	31.91
Mg	0.31
Si	0.4
P	0.0
S	0.0
Cl	0.18
K	4.49
Ca	0.7
Mn	0.0
Cu	1.69
Zn	1.07
Mo	0.0

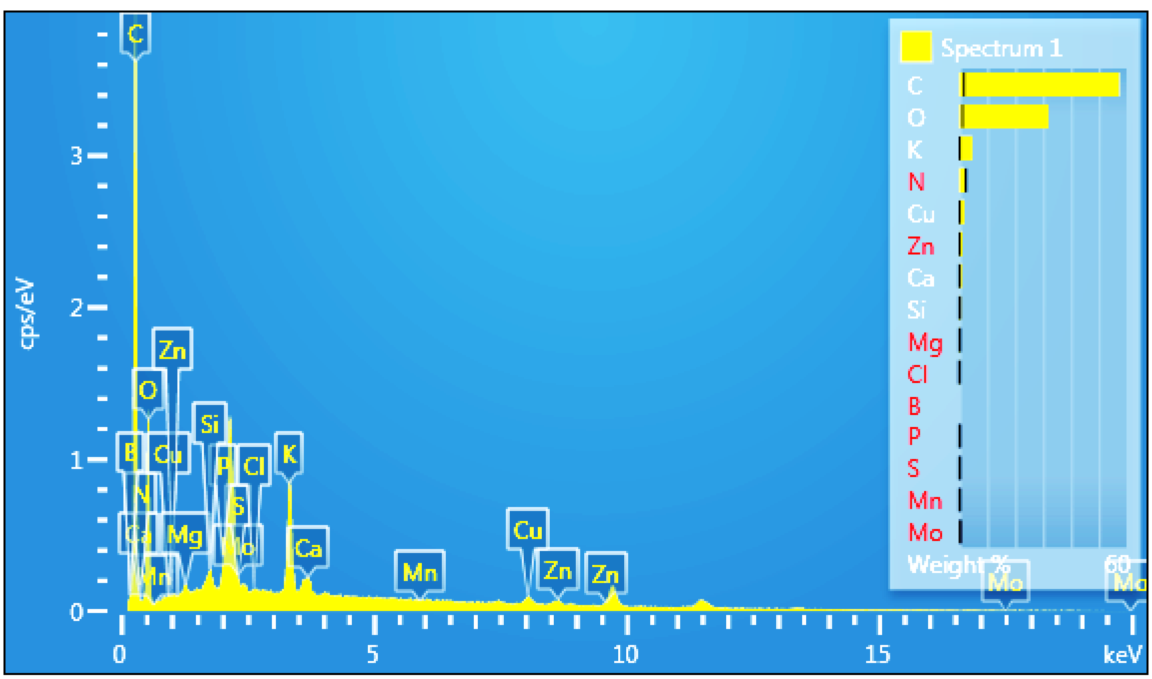


Fig 4.8. Effect of chemical mutagen on elemental composition of variety (SL744) treated with 0.4%SA.

4.7.3. The elemental composition of soybean variety (SL958) untreated control

The results show that Carbon (C) is 54.64%, Nitrogen (N) is 0.48%, Oxygen (O) is 34.02%, Magnesium (Mg) is 0.6%, Silicon (Si) is 1.37%, Phosphorus (P) is 0.0%, Sulfur (S) is 0.0%, Chlorine (Cl) is 0.09%, Potassium (K) is 4.51%, Calcium (Ca) is 1.36%, Manganese (Mn) is 0.09%, Copper (Cu) is 1.69%, Zinc (Zn) is 1.15%, and Molybdenum (Mo) is 0.0%.

The elemental composition of soybean material is rich in carbohydrates and possibly cellulose, with structural reinforcement from silicon and calcium. The absence of phosphorus in this data is unusual, as it is essential for ATP, DNA, and membrane phospholipids. This could be due to the phosphorus content being below detectable levels in this specific analysis, or it could be due to disruptions in phosphorus uptake, metabolism, or the plant's ability to synthesize nucleotides and ATP efficiently caused by mutagens. The absence of molybdenum could indicate a compromised nitrogen fixation process, potentially harmful to long-term growth but not immediately apparent in early metabolic measurements. The relatively high magnesium, calcium, and silicon levels point to a plant with structural adaptations or a mineral-rich environment. The high copper and zinc levels help in enzymatic or catalytic roles.

Table 4.16. The elemental composition of soybean variety SL958 (untreated control).

Elements	Percentage (%)
B	0.0
C	54.64
N	0.48
O	34.02
Mg	0.6
Si	1.37
P	0.0
S	0.0
Cl	0.09
K	4.51
Ca	1.36
Mn	0.09
Cu	1.69
Zn	1.15
Mo	0

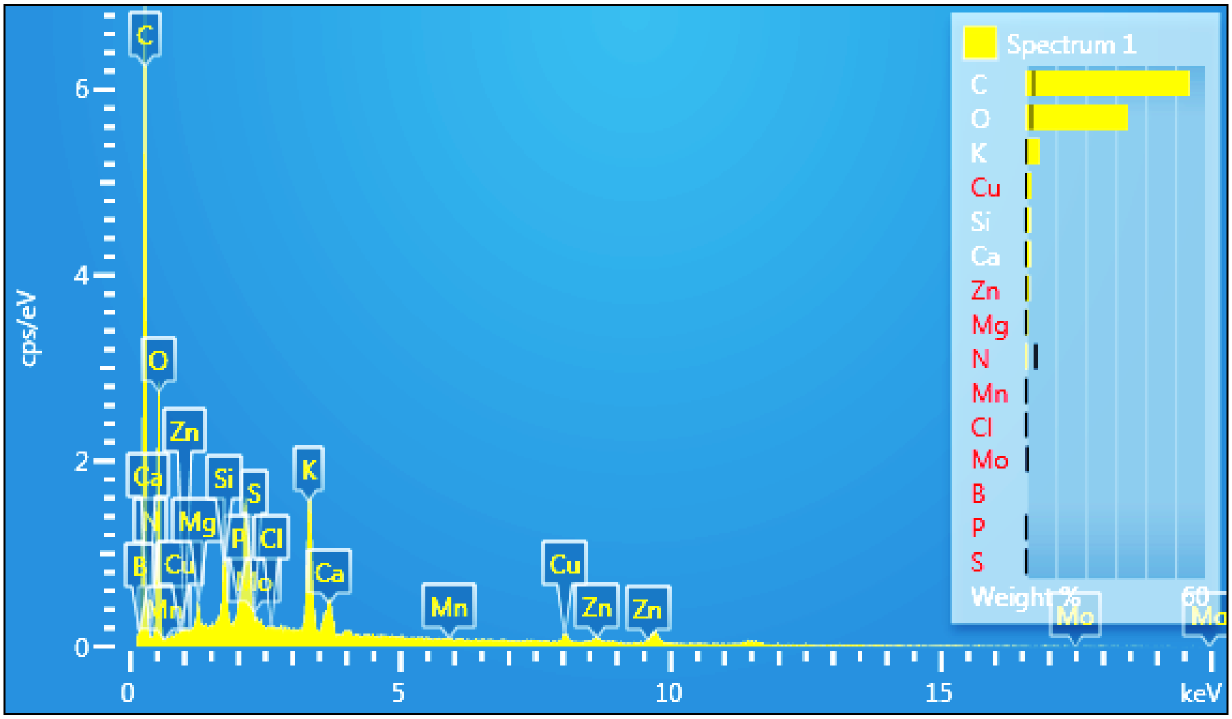


Fig 4.9. Effect of chemical mutagen on elemental composition of soybean variety (SL958) untreated control.

4.7.4. The elemental composition of soybean variety (SL744) untreated control

The results show that Carbon (C) is 51.23%, Nitrogen (N) is 0.0%, Oxygen (O) is 38.5%, Magnesium (Mg) is 1.28%, Silicon (Si) is 2.18%, Phosphorus (P) is 0.0%, Sulfur (S) is 0.0%, Chlorine (Cl) is 0.08%, Potassium (K) is 2.36%, Calcium (Ca) is 2.67%, Manganese (Mn) is 0.04%, Copper (Cu) is 1.08%, Zinc (Zn) is 0.59%, and Molybdenum (Mo) is 0.0%. The elemental composition of soybean material is rich in carbohydrates and possibly cellulose, with structural reinforcement from silicon and calcium. The absence of phosphorus in this data is unusual, as it is essential for ATP, DNA, and membrane phospholipids. This could be due to the phosphorus content being below detectable levels in this specific analysis, or it could be due to mutagen-induced disruptions in phosphorus uptake, metabolism, or the plant's ability to synthesize nucleotides and ATP efficiently. The relatively high magnesium, calcium, and silicon levels point to a plant with structural adaptations or a mineral-rich environment. The high copper and zinc levels help in enzymatic or catalytic roles. The lack of molybdenum may signal a compromised nitrogen fixation process, which could be detrimental to long-term growth but not immediately evident in early metabolic measurements.

Table 4.17. The elemental composition of soybean variety (SL744) untreated control.

Elements	Percentage (%)
B	0.0
C	51.23
N	0.0
O	38.5
Mg	1.28
Si	2.18
P	0.0
S	0.0
Cl	0.08
K	2.36
Ca	2.67
Mn	0.04
Cu	1.08
Zn	0.59
Mo	0.0

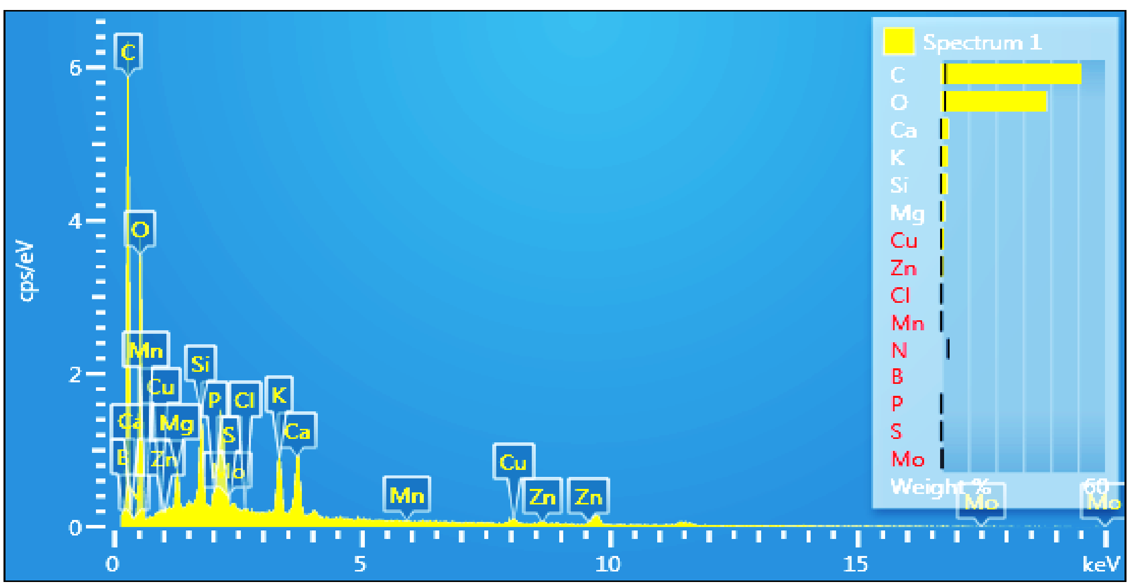
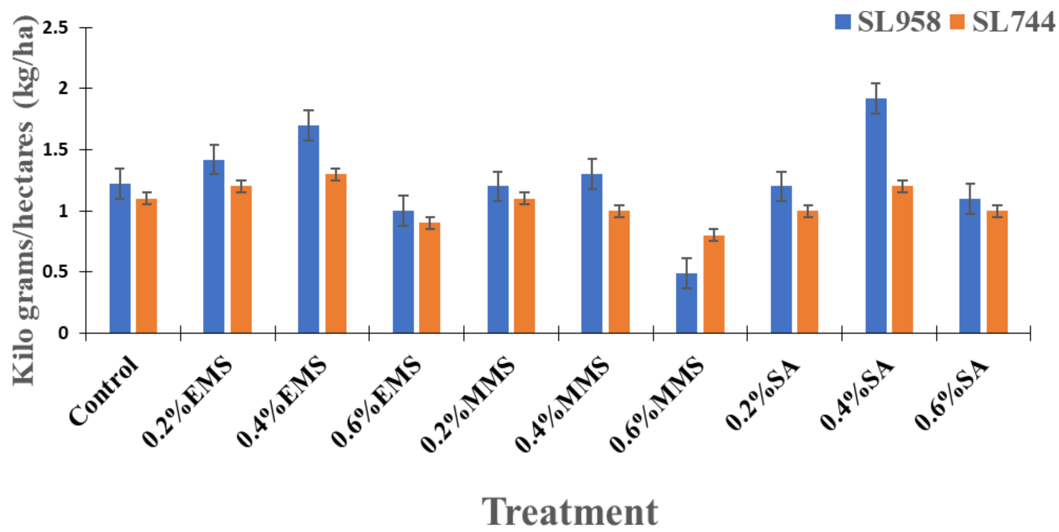


Fig 4.10. Effect of chemical mutagen on elemental composition of soybean variety (SL744) untreated control.

4.8. Effect of chemical mutagens on biological yield in M_1 and M_2 plant

The results show that 0.4% EMS exhibits an increasing trend up to 1.42 to 1.7 g, followed by a sharp drop at 0.6% EMS to 1 g in SL958, while SL744 displays a similar increasing trend up to 0.4% EMS (1.2 to 1.3 g), followed by a decrease at 0.6% EMS to 0.9 g. This indicates a possible optimal concentration at 0.4% EMS for both varieties, beyond which EMS becomes more effective. However, SL958 maintains stability from 0.2% MMS to 0.4% MMS (1.2 to 1.3 g), but experiences a significant decline at 0.6% MMS (0.49 g). Conversely, SL744 exhibits a slight decline from 0.2% MMS to 0.4% MMS (1.1 to 1 g), which is followed by a larger drop at 0.6% MMS (0.8 g). MMS appears to have a more negative impact at higher concentrations (0.6% MMS) for both varieties. However, SL958 exhibits an increase at 0.4% SA (1.2 to 1.92 g), followed by a drop at 0.6% SA (1.1 g), whereas SL744 maintains a relatively stable level across all concentrations (1 to 1.2 to 1 g). While SA at 0.4% appears beneficial for SL958, it has little negative effect on SL744 during the first year of the 2023 experiment compared to the control (SL958: 1.22 g, SL744: 1.1 g). Moreover, in the second year of the 2024 experiment, the SL958 variety gradually increased from 0.2% EMS to 0.4% EMS (1.52 g to 1.60 g), followed by a decrease at 0.6% EMS (1.10 g). EMS improves response at lower concentrations (0.2% to 0.4%) but has a negative effect at higher concentrations (0.6%). The SL744 variety exhibits a similar pattern: an increase from 0.2% to 0.4% EMS (1.20g to 1.30g), followed by a decrease at 0.6% EMS (0.96g). On the other hand, the MMS, SL958 variety, shows a slight increase from the control to 0.4% MMS (1.13g to 1.30g), followed by a sharp drop at 0.6% MMS (0.82g). Meanwhile, the SL744 variety demonstrates stability at 0.2% and 0.4% MMS (1.10 g), followed by a slight drop at 0.6% MMS (1.00 g). In comparison to the control (SL958: 1.24g, SL744: 1.00g), the SL958 variety gradually increased from 0.2% to 0.4% SA (1.40g to 1.94g), followed by a decline at 0.6% SA (1.20g), while the SL744 variety slightly increased at 0.2% SA and 0.4% SA (1.17g), followed by a slight decrease at 0.6% SA (1.10g). SA at 0.4% is highly effective for SL958 but has minimal impact on SL744.

A



B

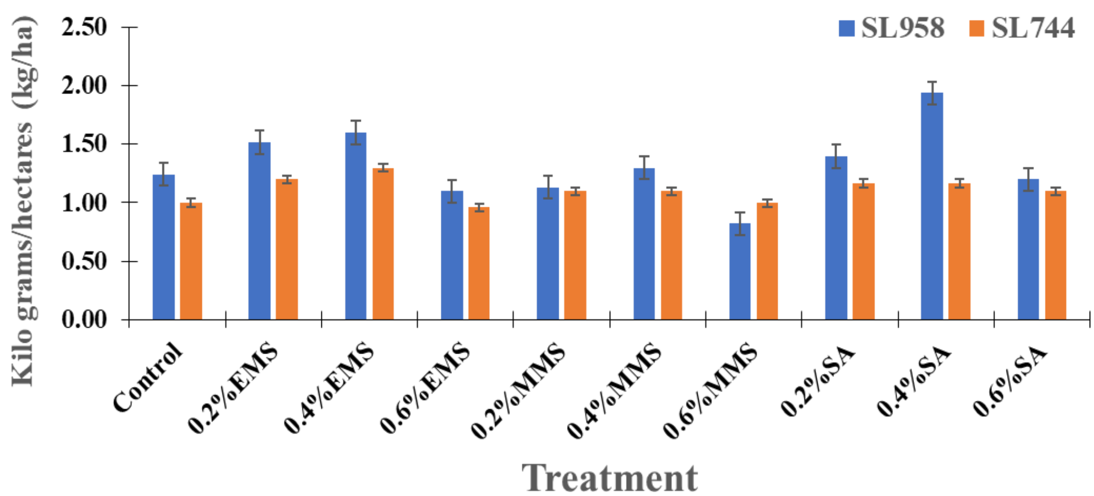


Fig 4.11. Biological yield in M_1 and M_2 plant: **(A)** Biological yield for 2023, **(B)** Biological yield 2024.

4.9. Effect of chemical mutagens on grain yield in M_1 and M_2 plant

Creating varieties with a high seed output is the ultimate goal of variety development. High yield is a polygenic trait that is regulated by numerous genes. There was a significant variation in the population (0.31 g), with SL744 showing the highest variation when treated with 0.4% SA and the lowest variation when treated with 0.6% EMS, 0.6% MMS, and 0.6% SA (0.1 g). However, during the 2023 year of the experiment, SL958 recorded the highest value (0.89 g) when treated with 0.4% SA and the lowest value (0.10 g) when treated with 0.6% MMS, compared to the control group (SL744: 0.1 g, SL958: 0.2 g). In the 2024 trial, it was seen that SL744 had the highest value (0.25 g) when treated with 0.4% SA and the lowest value (0.13 g) when treated with 0.6% MMS. On the other hand, SL958 had the highest value (0.92 g) when treated with 0.4% SA and the lowest value (0.10 g) when treated with 0.6% MMS compared to the control. In the data observations of the M1 and M2 populations, grain production per plant in the SL958 variety treated with 0.4% SA was revealed to be more effective, recording the highest value (0.89 g in 2023 and 0.92 g in 2024, respectively). This suggests that the seed yield per plant was influenced by non-additive gene action, and a simple selection model would not yield the desired genetic gain.

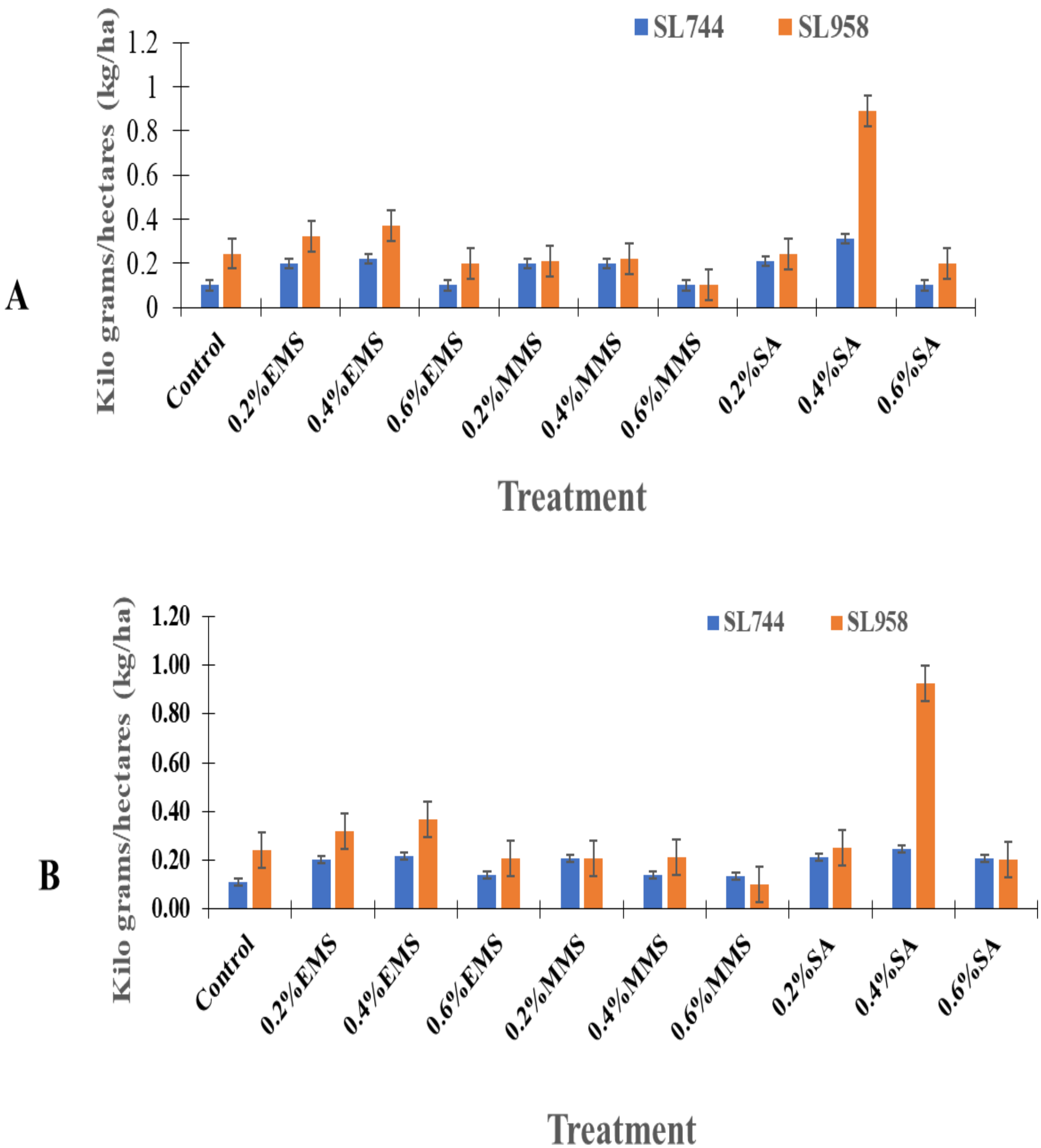


Fig 4.12. Grain yield in M_1 and M_2 plant: **(A)** Grain yield for 2023, **(B)** Grain yield 2024.

4.10. Effect of chemical mutagens on harvest index in M_1 and M_2 plant

The result indicated that chemical mutagens had a significant effect on the soybean harvest index. The maximum value (0.25 g) was recorded in SL744 treated with 0.4% SA, and the minimum value (0.11 g) was observed treated with 0.6% MMS. In SL958, we treated the highest value (0.81 g) with 0.4% SA and the lowest value (0.16 g) with 0.4% MMS, compared to the control group (SL744: 0.09 g, SL958: 0.19 g) during the first year of the 2023 experiment. However, during the 2024 trial, the highest value (0.21 g) was observed in SL744 treated with 0.4% SA, and the least value (0.12 g) was recorded treated with 0.4% MMS. Moreover, SL958 value (0.47 g) was observed to be high and least value (0.12 g) treated with EMS as compared with control (SL744: 0.11 g, SL958: 0.19 g). Some mutants may exhibit a lower harvest index than the normal soybean plant, meaning that a lower percentage of biomass will be given to seeds. Mutations that impact genes related to seed filling, nutrition partitioning, or reproductive development may result in these lower harvest index mutants.

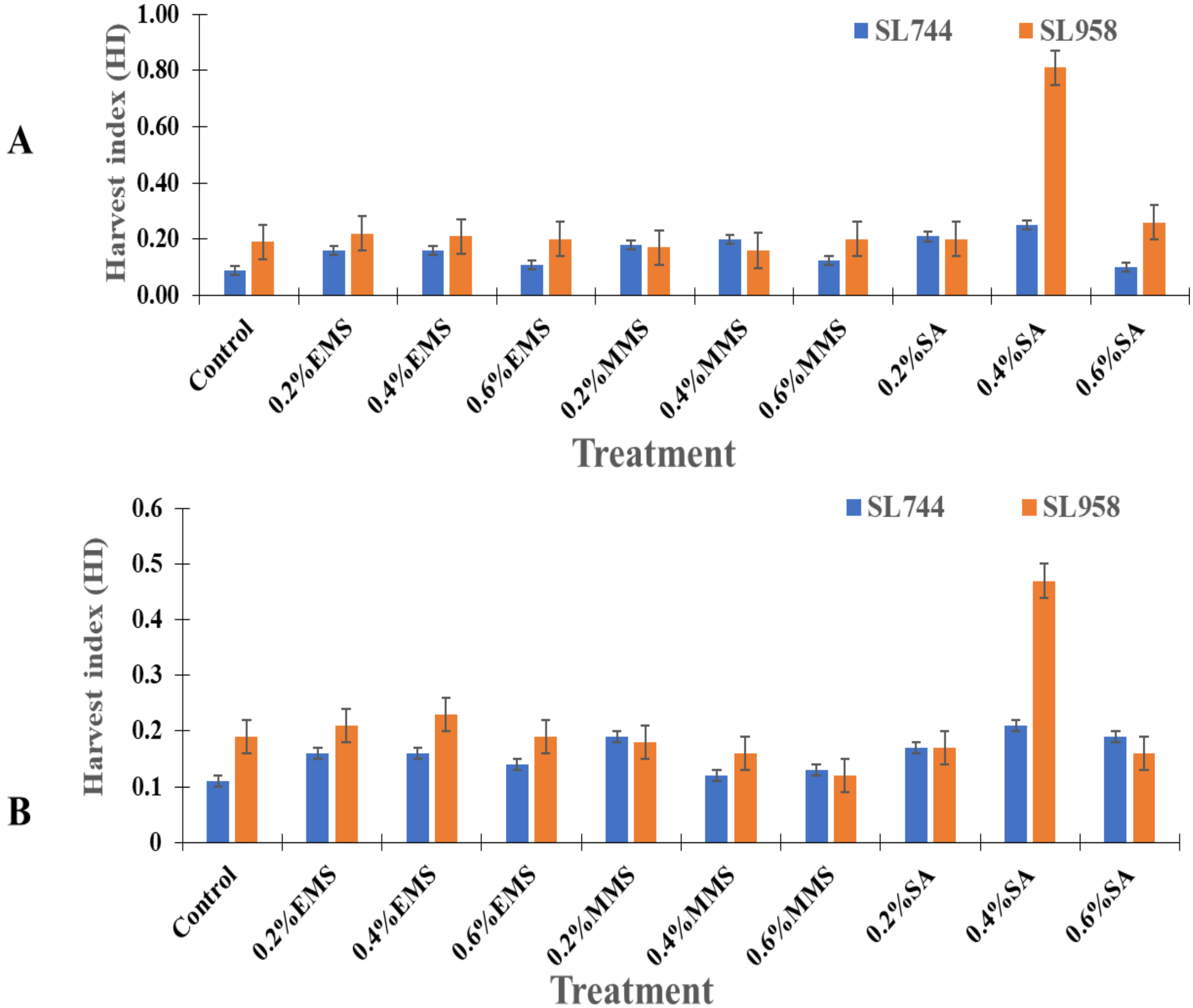


Fig 4.13. Harvest index in M_1 and M_2 plant: **(A)** Harvest index in M_1 2023, **(B)** Harvest index in M_2 2024.

4.11. Effect of chemical mutagens on chlorophyll content (nmol/cm^2)

Table 4.18. shows the effects of chemical mutagens on plant chlorophyll content at 4, 8, and 12 WAS. At 4 WAS, there was significant difference in chlorophyll content in the M_1 plants of the SL744 variety, while for the SL958 variety, there was no significant difference among the T_2 and T_3; T_6, T_5, and T_7; and T_4, T_8, T_9, and T_{10} plots. At 8 WAS, the chlorophyll content was found to be the highest (47.25 ± 3.07 nmol/cm^2) in the T_2 M1 plants (0.2% EMS) and the lowest (41.06 ± 4.06 nmol/cm^2) in the T_{10} (0.6% SA) M_1 plants of the SL744 variety. Meanwhile, in SL958, the highest chlorophyll content (45.76 ± 1.02 nmol/cm^2) was found in the T_9 plot and the lowest (40.97 ± 0.51 nmol/cm^2) in the T_{10} plot. However, at 12 WAS, among both of the varieties, the chlorophyll content was the highest (48.10± 0.71 nmol/cm^2) in the T_2 (0.2% EMS) plot of the SL958 variety and the lowest (41.33 3.63 nmol/cm^2) in the T_7 (0.6% MMS) plot of the SL958 variety, which differed substantially from the control plants (39.54 nmol/cm^2), during the first-year study. In the second-year experiment, T_9 records the highest chlorophyll content (55.18±1.86 nmol/cm^2) for the SL 958 treated with 0.4% SA. Meanwhile, the T_7 (0.6% MMS) having the least chlorophyl content (38.82±2.09 nmol/cm^2) 12WAS.Moreover, for the SL744 variety at 12 WAS, the T_2 (0.2% EMS) treated plot exhibited the highest chlorophyl content (51.54±2.19 nmol/cm^2), and the lowest chlorophyll was recorded for T_7 (41.04±0.81 nmol/cm^2) treated with 0.6%MMS. The results, however, differed positively from untreated control plant (SL958: 45.32±2.21 nmol/cm^2 SL744: 46.66±1.78 nmol/cm^2).

Table 4.18. Effect of chemical mutagens on chlorophyll content (nmol/cm^2).

	Data taking periods											
	Treatments		2023/2024					2024/2025				
	SL744W4	SL958W8	SL744W8	SL958W8	SL744W12	SL958W12	SL744W4	SL958W8	SL744W8	SL958W8	SL744W12	SL958W12
T1(Control)	34.58±1.88^{cd}	37.75 ±4.29^{a}	45.27±1.61^{a}	41.27±0.55^{b}	45.78±2.10^{a}	39.54±0.55^{d}	35.686±1.10^{e}	40.06±2.42^{c}	45.374±1.55^{b}	43.76±1.46^{cd}	46.66±1.78^{b}	45.32±2.21^{c}
T2	42.09±1.70^{a}	39.61±0.61^{a}	47.25 ±3.07^{a}	44.21±0.71^{ab}	46.43 ±2.50^{a}	48.10±0.71^{abc}	49.8±1.69^{b}	42.48±2.35^{b}	49.12±1.55^{a}	43.66±1.18^{cde}	51.54±2.19^{a}	44.48±0.90^{c}
T3	41.04±0.87^{ab}	38.71±1.55^{a}	45.04 ±2.06^{a}	44.09 ±0.6 ^{ab}	44.10 ±1.63^{a}	45.17±0.65^{a}	52.84±1.48^{a}	45.38±2.18^{a}	45.7±2.11^{b}	45.92±2.35^{b}	47.58±1.81^{b}	46.18±1.75^{b}
T4	38.06±3.15^{abc}	35.95±2.30^{ab}	43.57 ±1.65^{a}	42.80 ±0.65^{ab}	44.01±3.37^{a}	44.83±0.65^{ab}	40.04±0.60^{e}	39.5±0.66^{c}	40.06±1.75^{d}	41.94±1.75d^{ef}	41.64±1.18^{de}	42.02±2.20^{d}
T5	35.27±2.28^{cd}	37.79±2.30^{b}	45.51±3.15^{a}	44.66 ±0.61^{ab}	46.47 ±3.08^{a}	44.28±0.61^{abc}	33±3.45^{f}	35.04±1.40^{d}	39.36±5.29^{e}	40.08±1.76^{f}	41.68±3.57^{ef}	40.8±2.65^{e}
T6	32.89±2.65^{cd}	34.89±0.75^{a}	43.56 ±1.21^{a}	42.97±1.71^{ab}	44.35 ±7.18^{a}	43.61±1.72^{abc}	31.9±1.89^{g}	30.94±1.27^{e}	42.54±2.56^{c}	40.3±2.19^{ef}	44.22±2.66^{cd}	42.6±1.32^{de}
T7	31.57±1.14^{d}	31.85±1.07^{b}	42.26±6.32^{a}	42.88 ±3.63^{ab}	44.32 ±0.94^{a}	41.33±3.63^{cd}	30.56±2.59^{g}	29.32±1.128^{f}	38.24±3.36^{e}	34.692±3.68^{g}	41.04±0.81^{f}	38.82±2.09^{f}
T8	35.02±3.58^{cd}	35.63 ±1.45^{ab}	42.83±3.38^{a}	43.19±0.61^{ab}	43.75 ±4.22^{a}	44.34c±0.6^{ab}	35.46±2.18^{e}	42.3±1.03^{b}	41.64±2.33^{d}	45.06±1.47^{c}	43.32±2.58^{bcd}	46.62±1.22^{bc}
T9	35.95±2.84^{bcd}	38.77±3.45^{ab}	42.90 ±1.48^{a}	45.76 ±1.02^{a}	44.31±0.74^{a}	45.38±1.02^{a}	42.56±1.89^{c}	47.04±1.41^{a}	43.38±1.98^{c}	50.35±1.61^{g}	44.78±2.00^{c}	55.18±1.86^{a}
T10	32.87±2.25^{cd}	35.76±3.19^{ab}	41.06 ±4.06^{a}	40.97±0.5^{b}	41.47 ±4.07^{a}	41.76±0.51^{bcd}	34.82±1.22^{f}	40.1±1.46^{c}	39.84±1.15^{e}	41.01±1.45^{f}	41.74±1.24^{de}	42.22±1.63^{de}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = Standard deviation ±, WAS = Weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.12. Effect of chemical mutagens on protein content (%)

The results show that the T_9 treatment (0.4% SA) recorded the highest protein percentage (47.04 ± 0.87%) in SL958 and lowest was recorded for the T_7 treatment (0.6% MMS) (37.13 ± 0.0.72%) compared to the untreated control (39.87 ± 0.96%). However, in the SL744 variety, the highest (46.14 ± 0.64) was recorded in T_9 (0.4% SA) and the lowest in T_{10} (0.6% SA) (39.82 ± 1.07%). This was significantly different compared to the untreated controls (36.64 ± 3.83%), during the first-year study. In the second-year experiment, T_9 records the highest protein percentage (45.5±5.06 %) for the SL 958 treated with 0.4% SA. Meanwhile, the T_5 having the least protein percentage (37.24±4.27 %). Moreover, for the SL744 variety, the T_9 (0.4% SA) treated plot exhibited the highest protein percentage (42.3±1.29 days to podding), and the lowest protein percentage was recorded for T_5 (33.4±6.00 %) treated with 0.4%MMS. The results, however, differed positively from untreated control plant (SL958: 38.33±5.32 % and SL744: 37.6±6.01%).

Table 4.91. Effect of chemical mutagens on protein content (%).

Data taking periods				
	2023/2024		2024/2025	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	36.64±3.83^{c}	39.87±0.96^{c}	37.6±6.01^{c}	38.33±5.32^{de}
T2	42.45±0.25^{b}	43.25±0.42^{b}	40.3±7.11^{b}	41.2±2.50^{bc}
T3	46.01±0.51^{a}	45.46±0.19^{a}	37.7±2.66^{c}	40.23±3.66^{c}
T4	41.36±1.04^{b}	40.34±0.63^{cd}	35.23±2.13^{d}	39.2±3.56^{d}
T5	41.72±0.44^{b}	40.91±0.19^{cd}	33.4±6.00^{e}	37.24±4.27^{de}
T6	40.49±0.48^{b}	41.66±0.47^{c}	41.5±3.01^{ab}	40.33±3.79^{c}
T7	39.89±0.72^{b}	38.13±0.81^{e}	40.4±5.77b	40.02±5.92^{c}
T8	41.00±0.47^{b}	45.93±0.99^{a}	41.7±2.64^{ab}	43.18±5.38^{b}
T9	46.14±0.64^{a}	47.04±0.87^{a}	42.3±1.29^{a}	45.5±5.06^{a}
T10	39.82±1.07 ^{b}	40.62±0.41^{cd}	41.8±1.04^{ab}	40.23±5.19^{c}

The same letters in the same column are not significantly different from each other (p < 0.05). SD = Standard deviation ±, WAS = Weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.13. Effect of chemical mutagens on lipid content (%)

The highest lipid percentage (22.34± 0.59%) was observed in the T_9 (0.4% SA) M_2 plants of the SL958 variety, while the lowest (19.87 0.47%) was recorded in the T_{10} plants (0.6% SA), as compared to the untreated controls (20.74± 0.64%). The highest lipid percentage (21.74 ± 0.77%) was observed in the T_8 (0.2% SA) M2 plants of the SL744 variety, while the lowest (18.69± 0.55%) was recorded in the T_7 plants (0.6% MMS) compared to the untreated controls (21.39± 0.37%). during the first-year study. In the second-year experiment, T_9 records the highest lipid percentage per plant (24.3±1.17 %) for the SL 958 treated with 0.4% SA. Moreover, for the T3 (0.4% EMS) &T8 (0.2% SA) are slightly similar (22.2±1.89 % & 22.4±1.19 %). Meanwhile, the T_7 having the least lipid percentage (13.33±2.06 %). Moreover, for the SL744 variety, the T_9 & T_3 (0.4% SA & 0.4% EMS), treated plot are statistically exhibited the highest lipid percentage (22.5±1.48 % & 22.2±1.19 %), and the lowest lipid percentage was recorded for T_7 (11.4±2.27 %) treated with 0.6%MMS. The results, however, differed positively from untreated control plant (SL958: 20.7±2.39 % SL744: 21.3±2.32 %).

Table 4.20. Effect of chemical mutagens on lipid content (%).

	Data taking periods			
	2023/2024		2024/2025	
Treatments	SL744	SL958	SL744	SL958
T1(Control)	21.39±0.37^{ab}	20.74±0.64^{abc}	21.3±2.32^{b}	20.7±2.39bc
T2	20.62±0.41^{bc}	21.20±0.05^{bc}	20.9±1.45^{b}	18.33±1.64^{c}
T3	20.76±0.14^{bc}	21.67±0.71^{a}	22.2±1.19^{ab}	22.2±1.89^{ab}
T4	20.63±0.31^{bc}	21.68±0.58^{a}	17.1±1.58^{c}	20.4±2.33^{bc}
T5	20.04±1.09^{bcd}	20.32±0.41^{bc}	14.2±4.17^{d}	19.02±2.41^{d}
T6	19.19±0.44^{de}	21.09±0.88^{abc}	12.5±2.24^{e}	17.3±2.32^{e}
T7	18.69±0.55^{e}	20.49±0.11^{abc}	11.4±2.27^{ef}	13.33±2.06^{f}
T8	21.74±0.77^{a}	21.73±0.38^{a}	20.4±1.87^{bc}	22.4±1.19^{ab}
T9	21.37±0.50^{ab}	22.34±0.59^{a}	22.5±1.48^{a}	24.3±1.17^{a}
T10	19.95±0.94^{cde}	19.87±0.47^{c}	20.44±2.05^{bc}	21.22±1.86^{b}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = Standard deviation ±, WAS = Weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.14. Effect of chemical mutagens on fiber content (%)

The highest fibre content was observed in the T_9 plants (0.4% SA), which was 18.23±0.12% in the M_2 seeds of SL958, while the lowest (16.59 ± 0.36%) was observed in the T_7 plants (0.6%MMS) in SL958 compared to the control (17.71±0.39%). The highest fibre content was observed in the T_8 plants (0.4% SA), which was 17.89 ±0.39% in the M2 seeds of SL744, while the lowest (16.23 ±0.93%) was observed in the T_6 (0.4% MMS) plants in SL744 compared to the controls (16.99%). during the first-year study. In the second-year experiment, T_9 &T_3 statistically records the highest fiber content (17.6±2.04% &17.21±2.80 %) for the SL 958 treated with 0.4% SA & 0.4% EMS respectively. Meanwhile, the T4 having the least fiber content (12.4±1.60 %) treated with 0.4% EMS. Moreover, for the SL744 variety, the T_1 (untreated control), plot exhibited the highest fiber content (18.1±2.11 %), and the lowest fiber content was recorded for T7 (9.4 ±2.06 %), treated with 0.6%MMS. The results, however, differed significantly from untreated control plant (SL958: 16.41±2.72 % SL744: 18.1±2.11 %).

Table 4.21. Effect of chemical mutagens on fiber contain (%).

	Data taking periods			
	2023/2024		2024/2025	
Treatments	**SL744**	**SL958**	**SL744**	**SL958**
T1(Control)	16.99 ± 0.30^{a}	17.71 ± 0.39^{ab}	18.1 ± 2.11^{a}	16.41 ± 2.72^{c}
T2	17.56 ± 0.27^{a}	17.69 ± 0.26^{ab}	15.2 ± 1.95^{c}	14.5 ± 2.59^{bc}
T3	16.54 ± 0.39^{a}	17.24 ± 0.22^{bc}	17.1 ± 2.41^{ab}	17.21 ± 2.80^{a}
T4	16.28 ± 0.15^{a}	17.14 ± 0.16^{bc}	11.6 ± 1.99^{e}	12.4 ± 1.60^{e}
T5	16.32 ± 0.27^{a}	16.90 ± 0.19^{c}	13.7 ± 1.61^{d}	14.3 ± 2.90^{bc}
T6	16.23 ± 0.93^{a}	16.73 ± 0.52^{c}	11.2 ± 1.13^{ef}	13.1 ± 1.52^{d}
T7	16.28 ± 0.09^{a}	$16.59^{c}\pm0.36$	9.4 ± 2.06^{g}	13.01 ± 1.14^{d}
T8	17.89 ± 0.39^{a}	17.18 ± 0.03^{bc}	16.7 ± 1.98^{b}	15.2 ± 1.17^{b}
T9	17.43 ± 0.05^{a}	18.23 ± 0.12^{a}	15.4 ± 1.65^{c}	17.6 ± 2.04^{ab}
T10	16.54 ± 0.53^{a}	17.12 ± 0.23^{bc}	11.8 ± 1.17^{ef}	13.6 ± 2.17^{d}

The same letters in the same column are not significantly different from each other ($p < 0.05$). SD = Standard deviation ±, WAS = Weeks after sowing, T = Treatment (T_1 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.15. Qualitative phytochemical analysis of the extracts from the selected soybean varieties

Many phytochemicals, including alkaloids, flavonoids, saponin, cardiac glycoside, and tannins compounds, were found in both extracts when the extract samples of the selected soybean varieties were screened qualitatively for phytochemicals. The details of the initial phytochemical screening results are presented in **Table 4.22.**

Table 4.22. Effect of chemical mutagens on phytochemical classes of the soybean seed.

Phytochemical tests	**SL 744**										**SL 958**									
	C_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	C_2	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Test for Alkaloids (Meyer's Test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids (Alkaline reagent test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Test for saponins (Form test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Test for Tannins (Ferric test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: present = (+), absent **= (-)**

T = Treatment (C_1 & C_2 = untreated control, T_2 = 0.2% EMS, T_3 = 0.4% EMS, T_4 = 0.6% EMS, T_5 = 0.2% MMS, T_6 = 0.4% MMS, T_7 = 0.6% MMS, T_8 = 0.2% SA, T_9 = 0.4% SA, T_{10} = 0.6% SA).

4.16. Effect of Chemical mutagens on the quantitative measurement of flavonoids in the methanol extract of two varieties of soybeans.

Figure **4.14.** depicts the effect of chemical mutagens on flavonoid content. The results indicate that the maximum value of 65.47 was recorded in the treatment with 0.2% SA, while the minimum value of 16.65 was noted in the 0.6% MMS in SL744. However, in SL958, the highest values (81.34) were treated with 0.4% AS, and the lowest values (21.38) were treated with 0.2% MMS, as compared with control SL744: 21.02 and SL958: 30.87. There may be a concentration-dependent threshold effect where certain treatments boost flavonoid levels at lower doses but inhibit them at higher doses. SA is the most effective chemical for increasing flavonoid content, particularly in SL958, which peaks significantly.

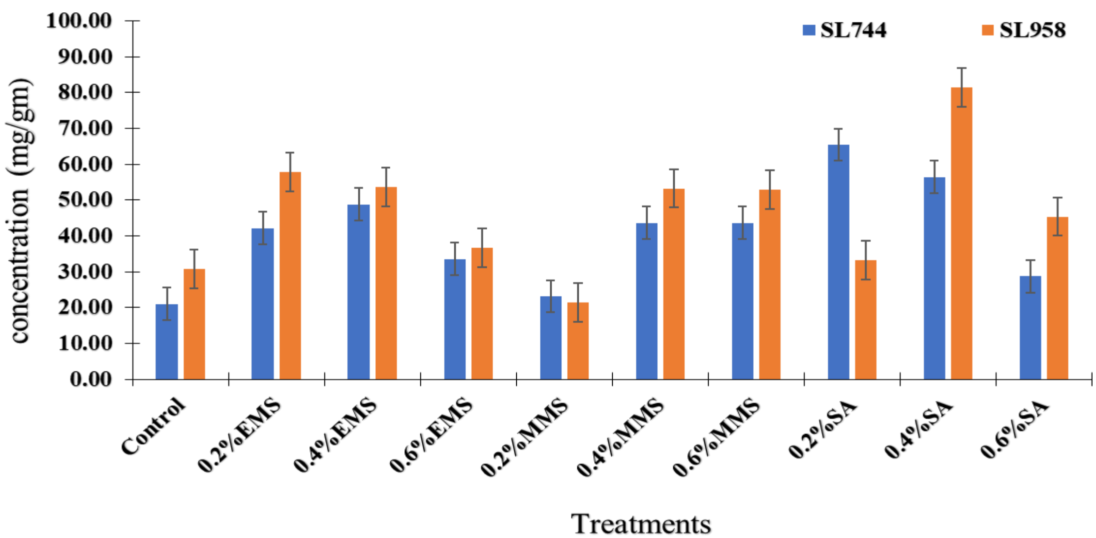


Fig 4.14. Quantitative measurement of flavonoids in the methanol extract of two varieties of soybeans.

4.17. Effect of chemical mutagens on quantitative determination of alkaloids in methanol extract of two soybean varieties

Fig 4.15. shows the effect of chemical mutagens on alkaloid content. The result shows that the highest value was observed (204.83) when treated with 0.2% SA and the lowest value (111.83) when treated with 0.6% SA in SL744. However, in SL958, the highest values (227.17) were treated with 0.2% EMS, and the lowest values (106.36) were treated with 0.4% MMS as compared with control (SL744: 222.24; SL958: 113.89).

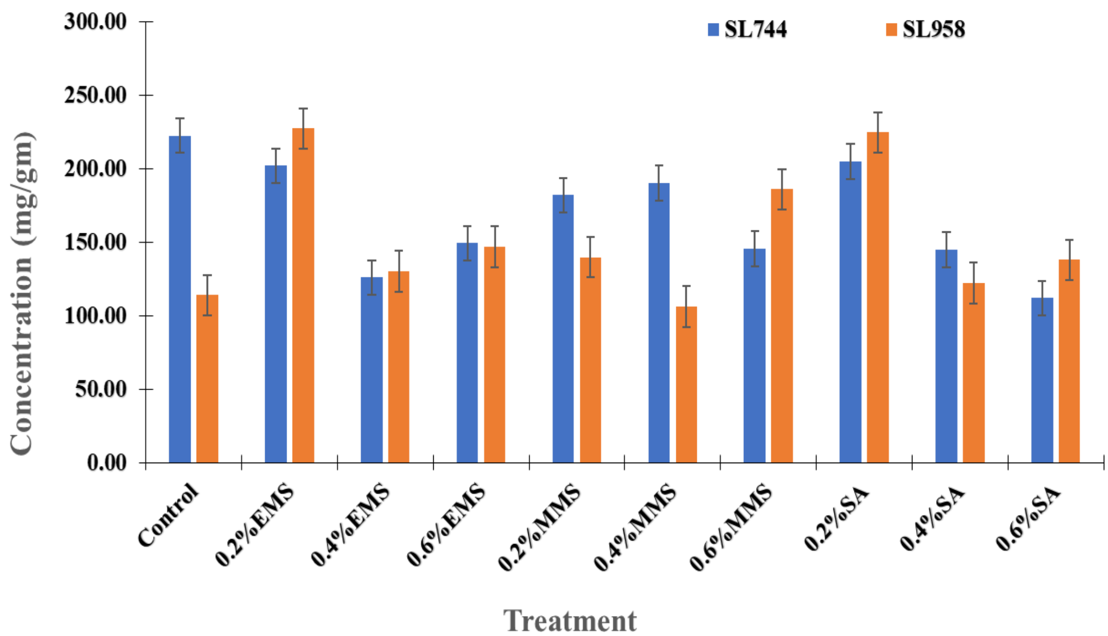


Fig 4.15. Quantitative measurement of alkaloids in the methanol extract of two varieties of soybean.

4.18. Impact of chemical mutagens on the quantitative determination of saponin in the methanol extract of two soybean varieties.

Fig 4.16. shows the impact of chemical mutagens on saponin content. The result shows that the highest value was observed (85.27) in the treatment with 0.4% MMS and the lowest value (28.20) in the treatment with 0.2% EMS in SL744. However, in SL958, the highest values (86.58) were treated with 0.6% MMS, and the lowest values (35.59) were treated with 0.6% EMS as compared with control SL744: 64.08; SL958: 72.88. MMS is the most effective treatment for increasing saponin content in both SL744 and SL958. In contrast, EMS consistently reduces saponin levels, while SA shows variable effects depending on concentration. Further statistical analysis would confirm the significance of these trends.

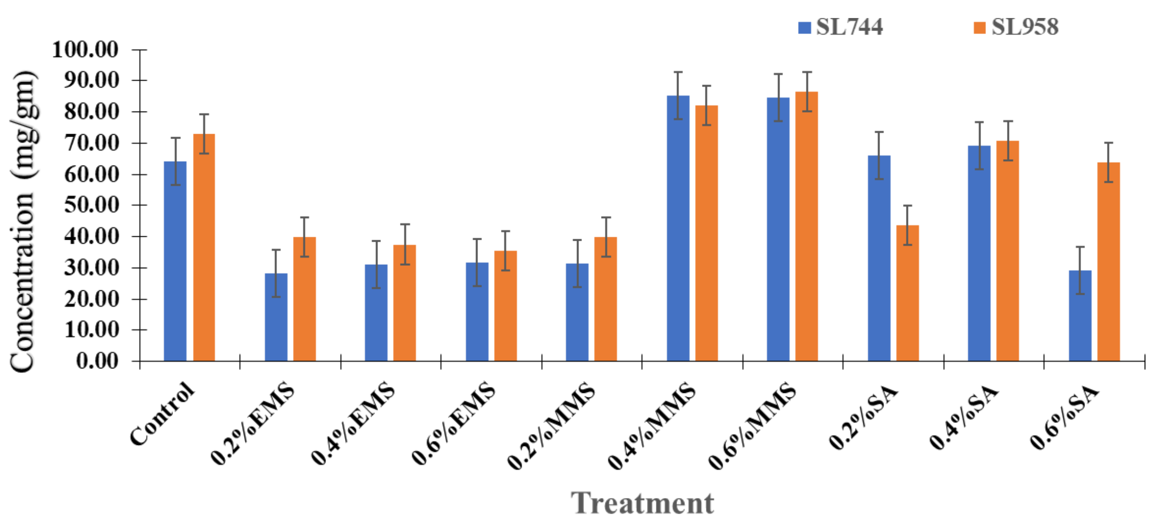


Fig 4.16. Quantitative measurement of saponins in the methanol extract of two varieties of soybeans.

4.19. Effect of chemical mutagens on quantitative determination of tannin in the methanol extract of two soybean varieties

Fig 4.17. shows the impact of chemical mutagens on tannin content. The result shows that the highest values were observed (91.27) in those treated with 0.4% EMS and the lowest values (44.52) in those treated with 0.2% EMS in SL744. However, in SL958, the highest values (94.39) were treated with 0.4% EMS, and the lowest values (68.44) were treated with 0.6% SA as compared with control SL744 (76.63) and SL958 (84.56). EMS and SA stimulate tannin biosynthesis pathways up to a threshold concentration, after which they become inhibitory. MMS appears to maintain tannin levels; it may have a milder or regulatory effect on tannin production.

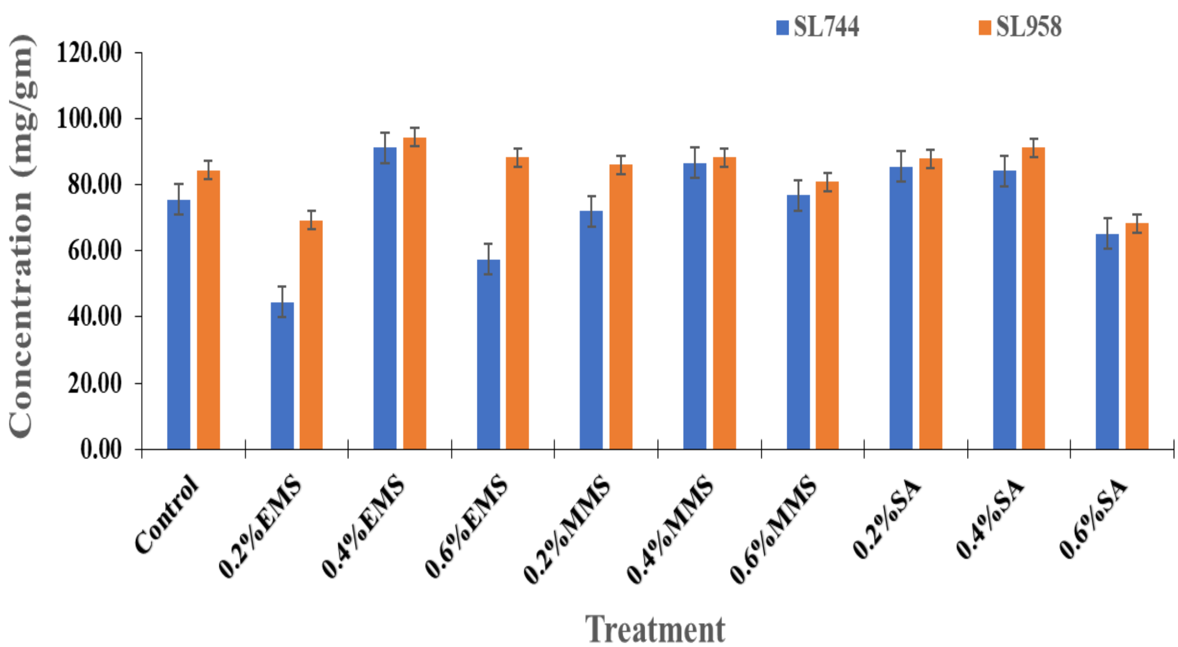


Fig 4.17. Quantitative measurement of tannins in the methanol extract of two varieties of soybeans.

CHAPTER 5

Discussion

5.0. DISCUSSION

The current findings indicate that induced mutagenesis is significant for enhancing the genetic composition of crop plants to support the growing population of billions. Chemical mutagens caused the appearance of leaf variations including unifoliate, bifoliate, tetrafoliate, and pentafoliate characteristics. The efficacy of breeding by mutagenesis primarily relies on the efficiency and effectiveness of the specific mutagen employed (Arisha et al. 2015). Nevertheless, variations in mutagens exhibit differing effects depending on the concentration employed or the materials utilized. It is essential to optimize the mutagen concentration prior to material treatment to ensure a high mutation frequency and sufficient viable seeds. High doses of mutagens adversely affect plants; nevertheless, increased mutation frequency may also result from higher concentrations of the mutant used (Shah et al. 2016). In our study, the seed germination percentage increased with lower doses of SA, EMS, and MMS (0.4%) and decreased with increasing dose (0.6%) compared to the untreated controls. Every living cell requires energy in the form of ATP molecules to carry out all biological reactions. When ATP levels are low, the rate of biological reactions inside the cell decreases (Aishatu 2015). Higher doses of SA (0.6%) can hinder ATP biosynthesis, resulting in a decreased availability of ATP molecules; as a result, the germination rate may slow down. Our findings are in consonance with those of Apparao (2005), who reported that there was a reduction in germination percentage with higher doses of SA (25%) and an increase in germination with 5% SA in cowpea seeds. The decrease in seed germination count was observed in sesame seed with higher doses of SA (0.0776%) and an increased germination count was observed with lower doses (0.0473%) (Mensah et al. 2007). Furthermore, Aliyu et al. (2018), reported that a nonlinear decline in mutagenic efficiency in *Sesamum indicum* was observed with increasing concentration and dose of sodium azide, and the best growth was recorded in terms of morphological and yield traits at a lower (0.5 mM) concentration of sodium azide. The decline in mutagenic effectiveness with increasing concentration of sodium azide might be attributed to increasing chromosome damage with increasing concentration of the mutagen.

Increased plant height was observed with lower doses (0.4%) of SA (37.8 cm) in the SL958 soybean variety and also with lower doses (0.2%) of EMS (24.80 cm) in the SL744 variety. This might be attributed to the effect of chemical mutagens on plant growth hormones. An increase in mutagen concentration often results in more physiological damage and direct DNA damage, as well as biochemical disturbances, auxin destruction, and changes in ascorbic acid content. These damages are phenotypically reflected in plant growth. The reduction in plant height caused by chemical mutagens can be attributed to any of the aforementioned factors. Our results are in agreement with the findings of Markeen et al. (2007) in 'urd bean', who reported a decrease in plant height with higher doses of EMS (12%), attributed to gross injury at the cellular level. Our findings are also in consonance with the observations of OlaOlorun et al. (2021. In wheat plants, their results indicated that the plant height increased by 21.2 cm with 0.1% EMS and decreased by 16.3 cm with 0.7% EMS.

There was an increase in leaf number (229/234 leaves per plant) at a low dose of EMS (0.2%) and SA (0.4%), respectively, and a decrease in leaf number (164/220 leaves/plant) with high doses of EMS (0.6%) and SA (0.6%) in the M_1 plants of the SL958 variety. Mean- while, in the SL744 variety, the leaf number (199.33 leaves per plant) was higher with lower doses of SA (0.4%), and there was a decrease in leaf number (135.53 leaves/plant) with high doses of EMS (0.6%). Our observations are in consonance with the findings of Gupta et al. (2023) who reported an increase in leaf number in soybean plants with lower doses of EMS (0.4%) and decrease in leaf number with higher doses of EMS (6%). However, it was also reported by Habib et al (2021). that there was a gradual reduction in the morphological characteristics of sunflower seedlings with the increasing dose of EMS in comparison to non-treated control seedlings.

In our findings, the highest leaf area (3625.8 cm^2) was observed in response to T_9 (0.4% SA) in SL958, and the leaf area decreased (2703.4 cm^2) with higher doses (0.6% SA). However, in the SL744 variety, the leaf area (2311.03 cm^2) was higher with lower doses of SA (0.4%), and there was a decrease in leaf number (731.54 leaves/plant) with high doses of SA (0.6%). This result is similar to the findings of Al-Qurainy. Al-Qurainy (2009) reported that in *Eruca sativa* plants, with lower doses of SA (3%), the leaf area increases, and with higher doses (5%), the leaf area decreases. Jabeen and Mirza (2004), found that in *Capsicum annum*, the leaf area increases with lower EMS doses (0.01%) and declines with higher doses (0.5%).

This study identified changes in various morphological traits, specifically in leaf morphology, which is very visible in the narrow/lanceolate leaves, tetrafoliate and pentafoliate forms, and leaves that display a variety of green color, with some plants even displaying chlorosis. The flowers were predominantly purple, with a few plants exhibiting white corollas. These observations align with the findings of Espina et al. (2018), which noted various phenotypic differences in leaf shape, including thin leaves, tetra-foliate, and penta-foliate forms. The leaf, recognized as the primary site of photosynthesis, indicates that an increase in leaf quantity leads to a greater leaf surface area, hence enhancing the rate of photosynthesis due to optimal solar absorption. Changes in the physiological processes of leaf development caused by mutagenesis can explain why some treatments produce different leaf shapes, ranging from ovate to intermediate and lanceolate. The observations are similar to what Shilpashree et al. (2021) found. They said that leaf shapes can vary from lanceolate to ovate, leaf colors can range from normal to dark green, and some varieties have flowers that are white or purple. According to Zhou et al. (2019), chemical mutagens in several mutants caused a wide range of morphological abnormalities, such as chlorotic leaves and multiple leaflets at a single node. In contrast, most wild-type soybean leaves were trifoliate. In our study, broad leaves were observed with lower doses (0.2% SA pentafoliate leaves and 0.4% SA tetrafoliate leaves) in SL958, and narrow leaves were observed with higher doses (0.6%). Meanwhile, in SL744, broad leaves were observed with lower doses (0.2% EMS tetrafoliate leaves and 0.6% EMS tetrafoliate leaves), and narrow leaves were observed with higher doses (0.6%) of EMS. This is in consonance with the findings of Khursheed et al. (2019), who reported that with lower doses of EMS (0.23%), broad leaves were observed, and with higher doses of EMS (0.25%), narrow leaves were observed in faba bean plants. It was also reported that with a much higher dose of EMS (0.3%), trifoliate leaves were observed, which is similar to our findings (0.6% EMS: trifoliate leaves). Ahire et al. (2005), reported that there was an increased number of pentafoliate leaves (3) with lower doses EMS (0.2%) and decreased number of pentafoliate leaves (1) with higher doses (0.4%). No tetrafoliate leaves were observed with either of the doses (2% EMS or 0.4% EMS) in soybean plants. Espina et al., 2018, reported that with higher doses of EMS (0.23%), tetrafoliate leaves were observed, and with lower doses of EMS (0.14%), pentafoliate leaves were observed in soybean plants.

In our study, the number of days to 50% flowering increased with increasing dose and decreased with decreasing dose of the chemicals. The T_9 (0.4% SA)-treated plants in SL958 took the least time to flower (39.67 days) and the T_4-treated plants (0.6% EMS) took the most time (51 days) in the SL958 variety. However, in the SL744 variety (51.33 days), the time was higher with lower doses of EMS (0.2%), and there was a decrease in the number of days (46.67 days) with the doses of SA (0.4%). Our results are in consonance with those of Kumar and Pandey (2019), who investigated the impact of EMS on *Coriandrum sativum* L. seeds by exposing them to various concentrations (0.1%, 0.3%, and 0.5%) for 3 and 5 hours, respectively. They found that as EMS concentrations increased, there was a delay in the amount of time to 50% flowering compared to a control (Kumar and Pandey 2019). Samadi et al. (2024), while working with corn, reported a similar observation (Siddique et al. 2020). The plant chlorophyll content was observed to be higher (48.10 nmol/cm^2) with lower doses of EMS (0.2%) and decreased (41.76 nmol/ cm^2) with increasing doses of MMS (0.6%) in the SL958 variety. Furthermore, in the SL744 variety, the chlorophyll content was observed to be higher (46.43 nmol/ cm^2) with lower doses of EMS (0.2%) and decreased (41.47 nmol/ cm^2) with increasing doses of SA (0.6%). Devi et al. 2021, reported that there was a progressive increase in chlorophyll content with lower doses of 10% EMS and 10% SA and a decrease in chlorophyll content with higher doses of EMS (40%) and SA (40%) in chili (*Capsicum annuum* L.). Dinkar et al. (2020) reported a higher frequency of chlorophyll mutations in chickpeas treated with 0.5% EMS. Selvaraj et al. 2014 reported that lower doses of SA (5%) increase the chlorophyll content and higher doses (10%) decrease the chlorophyll content in rice plants.

Podding was observed to be higher (277.55 pods/plant) with 0.4% SA (SL958) and lower (112.22 pods/plant) with 0.2% EMS (SL744). Our results are contrary to the findings of Parchin et al. (2021), who reported that a higher yield was obtained from fenugreek plants (*Trigonella foenum-graecum*) that received a higher dose of EMS (0.5%) and a lower yield was obtained with a lower dose of EMS (0.1%). The results indicated that seed yield per plant was controlled by many genes, likely due to favorable gene mutations at several loci. Rai et al. (2013), Reddy et al. (2013), and Rafeeq et al. (2017) all had similar results when they studied soybean plants and found high-yielding mutants that produced more seeds per plant. The increase of seed yield per plant may result from a growth in the number of capsules, primary and secondary branches, and other associated features. Similar outcomes for high yield per plant were also reported by Tyagi et al. (2014), Chandrawati et al. (2016), Ahmed (2017), and Meena et al. (2020) in soybean. The number of pods per plant may be used for selection of high yielding mutant lines in later generations for development of superior cultivars. These were in conformity with earlier findings by Chandrawati et al. (2016) and Ahmed (2017) in their study showing that number of pods per plant could be used for selection of high yielding mutant varieties. Khan et al. (2013), reported that high doses of SA (2.5%) decreased the yield and lower doses of 2% SA increased the yield of barley plants. According to Etther et al. (2019), *Cajanus cajan* plants treated with EMS had an increased grain yield per plant and 100-seed weight, which might be as a result of a high amount of mutagenic frequency, mutagenic effectiveness, and mutagenic efficiency at low concentrations of the mutagen. Ningsih et al. (2019) noted that the maximum number of pods per plant was 62.31, contrary to the largest pod per plant. Olena et al. (2020) indicated that 138.73 is the maximum number of pods per plant. The disparities observed in these results may be attributed to variations in the genetic mix of the utilized variety and the soil composition.

In our studies the highest value for 100 seed weight in T_9 was recorded in SL958 (0.40 % SA) with 10.8 g and the lowest value T_{10} was recorded as 8.7 g in SL958, 0.60 % SA. The results were in line with previous studies conducted on soybean by Chandrawati et al. (2016), Ahmed (2017), and Meena et al. (2020). The 100-seed weight observed in this study varied from Saha and Matiul (2022), which reported a range of 8.39 g to 13.15 g. These findings closely align with Biswas et al. (2016) research, which indicated that the weight of 100 seeds among the varieties ranged from 10.55 to 16.34 g. These results align with the findings of Pushpa and Ketosawara (2013), which vary from 7.26 to 15.38 g. The small variation in the value range reported by different authors and this study may be due to the differences in the number of varieties used and the environment of the study

In our study, the least pod length was recorded in T_2, T_4, and T_8 (3.1, 3.1 and 3.1 cm) treated with (0.2% EMS, 0.4% EMS and 0.4% SA), and the longest pod was obtained inT_9 (0.40 % SA) with the value of (4.7 cm), so therefore pod length could be consider in selecting high yielding mutant variety for next generation. The results were similar to those of Chandrawati et al. (2016) and Ahmed (2017). They showed that the pod length on each plant can be used to choose high-yield mutant lines for future generations so that better soybean cultivars can be grown. This conclusion aligns with a study that indicated the pod length to be between 3.1 and 4.7 cm (Ningsih et al. 2019). Olena et al. (2020) reported an average pod length of 3.90 cm.

In our study, the number of seed per pod was highest in SL958 with the value of (4.04 seed per pod), treated with 0.4% SA in T_8. While the lowest values were recorded in T_7 (2.36 seed per pod) treated with 0.6% MMS. Similar results were reported by Kanwar et al. (2014), Tyagi et al. (2014), Singh et al. (2015), Chandrawati et al. (2016), Ahmed (2017) and Meena et al. (2020). In our studies the Null Hypotheses (H_o): The application of mutagens has no significant effect on the phenological parameters of soybean compared to untreated controls. We reject the null hypothesis and accept the alternate hypothesis.

In our studies the pollen grains treated with mutagens showed significant morphological diversity, with 0.4% EMS and 0.4% SA treatments producing healthier, more regular grains compared to 0.6% MMS. The EDS analysis provides insights into the chemical changes induced by the mutagens. However, mutagen-treated samples show variations in elemental composition, SL744 treated with EMS (0.4%) has high carbon content (51.52%) and moderate levels of oxygen and calcium, reflecting structural and compositional changes. SL958 treated with EMS (0.4%) exhibits an even higher carbon content (63.9%), further indicating the presence of organic material and potential mutagenic effects. Sodium azide (SA)-treated samples show similarly altered elemental compositions, with increasing roughness and structural damage. In our studies the Null Hypotheses (H_0): Different concentrations of mutagens have no significant effect on meiotic division in soybean pollen grains. We reject the null hypothesis and accept the alternate hypothesis.

In our study, the protein content of the M_2 seeds of SL958 increased (47.04% TSP) with the decreased dose of SA (0.4%) and decreased (40.62% TSP) with the increased dose (0.6% SA). Jephter et al. (2017), reported that there was an increase in the percentage of protein with lower doses of EMS (0.1%) and a decrease with higher doses of EMS (0.5%) in soybean seeds. The highest crude lipid content was observed (22.34%) with 0.4% SA, while lowest was recorded (19.87%) with 0.6% SA. Our findings are similar to that of Olorunmaiye et al. (2019), who reported that high doses of EMS (1.2%) decreased the lipid content and lower doses (0.25%) increased the lipid content. In our study, the highest fibre content (18.23%) was observed with 0.4% SA, while the lowest (16.90%) was observed with 0.2% MMS. Olorunmaiye et al. (2019), reported that high doses of EMS (1.25%) decreased the fibre content and lower doses (0.25%) increased the fibre content in groundnut seeds.

The present study demonstrates that chemical mutagens significantly influence the accumulation of secondary metabolites, alkaloids, flavonoids, saponins, and tannins, in the methanol extracts of two soybean (*Glycine max*) varieties, SL744 and SL958. The observed responses varied with mutagen type, concentration, and varietal background, corroborating earlier reports that chemically induced mutagenesis exerts dose-dependent and genotype-specific effects on plant secondary metabolism (Mensah et al., 2007; Kumar & Dwivedi, 2014).

Alkaloid accumulation in the present study exhibited a two distinct phases response to chemical mutagen treatment. In SL744, low-dose (0.2% SA) enhanced alkaloid content, whereas higher concentration (0.6% SA) resulted in substantial reduction. This phenomenon response is consistent with earlier studies indicating that low levels of chemical stress stimulate secondary metabolite biosynthesis as part of plant defense mechanisms, while higher doses disrupt enzymatic activity and cellular metabolism (Taiz et al., 2015; Isah, 2019).

In SL958, ethyl methane sulfonate (EMS) at 0.2% produced the highest alkaloid content, whereas methyl methane sulfonate (MMS) at 0.4% caused marked inhibition. EMS is known to induce point mutations that may enhance transcription of genes involved in alkaloid biosynthesis, whereas MMS, due to its stronger alkylating capacity, can lead to excessive DNA damage and metabolic suppression at moderate to high concentrations (Van Harten, 1998; Khan et al., 2011). The varietal differences observed in alkaloid accumulation agree with previous reports that genetic background determines plant sensitivity to mutagenic treatments (Mensah et al., 2005).

Saponin content was strongly enhanced by MMS treatment in both soybean varieties, with peak values recorded at 0.4% MMS in SL744 and 0.6% MMS in SL958. This finding suggests that MMS effectively stimulates the triterpenoid biosynthetic pathway, which is responsible for saponin production. Similar increases in saponin content following chemical mutagen treatment have been reported in legumes and medicinal plants (Francis et al., 2002; Aremu et al., 2012).

In contrast, EMS treatments consistently reduced saponin levels across both varieties. This reduction may be attributed to EMS-induced mutations that impair key enzymes involved in the mevalonate pathway or reduce precursor availability for saponin biosynthesis. Previous studies have shown that EMS can negatively affect complex secondary metabolites when applied beyond optimal concentrations (Kodym & Afza, 2003). The variable effects observed with SA further support its role as a stress signaling molecule, whose influence on secondary metabolism depends largely on concentration and exposure duration (Hayat et al., 2010).

Tannin accumulation was most strongly stimulated by EMS, particularly at 0.4% concentration in both soybean varieties. This enhancement suggests activation of the phenylpropanoid pathway, which governs tannin biosynthesis. EMS has been reported to upregulate enzymes such as phenylalanine ammonia-lyase, leading to increased synthesis of phenolic compounds under moderate stress conditions (Dixon & Paiva, 1995; Kumar et al., 2010). However, the decline in tannin content at higher concentrations of SA indicates a threshold beyond which chemical stress becomes inhibitory. Excessive SA may generate oxidative stress that interferes with phenolic metabolism and enzyme stability (Apel & Hirt, 2004). MMS treatments-maintained tannin levels close to those of the control, suggesting a milder or regulatory effect rather than strong stimulation, in agreement with reports describing MMS as inducing relatively stable metabolic adjustments (Van Harten, 1998).

In our studies the Null Hypotheses (H_o): The application of SA, EMS, and MMS at different concentrations does not significantly affect the quantitative or qualitative parameters of soybean at harvest. We reject the null hypothesis and accept the alternate hypothesis. Thus, the response to mutagens depends on the dose, plant type, and geographical location of the study. Therefore, the evaluation and elicitation of mutagens could be considered as an alternative method for the production of low-cost functional foods to diversify the soybean market. Moreover, it is important to identify elicitors that stimulate sprout growth and yield so as to improve plants' nutritional properties.

CHAPTER 6

Summary, Conclusion and Recommendation

CHAPTER SIX

6.0. SUMMARY, CONCLUSION AND RECOMMENDATION

6.1. Summary

The golden plant cultivar (*Glycine max* L.) like many other Fabaceae is of great agricultural importance in Punjab State, India. It is the most cultivated variety of the soybean mostly by small-scale subsistence farmers in the area in the last six decade. It has simple agronomic maintenance practices and produces large yield that are used as great source of income for a vast number of families in different parts of the state and country at large. This crop is sometimes cultivated along with some other cereals and the profits gained from both side and is conveniently used to upset the cost of other crops. In India, soybean is also used in various food products, including tofu, soya sauce, simulated milk, and meat products, and the oil is frequently used for cooking. Soybean meal is used as a supplement in livestock feed. Apart from this, it is used in industries for the production of biofuels and functional foods. The industrial use of soybeans ranges from the production of yeasts and antibodies to the manufacture of soaps and disinfectants etc. Farmers are struggling hard to protect their legumes including the soybean plant against the various biotic and abiotic constraints for improved production and profits as well. This study has determined the effect of different chemical mutagens for yield and quality traits on the soybean varieties under climatic condition of Punjab.

5.2. Conclusion

In conclusion the results obtained in the present study with regard to the application of chemical mutagens on soybean varieties have shown it is a simple and inexpensive method for improving agronomic characteristics (tetrafoliate leaf, pentafoliate leaf, early flowering, early maturing, pod size seed size, oil content, protein content, and resistance to diseases can be enhanced by selecting desirable mutants). Their mutagenic effects manifest shortly after sowing seeds and are observable. Thus, chemical mutagens have been used in various crops to improve their yield and quality traits, enhanced oil and protein content, stress resistance, and adaptation to the double-cropping system will be key to maximizing the success of these mutant varieties. The findings of this research have shown positive effects of SA treatment in SL958, which proved to be efficient in generating the highest leaf area (the tetrafoliate leaves were broad, but the pentafoliate leaves were narrow), and the highest number of pods/plants and protein content were recorded for the $M_1$and M_2 plants. Furthermore, SL744 showed positive effects from the EMS treatment in terms of yield and quality traits, which indicates the potential of EMS and SA to induce useful mutations in soybean. The potential mutants generated should be selected for further evaluation (for assessing the transfer of trait(s) in subsequent generations) in a soybean breeding programme for farmers in Punjab.

6.3. Recommendation

The following recommendations were raised:

A. Looking at the neglection of soybean production in Punjab, unlike rice and wheat, which are predominantly water consumers, which lead to the excess mining of underground water for irrigation, and considering the effectiveness and efficiency of chemical mutagens at low doses (sodium azide is more effective) should be employed among others chemicals such as ethyl methane sulfonate and methyl methane sulfonate for creating genetic variability in soybean, which might serve as a complement in terms of oil, protein, fiber, essential amino acid, and saturated fatty acid and for yield and quality traits as observed in the current research.

B. There is a need for urgent action and collaboration between the policymakers, scientists, and farmers to provide a lasting solution and awareness on the effects of chemical mutagens on soybean production in the area, as they are known to be safe, cost-effective, eco-friendly, and sustainable for improved crop productivity worldwide compared with physical mutagens.

C. The SA, and EMS mutant population should be looked into further in future studies that screen soybeans for amino acid routes, phytic acids, allergens, and other agronomic traits.

D. More research needs to be done on how to use chemical mutagens to improve the ability of soybean mutant lines. The good traits seen in these mutants can be used in future soybean breeding projects which can be essential for improving resistance to abiotic and biotic stresses.

CHAPTER 7
BIBLIOGRAPHY

Adamu AK, Aliyu H (2007) Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). Sci World J 2(4):9–12

Adeniyan ON, Akande SR, Balogun MO, Saka JO (2007) Evaluation of crop yield of African yam bean, maize and kenaf under intercropping systems. AEJAES 2(1):99–102

Ahire DD, Thengane RJ, Manjaya JG, George M, Bhide SV (2005) Induced mutations in soybean (*Glycine max.* (L.). Soybean Res (3):1–8

Ahloowalia BS, Maluszynski M, Nichterlein K (2004). Global impact of mutation-derived varieties. Euphytica 135:187–204

Ahoowalia BS (1967) Colchicine induced in polyploids in ryegrass *Lolium perenne* L. Euphytica 16:49–60

Ahmed E (2017) Genetic studies of yield and yield component of soybean (*Glycine max* (L.) Merrill). J Pharmacogn Phytochem (1): 872–875

Aishatu, GA (2015) Sodium azide: A chemical mutagen for enhancement of yield traits of sesame. J Bacteriol Parasitol 5:1–21

Alghamdi SS, Khan MA, El-Harty EH, Ammar MH, Farooq M, Migdadi HM (2018) Comparative phytochemical profiling of different soybean (*Glycine max* (L.) Merr) genotypes using GC–MS. Saudi J Biol Sci 25(1):15‒21

Aliyu RE, Aliyu A, Adamu AK (2018) Induction of genetic variability in sesame (*Sesamum indicum* L.) with sodium azide. FUW Trends Sci Technol J 3:96–100

Al-Qurainy F, Khan S (2009) Mutagenic effects of sodium azide and its application in crop improvement. Sci World J 6 (12):1589 –1601

Al-Qurainy F (2009) Effects of Sodium azide on growth and yield traits of *Eruca sativa* (L.). World Appl Sci 7(2):220–226

Amoanimaa, Dede H, Su C, Yeboah A, Zhou H, Zheng D, Zhu H (2022) Growth regulators promote soybean productivity. Peer J 4(10):1–56

Apparao BJ (2005) Effect of sodium azide on peroxidase isozyme profiles in *Vigna unguiculata* (L.) Walp. Adv Plant Sci 18:44–50

Association of official analytical chemists. international, official methods of analysis, 16th ed, AOAC International: Arlington, VA, USA, (1995) 23–27

Arisha MH, Shah, SN, Gong ZH, Jing H, Li C, Zhang HX (2015) Ethyl methane sulfonate induced mutations in M2 generation and physiological variations in M1 generation of peppers (*Capsicum annuum* L.). Front Plant Sci 6:1–11

Agyei D, Potumarthi R, Danquah MK (2015) Food-derived multifunctional bioactive proteins and peptides: applications and recent advances. In: Gupta, V.K., Tuohy, M.G., O’Donovan, A., Lohani, M. (Eds.), Biotechnology of Bioactive Compounds: Sources and Applications. Wiley Blackwell, Chichester, 507–524

Anantha swamy HN, Vakil UK, Sreenivasan A (1971) Biochemical and physiological changes in gamma-irradiated wheat during germination. Radiat Bot 11(1):1–12

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55:373–399

Remanandan P, Asiegbu JE (1993) Pigeon pea germplasm collection in Nigeria. Genetic Resources Progress Report. WJAS 1–79

Bado S, Forster BP, Nielen S, Ali A M, Lagoda P J, Till B J, Laimer M (2015). Plant mutation breeding: current progress and future assessment. Plant Breed 39:23–88

Bakshi S, Jambhulkar SJ, Kumar U, Bhati P (2020) Induced mutagenesis to sustain wheat production under changing climate. In: Improving Cereal Productivity through Climate Smart Practices. Woodhead Publishing Series in Food Science, Technology and Nutrition. Elsevier, Amsterdam the Netherlands pp. 37–63

Beyaz R, Yildiz M (2017) The use of gamma irradiation in plant mutation breeding. In: Plant Engineering. Jurić S (ed), Intech Open, Rijeka, Croatia, Ch. 3, ISBN 978-953–51-3608-8

Bremner JM, Mulvancy CS (1982) Total nitrogen. In: page, A L, Miller RH, Keeney DR (Eds) Methods of soil analysis, Part 2. Chemical and microbiological properties. American society of agronomy and soil science society of America, Madison Wisconsin Inc. pp. 594 –596

Bouma J (1976) The spring barley mutant cultivar 'Diamant', its economic importance and breeding value. Mutat Breed Newsl 72–73

Biswas P, Karim, MA, Kabir G, Haque MM (2016) Characterization and seed quality of some dwarf soybean genotypes. IJAAR Castro CM, Oliveira AC, Calvaho FF (2003) Changes in allele frequencies in colchicine treated Ryegrass population assessed with APD marker. Agrociencia 9(2):107–112

Chandirakala R, Subbaraman N (2010) Character association and path analysis for yield attributes in full sib progenies in Pigeon pea (*Cajanus cajan* (L.) Mill Sp.). Electron J Plant Breed 1:824– 827

Chandrawati N, Yadav VK, Kumar R, Kumar S, Yadav HK (2016) Genetic variability and interrelationship among morphological and yield traits in soybean (*Glycine max* (L.) Merrill.). Genetika 48:881–892

Chaturvedi S, Malik MY, Sultana N, Jahan S, Singh S, Taneja I, Wahajuddin M (2021) Chromatographic separation and estimation of natural antimalarial flavonoids in biological matrices. Proc Indian Natl Sci Acad 87:446–468

Chandrawati KS, Baig KS, Hashmi S, Sarang DH, Kumar A, Dumai PK (2017) Study on genetic variability, heritability and genetic advance in soybean. Int J Pure Appl Biosci 5(1):57–63

Chen CT, Lu CT, Tzen JTC, Yang CY (2021) Physiological properties and molecular regulation in different edamame cultivars under drought stress. Agronomy 11(5):1–12

Chen L, Duan L, Sun M, Yang Z, Li H, Hu K, Liu L (2023) Current trends and insights on EMS mutagenesis application to studies on plant abiotic stress tolerance and development.

Front Plant Sci 4(13):1–13

Chen T, Huang L, Wang M, Huang Y, Zeng R, Wang X, Zhang L (2020) Ethyl methyl sulfonate- induced mutagenesis and its effects on peanut agronomic, yield and quality traits. Agronomy 10(5):1–13

Chopra VL (2005) Mutagenesis: Investigating the process and processing the outcome for crop improvement. Curr Sci 89:353–359

Crawford LE, Williams MM (2019) Planting depth and seed size affect edamame emergence individually. Hort Sci 54:92–94

Chrispeels MJ, Varner JE (1967) Hormonal control of enzyme synthesis: on the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiol 42(7):1008–1016

Devi AS, Mullainathan L (2011) Physical and chemical mutagenesis for improvement of chilli (Capsicum annuum L.). World Appl Sci J 15(1):108–113

Devi J, Sagar V, Kaswan V, Ranjan JK, Kumar R, Mishra GP, Dubey RK, Verma RK (2021) Advances in breeding strategies of bell pepper (Capsicum annuum L. var). In: Advances in Plant Breeding Strategies: Vegetable Crops, Volume Fruits and Young Shoots. Al-Khayri JM, Jain SM, Johnson DV (eds), Springer International Publishing, Cham, Switzerland, pp. 3–58, ISBN 978-3- 030-66961-4

Dewi K, Meidiana G, Sudjino S, Suharyanto S (2016) Effects of sodium azide (NaN3) and cytokinin on vegetative growth and yield of black rice plant (Oryza sativa L.). AIP Conf Proc 1755(1), AIP Publishing

Dhaliwal DS, Williams MM II (2020) Economically optimal plant density for machine-harvested edamame. Hort Sci 55 368–373

Dinkar V, Kumar Verma B, Sanjay G, Arora A, Panwar R, Verma S (2020) Mutagenesis induced variability through gamma rays, EMS and combination treatments in chickpea genotypes. J Pharmacogn Phytochem 9:1139–1144

Dilawari R, Kaur N, Priyadarshi N, Prakash I, Patra A, Mehta S, Islam MA (2022) Soybean: A key player for global food security. Physiol Mol Biol Plants 1–46 Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. The Plant Cell 7:1085–1097

Dupare BU (2023) Improved technologies and recommendations for maximizing soybean productivity. ICAR-Indian Inst Soybean Res Publ Ext Bull 18 pp 1–74

Dwivedi SL, Kanwar L, Sahrawat H, Upadhyaya D, Mengoni A, Ortiz R (2015) Advances in host plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. Adv Agron 129:1–116

Eseigbe OB, Kamara AY, Miko S, Omoigui LO, Solomon R, Adeleke MA (2024) The impact of planting dates on the performance of soybean varieties [Glycine max (L.) Merr.] in the Nigerian Savannas. Agronomy 14(10):1–18

Espina MJ, Ahmed CMS, Bernardini A, Adeleke E, Yadegari Z, Arelli P, Pantalone V, Taheri A (2018) Development and phenotypic screening of an ethyl methane sulfonate mutant population in soybean. Front Plant Sci 9:1–12

Etther Y, Sj G, Akhare A, An P, Sj J, Gawande M (2019) Genetic variability induced by gamma radiation and ethyl methane sulphonate on quantitative characters in pigeon pea (Cajanus cajan). J Pharmacogn Phytochem 8:1903–1907

Farnsworth NR (1966) Biological and phytochemical screening of plants. J Pharm Sci 55(3):225– 276

FAO (FAOSTAT) (2013) Food and agriculture data. Available online at: http://www.fao.org/faostat/ en/#home, Accessed date: 21 August 2023

FAO (FAOSTAT) The statistical database (2023/2024) Annual soybean production by different countries Website http://www.fao.org/faostat/en/. Accessed 14 April 2024

Francis G, Kerem Z, Makkar HPS, Becker K (2002) The biological action of saponins in animal systems. British Journal of Nutrition 88:587–605

Garber B, Neill CL (2019) Edamame: Costs, revenues, and profitability. No. AAEC-189P, Virginia Cooperative Extension, Blacksburg, VA, USA 1–4

Garner A, Allard R (1920) Cajanaus cajan (L) Millsp. As a potential agroforestry crop in the Eastern Province of Zambia. Agroforesty Systems 9:127–140

Ghassemi-Golezani K, Taifeh-Noori M, Oustan S, Moghaddam M (2009) Response of soybean cultivars to salinity stress. J Food Agric Environ 7:401–404

Gill B, Kumar A, Dhillon S, Singh T (2015) SL958: A new high yielding and yellow mosaic resistant variety of soybean. Agric Res J 52–124

Girija M, Dhanavel D (2009) Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonates and their combined treatments in cowpea (Vigna unguiculata L. Walp). Int J Mol Sci 4(2): 68–75

Gupta S, Kumawat G, Agrawal N, Tripathi R, Rajesh V, Nataraj V, Maranna S, Satpute GK, Chandra S, Ratnaparkhe MB (2022) Photoperiod trait: Insight in molecular mechanism for growth and maturity adaptation of soybean (Glycine max) to different latitudes. Plant Breed 141: 483–500

Gutiérrez-Valencia J, Zervakis PI, Postel Z, Fracassetti M, Losvik A, Mehrabi S, Slotte T (2024) Genetic causes and genomic consequences of breakdown of distyly in Linum trigynum. MBE 41(5):23–87

Gulati A (2002) Challenges to Punjab agriculture in a globalizing world; IFPRI: Washington, DC, USA 1–9 Habib SH, Akanda MAL, Roy P, Kausar H (2021) Effect of different dosage of EMS on germination, survivability and morphophysiological characteristics of sunflower seedling. Helia 44:167–180

Hartman GL, Pawlowski ML, Herman TK, Eastburn D (2016) Organically grown soybean production in the USA: Constraints and management of pathogens and insect pests. Agronomy 6(1):1–16

Hamisu A, Koul B, Arukha AP, Al Nadhari S, Rabbee MF (2024) Evaluation of the impact of chemical mutagens on the phenological and biochemical characteristics of two varieties of soybean (Glycine max L.). Life 14 (7):1–17

Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of exogenous salicylic acid under changing environment. Environmental and Experimental Botany 68:14–25

He J, Hu Y, Li WC, Fu FL (2009) Drought tolerant mutant induced by gamma ray and sodium azide from maize calli. Maize Genet Coop Newsle 83:53–55

Helmke P A, Sparks D L (1996) Methods of soil analysis, Part 3. Chemical methods -soil science society of America. Book series No. 5. Madison, Wisconsin, USA

Hoeft RG, Nafziger ED, Johnson RR, Aldrich SR (2000) Modern corn and soybean production. 1st ed. MCSP Publishing, Champaign, IL, pp. 48–52

Hymowitz T, Harlan JR (1983) Introduction of soybeans to North America by Samuel Bowen in 1765. Econ Bot 37: 371–379

ICAR India Institute of Soybean Research (2023) Extension Bulletin 18:1–77

Ikhajiagbe B, Odigie EU, Okoh BE, Agho EE (2013) Effects of sodium azide on the survival, growth, and yield performance of rice (Oryza sativa, FARO-57 variety) in a hydrocarbon-polluted soil. Int J Biotechnol 2(1):28–41

Ilbas AI, Eroglu Y, Eroglu HE (2005) Effects of the application of different concentrations of NaN3 for different times on the morphological and cytogenetic characteristics of barley (Hordeum vulgare L.) seedlings. J Integr Plant Biol 47(9):1101–1106

Iwuchukwu JC, Beeior CT (2018) Constraints to USAID Market II soybean production project in Benue State Nigeria. J Agric Ext. 22(3):139–148

Isah T (2019) Stress and defense responses in plant secondary metabolites production. Biological Research 52:39

Jephter BFM, Mumba P, Bokosi JM (2017) Assessment of the agronomic productivity and protein content in 16 soybean genotypes. Afr J Food Agric Nutr Dev 17(4):12600–12613

Kanwar RR, Saxena RR, Ruth EE. (2014) Variability, heritability and genetic advance for yield and some yield related traits in soybean (Glycine max (L.) Merrill). Agric Sci Dig 34:154–156

Kataliko RK, Kimani PM, Muthomi JW, Wanderi WS, Olubayo FM, Nzuve FM (2019) Resistance and correlation of pod shattering and selected agronomic traits in soybeans. J Plant Stud 8(2):40– 45

Khan MH, Tyagi V (2009) Cytological effects of different mutagens in soybean (Glycine max (L.) Merrill). China Agric Font 3:397–401

Khan S, Al-Qurainy F, Anwar F (2009) Sodium azide: a chemical mutagen for enhancement of agronomic traits of crop plants. Environ We Int J Sci Tech 4:1–21

Kharkwal MC, Nagar, JP, Kala YK. (2005) BGM 547—A high yielding chickpea (Cicer arietinum L.) mutant variety for late sown condition in north western plain zone of India. Indian J Genet Plant Breed 65:229–230

Khursheed S, Raina A, Parveen K, Khan S (2019) Induced phenotypic diversity in the mutagenized populations of faba bean using physical and chemical mutagenesis. J Saudi Soc Agric Sci 18:113– 119

Kihlman BA (1959) The effect of respiratory inhibitors and chelating agents on the frequencies of chromosomal aberrations produced by X-rays in Vih. J Biophys Biochem Cytol 5:479–490

Krishna G, Shivshankar S, Nath J (1984) Mutagenic response of Rhodes grass (Chloris gayana Kunth.) to gamma rays. Environ Exp Bot 24:197–205

Kumar G, Pandey A (2019) Ethyl methane sulphonate induced changes in cyto-morphological and biochemical aspects of Coriandrum sativum L. J Saudi Soc Agric Sci 18:469–475

Kurata N, Miyoshi K, Nonomura KI, Yamazaki Y, Ito Y (2005). Rice mutants and genes related to organ development, morphogenesis and physiological traits. Plant Cell Physiol 46:48–62

Lokuruka MNI (2010) Soybean nutritional properties: The good and the bad about soy foods consumption. AJFAND 10(4):1–21

Lima JME, Smiderle OJ (2013) Potencial fisiológico de sementes de soja-hortaliça produzidas com diferentes adubações e armazenadas por doze meses 7:70–79

Lord N, Kuhar T, Rideout S, Sutton K, Alford A, Li X, Wu X, Reiter M, Doughty H, Zhang B (2021) Combining agronomic and pest studies to identify vegetable soybean genotypes suitable for commercial edamame production in the mid-atlantic U.S. Agric Sci 12:738–754

Lu WH, Wang XZ, Zheng Q, Guan SH, Xin P, Sun YQ (2008) Diversity and stability study on rice mutants induced in space environment. Genom. Proteom. Bioinform 6:51–60

Markeen KG, Suresh B, Lavanya GR (2007) Effect of mutagenesis on M1 population in urd bean. J. Food Legumes 20(1):109–110

Masuda T, Goldsmith PD (2009) World soybean production: area harvested, yield, and long-term projections. Int Food Agribus Manag Rev 12(4):1–20

Madhu M, Sailaja V, Satyadev TNVSS, Satyanarayana MV (2016) Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. J Pharmacogn Phytochem 5(2):25–29

Mathew BA, Sule, HA, Toluhi OJ, Idachaba SO, Ibrahim AA, Abuh SJ (2015) Studies on protein composition of pigeon pea (Cajanus cajan (L.) Millspaugh) treated with sodium azide and gamma radiation. J Pharm Biol Sci 10(1): 01–04

Meena AK, Kulhari S, Kumar M, Koli MN, Tak Y, Meena M, Meena N (2020) Studies on genetic variability and character association in soybean (Glycine max (L.) Merrill) genotypes. Int J Curr Microbiol Appl Sci 9:3949–3957

Mensah JK, Akomeah PA, Ekpekurede EO (2005) Gamma irradiation induced variation of yield parameters in cowpea (Vigna unguiculata (L.) Walp.) Glob J Pure Appl Sci 11(3):327–330

Mensah JK, Obadoni BO, Akomeah PA, Ajibolu J (2007) The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (Sesame indicum L.) Afr J Biotechnol 6(5):534–538

Mensah JK, Obadoni BO, Akomeah PA, Ikhajiagbe B, Ajibolu J (2007) The effects of sodium azide and colchicine treatments on morphological and yield traits of soybean. Afr J Biotechnol 6:1015–1020

Mishra R, Tripathi MK, Sikarwar RS, Singh Y, Tripathi N (2024) Soybean (Glycine max L. Merrill): A multipurpose legume shaping our world. PCBMB 25(4):17–37

Mir S, Faheem M, Sial MA, Ullah G, Leghari KA (2024) Mutagenesis: Exploring genetic diversity of industrial crop plants. AGP 73–100

Moloi MJ, Van der Merwe R (2021) Drought tolerance responses in vegetable-type soybean involve a network of biochemical mechanisms at flowering and pod-filling stages. Plants 10(8):1– 17

Motsara MR, Roy RN (2008) Guide to laboratory establishment for plant nutrient analysis. FAO of the UN Rome pp 407–412

Mullangie DP, Thiyagarajan K, Swaminathan M, Ramalingam J, Natarajan S, Govindan S (2024) Breeding resilience: Exploring lodging resistance mechanisms in rice. Rice Science. 31(6): 659–672

Nagar S, Singh M, Billore SD, Verma RK, Kuchlan P, Chandra S (2018) Soybean seed germination: factors affecting and ways to minimize germination loss. Pop. Kheti (ICAR) 6(2):13– 17

Nair RM, Boddepalli VN, Yan MR, Kumar V, Gill B, Pan RS, Somta P (2023) Global status of vegetable soybean. Plants 12(3):1–22

Naaz N, Choudhary S, Hasan N, Sharma N, Alharbi K, Abd El Moneim D (2024) Enhancing genetic variability in Trigonella species through sodium azide induction: morpho-physiological and chromosomal amelioration. Front Genet 15:1–14

Nakata Y, Ueno M, Kihara J, Ichii M, Taketa S, Arase S (2008) Rice blast disease and susceptibility to pests in a silicon uptake-deficient mutant lsi1 of rice. Crop Prot 27:865–868

Nelson D W, Sommers LW (1982) Total carbon, organic carbon and organic matter. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis. Part 2. Second edition. Chemical and
microbiological properties. American society of agronomy and soil science society of America. Madison, Wisconsin USA. Pp 303–300

Negi JS, Singh P, Rawat B (2010) Chemical constituents and biological importance of Swertia A review Curr Res Chem 3(1):1–15

Nillan RA, Pearson OW (1975) Lack of chromosome breakage by azide in embryonic shoots and microspores of barley. Barley Genet Newsl 5:33–35

Ningsih F, Zubaidah S, Kuswantoro H (2019) Diverse morphological characteristics of soybean (Glycine max L. Merill) pods and seeds germplasm. Earth Environ Sci 276(1):12–14

Nolen S, Zhang B, Kering MK (2016) Increasing fresh edamame bean supply through season extension techniques. J Hortic 3(170):2376–0354

Norsuwan T, Poungmanee J, Kasensoontron R, Utasuk K, Pongjanta W (2015) Application of yellow sticky traps and gap recommended insecticide for insect pest control in green soybean production in Chiang Mai province J Agric 31:319–327

Nuthalapati CS, Kumar A, Birthal PS, Sonkar VK (2024) Demand-side and supply-side factors for accelerating varietal turnover in smallholder soybean farms. J Clean Prod 1(447):1–14

Ogedengbe II, Bello EI (2018) Production, characterization and glycerine analysis of soybean oil and its biodiesel. J Energy Res Rev 1(2):1–10

OlaOlorun BM, Shimelis H, Laing M, Mathew I (2021) Morphological variations of wheat (Triticum aestivum L. em. Thell.) under variable ethyl methane sulphonate mutagenesis. Cereal Res Commun. 49:301–310

Olorunmaiye KS, Joseph GG, Animashaun DA, Oyedeji S (2019) Mutagenic components and dosage effects of ethyl methane sulphonate on Arachis hypogea (Samnut 24 VR). Ife J Sci

21(2):309–322

Opoku Gyamfi M, Eleblu JSY, Sarfoa LG, Asante IK, Opoku-Agyemang F, Danquah EY (2022) Induced variations of ethyl methane sulfonate mutagenized cowpea (Vigna unguiculata L. walp) plants. Front Plant Sci 5(13):1–17

Olena S, Sabine Z, Wilhelm C, Sabine G (2020) Seed yield, seed protein, oil content, and agronomic characteristics of soybean (Glycine max L. Merrill) depending on different seeding systems and cultivars in Germany. Agronomy 10 (7):10–20

Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (Eds) Methods of soil analysis. Part 2, Chemical and microbiological properties. Second edition. American Soc Agron Soil Sci Soc of America, Madison, Wisconsin USA. pp. 405–429

Parchin RA, Ghomi AA, Badi HN, Navabpour S, Mehrafarin A, Eskandari A (2021) Investigation effect of ethyl methane sulfonate (EMS) on some of morphophysiological and

phytochemical traits of fenugreek (Trigonella foenum-graecum l.). Ind Crops Prod 162:113–239 Pagano MC, Miransari M (2016) The importance of soybean production worldwide. Abiotic and Biotic Stresses in Soybean Production. Academic Press 1–26

Pagano MC, Covacevich F (2011) Arbuscular mycorrhizas in agroecosystems. In: Fulton SM (Ed.), Mycorrhizal Fungi: Soil, agriculture and environmental implications. New York, Nova Science Publishers 35–65

Pagano MC (2012) Plant and soil biota: crucial for mitigating climate change. In: Interactions of forests, climate, water resources, and humans in a changing environment. Br J Environ Clim Change 3(2):188–196

Pandey A, Malik P, Kumar A, Kaur N, Saini DK, Gill RK, Kashyap S, Kaur S (2024) Multi-GWAS reveals significant genomic regions for mung bean yellow mosaic India virus resistance in urdbean (Vigna mungoL.) across multiple environments. Plant Cell Rep 43(7):166

Patil AM, Wagh SG, Janvale GB, Pawar BD (2024) Daspute AA Viral delivery of CRISPR/Cas9 genome editing for rapid crop improvement: A promising approach to enhance crop resilience against biotic and abiotic stresses. Int J Adv Biochem Res (8):782–796

Pushpa R, Ketoswara R (2013) Genetic variability in soybean. IJPAES 1–35

Rafeeq MKM, Mogali SC, Patil KS, Leelavathi TM (2017) Isolation of high-yielding mutants through EMS-Induced mutagenesis in soybean (Glycine max (L.) Merrill.). IJCMAS 6:278–285

Rai A, Bornare SS, Prasad LC, Lal JP and Prasad R. (2013) Effect of different dose of gamma rays on two varieties of soybean crop [Glycine max (L.) Merrill.]. Int J Plant Res 26:368–371

Ram B, Hemaprabha G (2020) The sugarcane variety Co 0238—A reward to farmers and elixir to India's sugar sector. Curr Sci 118:1643–1646

Reddy MP, Reddy BN, Arsul BT and Maheshwari JJ (2013) Genetic variability, heritability and genetic advance of growth and yield components of soybean (Glycine max (L.) Merrill). Int J Curr Microbiol Appl Sci 2: 231–237

Robert JW (1986) The soybean solution: Meeting world food needs. NIT-College of Agriculture, University of Illinois at Urbana, Champaign, USA. I Bulletin 4–27

Samadi A, Derafshi M, Hassani A, Gholamhoseini M, Asgari Lajayer B, Astatkie T, Price GW (2024) Effects of biofertilizers and potassium sulfate on nutrients uptake and physiological characteristics of maize (Zea mays L.). J Crop Health 76(1):209–218

Sato M, Gaul H (1967) Effect of ethyl methane sulfonate on the fertility of barley. Radiation Botany 7(1):7–15

Shah D, Kamili AN, Wani AA, Nazir N, Sajad N, Khan,I, Shah S (2016) Mutagenic action of ethyl methane sulphonate (EMS) J. Res. Dev 16: 63–68

Saha B, Matiul L (2022) Assessment of yield performance and related attributes of selected soybean (Glycine max L. Merrill) genotypes in southwest coastal region of Bangladesh. Bulg. J. Agric. Sci 28 (1):76–88

Shea Z, Singer WM, Zhang B (2020) Soybean production, versatility, and improvement. Legume crops 29–50

ShilpaSshree N, Devi SN, Manjunathagowda DC, Muddappa A, Abdelmohsen SA, Tamam N, Janhavi V (2021) Morphological characterization, variability and diversity among vegetable soybean (Glycine max L.) genotypes. Plants, 10(4):671

Siddique MI, Back S, Lee JH, Jo J, Jang S, Han K, Venkatesh J, Kwon JK, Jo YD, Kang BC (2020) Development and characterization of an ethyl methane sulfonate (EMS) induced mutant population in Capsicum annuum L. Plants 9:380–396

Song M, Fan S, Pang C, Wei H, Liu J, Yu S (2015) Genetic analysis of fiber quality traits in short season cotton (Gossypium hirsutum L.). Euphytica, 202:97−108

Sedibe M.M, Mofokeng AM, Masvodza DR (2023) Soybean production, constraints, and future prospects in poorer countries: A Review. In Production and utilization of legumes—progress and prospects; Hasan Uzza man, M., Ed.; Intech Open: Rijeka, Croatia, p. Ch. 4, ISBN 978-1- 83768- 647-6

Sugiyama A, Ueda Y, Takase H, Yazaki K (2015) Do soybeans select specific species of Brady rhizobium during growth. Commun Integr Biol 8(1):27–34

Simard S, Austin ME (2010) The role of mycorrhizas in forest soil stability with climate change. In: Simard, S., Austin, M.E. (Eds.), Climate Change and Variability. Intech Open Access Publisher, 275–302

Tyagi AK, Sharma MK, Mishra SK, Kerkhi SA and Chand P (2014) Estimates of genetic variability, heritability and genetic advance in soybean (Glycine max (L.) Merrill) germplasm. Progress Agric 14:37–48

Tamilzharasi M, Dharmalingam K, Venkatesan T, Jegadeesan S, Palaniappan J (2022) Mutagenic effectiveness and efficiency of gamma rays and combinations with EMS in the induction of macro mutations in blackgram (Vigna mungo (L.) Hepper). Appl Radiat Isot 188:110382

Ulafić A, Varga I, Stošić M, Iljkić D, Antunović M (2020) Analysis of soybean growth in regard to different row-spacing. BJAS 26(3):1–7

Van Harten AM (1998) Mutation breeding: theory and practical applications. Cambridge University Press, Cambridge Vivian R, Reis A, Kálnay PA, Vargas L, Ferreira AC, Mariani F (2013) Weed management in soybean. Soyb Pest Resist 50–84

Yang YJ, Yang SJ, Yang YH, Zeng JM (2003) The breeding of an early sprouting and high-quality new clone suitable for fine green tea. J. China Tea 25:13–15

Walkley A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29– 37

CHAPTER 8

Appendixes

List of publication

1. **Hamisu A**, Koul B, Arukha AP, Al Nadhari S, Rabbee MF (2024) Evaluation of the Impact of Chemical Mutagens on the Phenological and Biochemical Characteristics of Two Varieties of Soybean (*Glycine max* L.). Life 14 (7):1–17 (IP: 3.2).

2. **Anas H**, Bhupendra K, Subhra S, Pabitra A, Rajib D (2025) Effect of physical and chemical mutagens on the growth performance of two soybean (TGX 1987-62F and TGX 1835-10E) varieties in the M1 generation. Plant Sci.12 (4):1–9 (IP: 0.9)

life

MDPI

Article

Evaluation of the Impact of Chemical Mutagens on the Phenological and Biochemical Characteristics of Two Varieties of Soybean (*Glycine max* L.)

Anas Hamisu [1], Bhupendra Koul [1,*], Ananta Prasad Arukha [2], Saleh Al Nadhari [3] and Muhammad Fazle Rabbee [4,*]

[1] Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144411, Punjab, India; anashamisu213@gmail.com
[2] Department of Nephrology and Hypertension, Mayo Medical Sciences, Rochester, MN 55902, USA; ananta.arukhaa@gmail.com
[3] Deanship of Scientific Research, King Saud University, Riyadh 11451, Saudi Arabia; salnadhari@ksu.edu.sa
[4] Department of Biotechnology, Yeungnam University, Gyeongsan 38541, Republic of Korea
* Correspondence: bhupendra.18673@lpu.co.in (B.K.); rabbi.biotech@gmail.com (M.F.R.)

Abstract: Mutagenic effectiveness and efficiency are the most important factors determining the success of mutation breeding, a coherent tool for quickly enhancing diversity in crops. This study was carried out at Lovely Professional University's agricultural research farm in Punjab, India, during the year 2023. The experimental design followed a randomized complete block design (RCBD) with three replications. The experiment aimed to assess the effect of three chemical mutagens, sodium azide (SA), ethyl methyl sulphonates (EMSs), and methyl methane sulfonate (MMS), at three different concentrations (0.2%, 0.4%, and 0.6%), in SL958 and SL744 soybean varieties to select the mutant exhibiting the highest yield. The data were collected and analysed using a two-way ANOVA test through SPSS software (version 22), and the means were separated using Duncan's multiple range test (DMRT) at the 5% level of significance. Between the two varieties, the highest seed germination percentage (76.0% seedlings/plot) was recorded in SL958 (0.4% SA), while the lowest (30.33% seedlings/plot) was observed in 0.6% MMS as compared to the control (53% and 76% in SL744 and SL958 at 10 days after sowing, respectively). Several weeks after sowing, the average plant height was observed to be higher (37.84 $\pm$ 1.32 cm) in SL958 (0.4% SA) and lower (20.58 $\pm$ 0.30 cm) in SL744 (0.6% SA), as compared to the controls (SL958: 26.09 $\pm$ 0.62 cm and SL744: 27.48 $\pm$ 0.74 cm). The average leaf count was the highest (234.33 $\pm$ 3.09 tetrafoliate leaves/plant) in SL958 (0.4% SA) while it was the lowest (87 leaves/plant) in 0.6% MMS as compared to the control (SL744 180.00 $\pm$ 1.63 and SL958 160.73 $\pm$ 1.05). The highest total leaf areas recorded in the SL958 and SL744 M1plants were 3625.8 $\pm$ 1.43 cm^2 and 2311.03 $\pm$ 3.65 cm^2, respectively. Seeds of the SL958 variety treated with 0.4% SA resulted in the development of tetrafoliate leaves with a broad leaf base and the maximum yield (277.55 $\pm$ 1.37 pods/plant) compared to the narrow pentafoliate leaves obtained through the treatment with EMS. Meanwhile, in the SL744 variety, the same treatment led to tetrafoliate leaves with a comparatively lower yield of 206.54 $\pm$ 23.47 pods/plant as compared to the control (SL744 164.33 $\pm$ 8.58 and SL958 229.86 $\pm$ 0.96). The highest protein content (47.04 $\pm$ 0.87% TSP) was recorded in the SL958 (0.4% SA) M2 seeds followed by a content of 46.14 $\pm$ 0.64% TSP in the SL744 (0.4% SA) M2 seeds, whereas the lowest content (38.13 $\pm$ 0.81% TSP) was found in SL958 (0.6% MMS). Similar observations were recorded for the lipid and fibre content. The 0.4% SA treatment in SL958 proved to be efficient in generating the highest leaf area (tetrafoliate leaves) and a reasonable yield of M1 (the first generation after mutation) plants.

Keywords: mutagens; *Glycine max*; crop improvement; induced mutation

Citation: Hamisu, A.; Koul, B.; Arukha, A.P.; Al Nadhari, S.; Rabbee, M.F. Evaluation of the Impact of Chemical Mutagens on the Phenological and Biochemical Characteristics of Two Varieties of Soybean (*Glycine max* L.). *Life* **2024**, *14*, 909. https://doi.org/10.3390/life14070909

Academic Editors: Balazs Barna, Katalin Magyar-Tábori and Nóra Mendler-Drienyovszki

Received: 27 May 2024
Revised: 5 July 2024
Accepted: 18 July 2024
Published: 22 July 2024

PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.9136

eISSN 2348-1900

RESEARCH ARTICLE

Effect of physical and chemical mutagens on the growth performance of two soybean (TGX 1987-62F and TGX 1835-10E) varieties in the M_1 generation

Anas Hamisu[1,2], Bhupendra Koul[3*], Subhra Shil[4], Pabitra Adhikary[5] & Rajib Das[6]

[1]Department of Botany, Lovely Professional University, Phagwara 144 411, Punjab, India
[2]Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Private Mail Bag address 1144, Kebbi, Nigeria
[3]Department of Biotechnology, Lovely Professional University, Phagwara 144 411, Punjab, India
[4]Department of Vegetable Science, Indian Agricultural Research Institute, Dhirpai, Gogamukh, Dhemaji 787 035, Assam, India
[5]Department of Agronomy, Krishi Vigyan Kendra, Ashoke Nagar, Haripur 743 223, West Bengal, India
[6]Department of Agricultural Extension, Krishi Vigyan Kendra, Khowai 799 207, Tripura, India

*Correspondence email - bhupendra.18673@lpu.co.in

Received: 27 April 2025; Accepted: 11 August 2025; Available online: Version 1.0: 07 September 2025

Cite this article: Anas H, Bhupendra K, Subhra S, Pabitra A, Rajib D. Effect of physical and chemical mutagens on the growth performance of two soybean (TGX 1987-62F and TGX 1835-10E) varieties in the M_1 generation. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9136

Abstract

Mutational breeding is a cost-effective and time-efficient technique that enhances genetic variability in crops by inducing mutations using physical agents like gamma rays or chemical agents like sodium azide, without involving genetic engineering. The aim of the study was to analyse the effect of gamma radiation and sodium azide (SA) on the growth performance of two soybean varieties (TGX 1987-62F and TGX 1835-10E). Growth parameters included germination percentage, plant height, number of leaves per plant, number of pods per plant, days to 50 % flowering, chlorophyll content, 100-seed weight and total grain yield. The experiment included 20 treatments and was laid out in a randomized complete block design (RCBD). Statistical analysis showed that among the two varieties, the highest rate of germination (60.0 % seedling/plot) was found in TGX 1835 (15Gy) while, 19.67 % seedling/plot was observed at 0.0.2 % SA. Average plant height (64.40 cm) was observed in TGX 1835 irradiated with 10Gy, as compared to the controls (TGX 1987: 55.46 cm and TGX 1835: 60.93 cm). Highest average leaf count in TGX 1987 was 125.67 leaves/plant, irradiated with 10Gy while, 183.33 leaves/plant, treated with 0.02 % SA. The highest total leaf area of 163.19 cm^2 was recorded in TGX 1835 in M1 (mutation 1 generation) plants and 163.56 cm^2, treated with 0.02 % SA. The gamma ray's treatment at 10Gy in TGX 1835 affirmed its potential in generating highest yield in M1 plants. Future breeding programs should focus on stabilizing these mutants (M1 and subsequent generations) to develop commercially viable high-yield varieties, for food security.

Keywords: gamma rays; *Glycine max*; mutation; physical mutagens; sodium azide

Introduction

The steadily growing global population is expected to surpass 9 billion by 2050 which presents a major challenge to food security. To meet this rising demand, crop production must be increased by 50-60 % over the next 25 years to feed the teeming millions (1). Heritable changes in a plant's genetic makeup that occur at the gene or chromosomal level are referred to as plant mutations. Mutation breeding serves as an effective strategy for enhancing both quality and yield-related traits in crops. Quality improvements include better disease resistance, superior fruit characteristics, early maturation and greater tolerance to stress conditions such as salinity, cold and heat. On the other hand, quantitative gains involve increases in seed yield, seed weight, total biomass and oil content. These genetic alterations act as powerful tools in modern crop improvement efforts. Thus, mutations are crucial for the genetic advancement of crops that are significant to the economy (2, 3). Mutation breeding, sometimes referred to as variant breeding, is a process that uses physical or chemical means to produce spontaneous genetic diversity in order to create new crop types. Mutation breeding is a simple, reliable, cost-effective, non-hazardous method for developing quality trait which are heritable. Nevertheless, this technique is not free from constraints, which include stringent screening of mutants exhibiting the desired characteristics, possibility of unpredictable random genetic recombination. Moreover, the effectiveness of this process relies on desirable genetic traits being present in the gene pool (4, 5). Mutagens are mainly classified into physical and chemical types. Physical mutagens include ionizing radiation like alpha, beta and gamma rays and non-ionizing ones like UV rays. Chemical mutagens involve agents such as alkylating compounds (e.g., EMS, MMS), deaminating agents (e.g., nitrous acid) and base analogues. Mutation breeding, using these mutagens, has been instrumental in enhancing crop traits, improving yields and developing better varieties, especially through crossbreeding programs (6, 7).

List of conferences attended

1. **Anas Hamisu**, Bhupendra Koul, Anita Jaswal (2024) Evaluation of the impact of chemical mutagens on the phenological and biochemical characteristics of two varieties of soybean (*Glycine max* L.). 30th Annual conference Botanical society of Nigeria
2. **Anas Hamisu,** Shabnam Ansari, Anita Jaswal (2023) Morphological characterization of soybean treated with gamma radiation for mutation induction: 2nd ICPPB conference, April 2023 at LPU, India.
3. **Anas Hamisu** (2023) The 4th international conference on recent advances in bioenergy research, October 2023 at Sardar Swaran Singh National Institute of Bioenergy, Kapurthala, Punjab, India.

LOVELY PROFESSIONAL UNIVERSITY

SoilResLab Solutions for Research Laboratories

ICPPB

CERTIFICATE

This is to certify that Prof./Dr./Mr./Ms. ANAS HAMISU of Lovely Professional University has participated in Poster Presentation/~~Oral Presentation~~ on the topic entitled Morphological characterization of soybeans (Glycine max L.) treated with gamma rays for induction of mutation in 2nd International Conference on Plant Physiology and Biotechnology (ICPPB) held from 20-21 April 2023 organized by School of Bio-engineering and Biosciences, under the aegis of Lovely Professional University, Punjab.

Dr. Vijay Kumar
Organizing Secretary

Dr. Umesh Goutam
Co-Convener

Dr. Neeta Raj Sharma
Convener

BOTANICAL SOCIETY OF NIGERIA

PAPER PRESENTATION CERTIFICATE

This is to certify that

Anas Hamisu, Bhupendra Koul and Anita Jaswal

Presented a Paper (Oral/Poster/Online) titled EVALUATION OF THE IMPACT OF CHEMICAL MUTAGENS ON THE PHENOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF TWO VARIETIES OF *GLYCINE MAX* L. (SOYBEAN)

30th ANNUAL CONFERENCE & GENERAL MEETING

Theme: PLANTS FOR A SUSTAINABLE AND HEALTHIER EARTH

HOSTED BY: DEPARTMENT OF PLANT SCIENCE AND BIOTECHNOLOGY
KEBBI STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY ALIERO, NIGERIA

Date: 2nd - 6th June, 2024 @ Multipurpose Hall, Kebbi State University of Science and Technology, Aliero

Chairman
Prof. Jibrin Naka Keta

Secretary
Dr. Danladi Garba Hani

MINISTRY OF NEW AND RENEWABLE ENERGY

G20 2023 INDIA

THE 4TH INTERNATIONAL CONFERENCE on RECENT ADVANCES IN BIO-ENERGY RESEARCH

October 09-12, 2023

Organized by

Sardar Swaran Singh National Institute of Bio-Energy, Kapurthala, Punjab, India

(An autonomous Institution of Ministry of New and Renewable Energy, Government of India)

CERTIFICATE OF PATICIPATION

is awarded to

Anas Hamishu

for his/her Participation/Presentation (Oral/Poster) entitled

in the 4th International Conference on Recent A... ...Bio-Energy Research.

Dr. Anil Kumar Sarma
(Co-Chair, ICRABR 2023)

(ICRABR-2023)

Dr. G. Sridhar
(Chairman, ICRABR 2023)

Work shop attended

1. **Anas Hamisu** (2023). Combatting microplastics challenge in the global context: A workshop, April 2023 at LPU

CERTIFICATE

This is to certify that

Anas Hamisu

has successfully participated in the program of

international workshop

«Combatting Microplastics Challenge in the Global Context»

Including

Microplastics an emerging contaminant: Challenges to analysis and mitigation

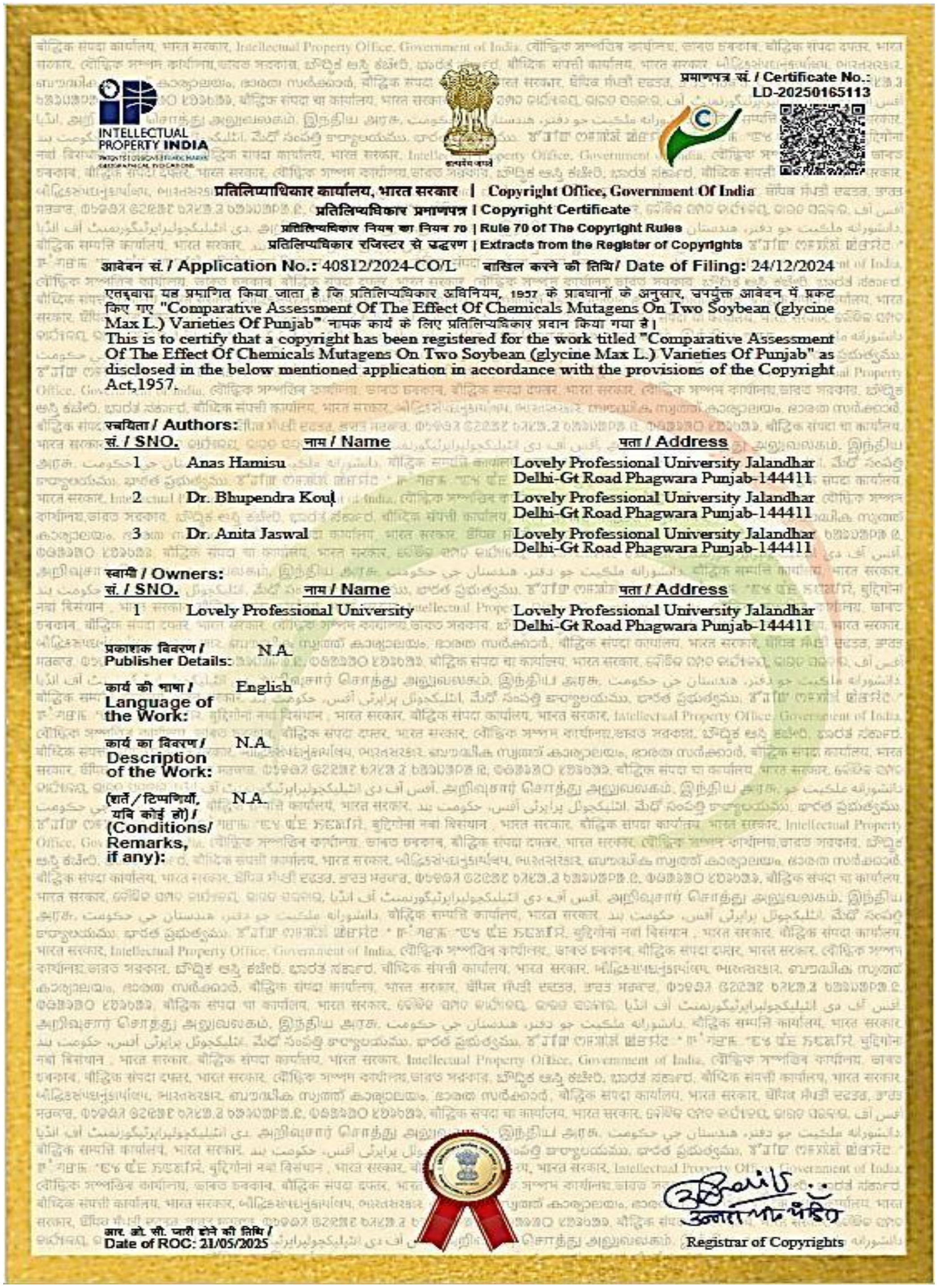
Microplastics in fresh waters: impact on the ecosystem and ways to reduce it

Analysis of microplastics in the integral water cycle: wastewater, sludge and drinking water by Micro-FTIR infrared spectroscopy

April 14, 2023

Copyright

1. **Anas Hamisu**, Bhupendra Koul, Anita Jaswal (2024) Comparative assessment of the effect of chemicals mutagens on two soybean (*Glycine max* L.) varieties of Punjab.



INTELLECTUAL PROPERTY INDIA

प्रमाणपत्र सं. / Certificate No.: LD-20250165113

प्रतिलिप्याधिकार कार्यालय, भारत सरकार | Copyright Office, Government Of India

प्रतिलिप्यधिकार प्रमाणपत्र | Copyright Certificate

प्रतिलिप्यधिकार नियम का नियम 70 | Rule 70 of The Copyright Rules

प्रतिलिप्यधिकार रजिस्टर से उद्धरण | Extracts from the Register of Copyrights

आवेदन सं. / Application No.: 40812/2024-CO/L   दाखिल करने की तिथि/ Date of Filing: 24/12/2024

एतद्द्वारा यह प्रमाणित किया जाता है कि प्रतिलिप्यधिकार अधिनियम, 1957 के प्रावधानों के अनुसार, उपर्युक्त आवेदन में प्रकट किए गए "Comparative Assessment Of The Effect Of Chemicals Mutagens On Two Soybean (glycine Max L.) Varieties Of Punjab" नामक कार्य के लिए प्रतिलिप्यधिकार प्रदान किया गया है।

This is to certify that a copyright has been registered for the work titled "Comparative Assessment Of The Effect Of Chemicals Mutagens On Two Soybean (glycine Max L.) Varieties Of Punjab" as disclosed in the below mentioned application in accordance with the provisions of the Copyright Act,1957.

रचयिता / Authors:

सं. / SNO.	नाम / Name	पता / Address
1	Anas Hamisu	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
2	Dr. Bhupendra Koul	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411
3	Dr. Anita Jaswal	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

स्वामी / Owners:

सं. / SNO.	नाम / Name	पता / Address
1	Lovely Professional University	Lovely Professional University Jalandhar Delhi-Gt Road Phagwara Punjab-144411

प्रकाशक विवरण / Publisher Details: N.A.

कार्य की भाषा / Language of the Work: English

कार्य का विवरण / Description of the Work: N.A.

(शर्तें / टिप्पणियाँ, यदि कोई हो) / (Conditions/ Remarks, if any): N.A.

आर. ओ. सी. जारी होने की तिथि / Date of ROC: 21/05/2025

Registrar of Copyrights