

Genetic Studies in Lentil (*Lens culinaris* Medik.) through Induced Mutagenesis

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UTTAR BANGA KRISHI VISWAVIDYALAYA
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In

GENETICS AND PLANT BREEDING

By

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PUNDIBARI, COOCHBEHAR
WEST BENGAL, INDIA**

September, 2022



DEDICATION

Every challenging work needs selfless efforts as well as guidance of our elders especially those who are close to our heart.

My humble effort, I would love to dedicate this work to my beloved parents Mr. Snehasish Chakraborty and Mrs. Banani Chakraborty;

and to my Guide

Prof. Arup Sarkar

Whose Affection, Encouragement and Sincere efforts made me able to complete this work and get me Success and Honour.



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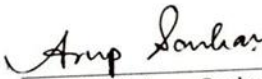
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CERTIFICATE

This is to certify that the work recorded in the thesis entitled “**Genetic Studies in Lentil (*Lens culinaris* Medik.) through Induced Mutagenesis**” submitted by Ms. Imon Chakraborty, B.Sc. Hons. (Ag.) in partial fulfilment of the requirement for the Degree of Master of Science (Agriculture) in Genetics and Plant Breeding of the Uttar Banga Krishi Viswavidyalaya is a faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received from various sources during the course of investigation have been duly acknowledged.


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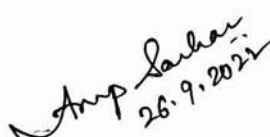
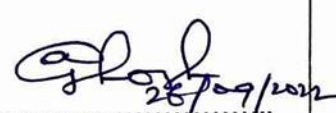
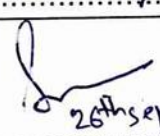
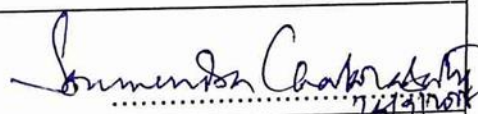
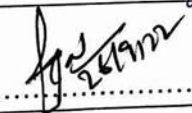
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**APPROVAL OF EXAMINERS FOR THE AWARD OF THE
DEGREE OF MASTER OF SCIENCE (AGRICULTURE) IN
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We, the undersigned, having been satisfied with the performance of Ms. Imon Chakraborty, in the viva-voce examination on Final evaluation of the thesis, conducted today, the ~~26th September~~ 2022, recommended that the thesis be accepted for the award of the degree of Master of Science (Agriculture) in Genetics and Plant Breeding.

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Place: Pundibari
Date: 20.09.2022

Iman Chakraborty

Signature

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List of Abbreviations

ANOVA: Analysis of variance

C.D: Critical Difference

C.V.: Coefficient of variation

DAS: Days after sowing

d.f: Degrees of freedom

EMS: Ethyl Methane Sulphonate

GA: Genetic Advance

GCV: Genotypic Coefficient of Variation

H² (BS): Heritability (broad sense)

IBPGR: International Board for Plant Genetic Resources

MMS: Methyl Methane Sulphonate

MSS: Mean Sum of Squares

NMC: Nitrozo Methyl Carbonate

PCV: Phenotypic Coefficient of Variation

SA: Sodium Azide

SE: Standard Error

Abstract

Lentil (*Lens culinaris* Medik.), a diploid species with a chromosome number $2n=2x=14$, is grown mainly in India and Canada besides USA, Nepal, Bangladesh, Turkey, Australia and China. In India, grown principally as a rabi crop, the productivity is quite low compared to other countries. The traditional breeding methods for a long period may have narrowed down the available genetic variability because of which it has been difficult to develop high yielding varieties using the limited germplasms available. Mutation breeding under such situation can be an important possible route to broaden the genetic variability in lentil (Erskine *et al.*, 1998).

Considering all these, an experiment was undertaken in the institutional farm of Uttar Banga Krishi Viswavidyalaya by raising the M₂ seeds of five different varieties of lentil earlier treated with three different doses of chemical mutagen Ethyl Methane Sulphonate (EMS) viz., 0.1%, 0.2% and 0.3% with the objectives, viz., i) To study the genetic variability of different characters and their association in lentil under induction of mutation in M₃ generation. ii) To study the spectrum of mutations for different characters of lentil in M₃ generations.

The analysis of variance for different characters in lentil varieties showed highly significant differences among the varieties in M₃ generation for all the characters except primary branches per plant. Only four characters viz., number of pods per plant, pod weight per plant, yield per plant, and cooking time, showed highly significant differences among the treatments. Highly significant genotype versus treatment interaction was noted for the characters viz., number of pods per plant, pod weight per plant, days to 90% maturity, yield per plant, and cooking time. Plant height, days to 90% maturity, number of seeds per pod, and days to 50% flowering were shown to have the high heritability estimates.

Study on the shift in mean values for days to 50% flowering revealed that in the variety HUL 57 earliness in flowering was observed at 0.3% EMS and it was found to be significant. The mutagen did not have significant mutagenic effect on inducing the mutation in the desired direction for primary branches per plant. On the contrary, in varieties HUL 57, IPL 406 and WBL 77 a positive shift in mean values were observed in M₃ generation for secondary branches per plant. However, in none of the varieties

and in none of the treatments significant shift in mean values was observed. Similar such significant shift in positive direction was observed for pods per plant, pod weight per plant and seeds per pod. Like M2 generation no significant shift was observed for plant height though heritability value was high. Hence the mutagen does not seem to have any effect on the character. For days to 90% maturity, the heritability value was high and significant positive shift in mean values were observed in WBL 77 at 0.1% EMS, and in Pusa Ageti at 0.1% EMS; thus, the mutagenic effect at the respective concentration in the stated varieties can be said to have taken place in the generation. Significant positive shift in mean values were observed in HUL 57 at 0.2% and 0.3% EMS concentration in M3 generation for the character 1000 seed weight. The heritability value was moderate compared to that in M2. Hence, the real effect of the mutagen for the character needs to be studied further in coming generation (M4 onwards). Only pod weight per plant had significant positive correlation (0.669) with yield while traits like days to 50 % flowering, secondary branches per plant, number of seeds per pod, plant height at maturity, days to maturity and seed weight had shown negative correlation with yield among which only the character i.e., days to 50 % flowering had shown significant negative correlation (-0.578) with yield. Traits like pod weight, days to 50% flowering and days to maturity are interlinked and they are indirectly associated with yield. Hence, apart from direct selection indirect selection can also be used for crop improvement. The seeds of the variety IPL 406 raised through treatment at 0.2% EMS was significantly found to be taking lesser time that the others in the study to get cooked.

INTRODUCTION

Introduction

Pulses are considered to be a good source of protein, dietary fibre and essential minerals and therefore, have the potential to be used as a crop for eradication of hidden hunger. Worldwide the most serious malnutrition problem is that of protein deficiency, particularly among the children in the developing countries and thus they have to rely upon pulses being an important source of protein (**Amjad et al., 2006**)

Given the fact that qualitatively protein nutrition has many facets, lentil is considered to be an inexpensive, sustainable and affordable source of nutrition in having a good source of protein, minerals and vitamins in addition to iron, zinc, selenium, folates and carotenoids. Wide range of protein content and same of essential minerals and dietary fibres have been reported by **Hamdi et al., (1991)**, **Wang (2003)**, **Burstin et al. (2011)** and **Roy et al. (2010)**. Among the amino acids, lentil is rich in lysine and leucine and methionine being the limiting amino acid, the pulse is recommended along with such methionine rich food as wheat, rice and corn. (**Chatterjee and Abrol, 1975; Bhatta and Slinkard, 1979; Shekib et al., 1986**).

Lentil (*Lens culinaris Medikus subsp. culinaris*), one of the first crops domesticated, has grown to be a significant food legume crop in the farming and food systems of many nations across the world. Its straw is prized as animal feed, and its seed is a rich source of protein, minerals, and vitamins for human nutrition. Its capacity to store carbon and nitrogen enhances the nutrient status of the soil, resulting in the sustainability of production systems. (**Sarker and Erskine, 2006**). Differences in the concentration of protein in green and red lentils have also been reported. (**Boyle et al., 2010**).

It is one of the ancient cultivated crops among the legumes which was domesticated during the Neolithic period in the Near East stretching from Jordan to Turkey Northward and to Iran towards Southeast, the area designated as the Cradle of Agriculture. (**Kislev and Bar-Yosef, 1988**). Like Einkorn and Emmer wheat, barley and pea, lentil is one among the few early domesticated crops (**Harlan, 1992**). The archaeological evidence suggests that the crop is indigenous to south western Asia and the Mediterranean region with a chromosome number of $2n=2x=14$ and a haploid genome size of 4063 Mbp. (**Choudhary et al., 2017**). It is mostly grown in Canada, India, besides Nepal, Australia, Turkey, USA and China.

The genetic resources including germplasms, landraces and wild relatives cause high genetic variability for various agronomic and qualitative traits (**Kumar *et al.*, 2016**) but being a protein rich crop, the variability needs to be explored for higher seed protein content and essential amino acids. Considerable improvement has been made in lentil through conventional breeding, however, productivity of the crop has been found to be stagnant which offers the scope for further improvement of yield potential of the cultivars. The yield stagnation is primarily due to narrow genetic base of the germplasm resources leading to repeated use of the same genotype in breeding program. (**Ferguson *et al.*, 1998; Kumar *et al.*, 2013**).

Though incidence of diseases like vascular wilt, Stemphylium blight, collar rot, root rot, rust, powdery mildew, downy mildew, etc. have been identified as the reasons for low productivity (**Khalequzzaman, 2016**) non-availability of short duration varieties in areas having shortage of water is also another major constraint in enhancing the productivity of the crop in the desired direction. Thus, it has been suggested to broaden the genetic base in lentil particularly in south Asia where it is considered to be a crop of prominence so that the diverse genotype can be effectively utilised in furthering the cause of crop improvement. (**Ladizinski *et al.*, 1984; Erskine, 1997; Erskine *et al.*, 1998; Rahman *et al.*, 2009**).

Thus, the above discussion is indicative of a well-directed breeding approach with a desired objective. Lentil being a self-pollinated crop, the conventional breeding methods like pure line selection, bulk method, pedigree method, single seed descent or its modification have not been too effective to improve the yield potential primarily due to shortage of new sources of variation and/or germplasm. According to **Jain (1973)** natural selection has played a greater role in lentil genotypes being principally raised under poor conditions of growth with very little fertilizers and inputs like water and the genotype which might respond to inputs like fertilisers and irrigation have been gradually lost thereby making it difficult to develop improved genotypes using such limited germplasms available. Thus, there is a need to harness the artificial means to generate the desired variability and mutation breeding is considered to be one of the most important possible approaches to broaden the genetic bases in lentil under such conditions. (**Erskine *et al.*, 1998**). Mutation breeding also has the advantage of generating otherwise unexplored sources of genetic variability (**Swaminathan, 1969; Blixt and Gottschalk, 1975; Khan and Siddiqui, 1988**). Artificial induction of

mutation eliminates the necessity to wait for occasional mutation and thereby opens up new possibility like introduction of new characters to a species, breaking the undesirable linkage between the desirable and undesirable characters and finally increasing the variability present in the population for further selection to happen.

Frey (1969) reported that mutation induced for quantitative traits in crop plants is heritable and thus response to selection is good. Thus, the frequency and spectrum of mutations in the resultant mutants would not only broaden the genetic base but also is a boon for plant breeders for developing an improved genotype of a crop.

With this background information an investigation was undertaken in the instructional farm of Uttar Banga Krishi Viswavidyalaya during 2021-22 in M3 generation of five varieties of lentil, raised through the treatment with three different doses of the chemical mutagen Ethyl Methane Sulphonate (EMS) with the following objectives:

1. To study the genetic variability in different characters in lentil under induction of mutation in M3 generation.
2. To study the spectrum of mutations for different characters in lentil in M3 generation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Lentil (*Lens culinaris* Medik.) is one of the ancient legumes cultivated in the world (**Abraham, 2015**). Being the second producing country the progress of lentil production in India has been far from satisfactory as the production cannot cope with the dietary demand. With the use of traditional breeding method and concomitant narrowing down of available variability there is a need to broaden the genetic base for further crop improvement.

Of the different other approaches mutation breeding is considered to be one of the important possible routes to broaden the desired genetic base under such bottleneck conditions (**Erskine *et al.*, 1998**). Mutation can lead to a greater variability in the polygenic traits (**Singh *et al.*, 2001**) and can thus be used to complement and supplement the existing germplasm resources (**Konzak *et al.*, 1975**).

Of the several chemical mutagens used for induction of mutation Ethyl Methane Sulphonate (EMS) has been found to increase the genetic variability in the plant population (**Lee *et al.*, 2003**) and has thus been found to be more efficient and effective than gamma rays in inducing chlorophyll and morphological mutation in lentil (**Ravi *et al.*, 1980**).

Changes in the characters namely viz., plant height, growth habit, branching and stem structure, leaf morphology, inflorescence, calyx, flower, pod characters, fertility and seed colour due to mutagenic treatments have been reported in lentil by **Tyagi and Gupta (1991)**, **Ashutosh and Dubey (1992)**, **Vandana *et al.*, (1994)**, **Ramesh and Dhananjay (1996)**, **Tyagi and Ramesh (1998)** and **Jeena and Singh (2000)**.

Physiological, biochemical, metabolic and genetic abnormalities in lentil varieties have been observed by **Amin *et al.* (2015)** due to action of the chemical mutagen Methyl Methane Sulphonate (MMS) alone and in combination with Dimethyl Sulphoxide (DMSO), resulting in substantial bio morphological and quantitative differences.

In an experiment with lentil varieties **Alka (2008)** reported about differential response to mutagenic treatment for the quantitative characters viz., plant height, days to flowering, days to maturity, number of fertile branches, number of pods etc. Of these

characters, plant height, days to flowering, days to maturity, seeds per pod and 100 seed weight were found to decline with the effect of the mutagen used.

In an experiment with lentil genotypes, **Sharma and Sharma (1984)**, reported that mean values for the characters namely days to flowering and plant height dropped in M3 generation but pods per plant and seed yield per plant was found to be higher compared to the corresponding controls. Early flowering plants identified in M2 appeared to be the greatest producing plant and bred true in M3 generation. A substantial rise in the negative correlation of days to flowering with pods and seed yield per plant was also observed in all the treatments.

Sharma and Sharma (1986), while investigating the polygenic variability in lentil seeds by treating them with gamma rays and NMV alone or in combination found that days to maturity had the highest heritability followed by plant height, test weight, grain production and pods per plant.

A dwarf mutant in M2 was identified in *Vigna radiata* using gamma rays and EMS which bred true in M3 generation. The mutant generated was identified to have more number of seeds per pod resulting in a greater seed yield and the mutant matured one week early than the corresponding control.

Intervarietal variation in test weight was observed in lentil varieties K-75 and K-303 when the seeds were treated with mutagen. (**Tripathi and Dubey, 1992**)

In an experiment with lentil variety an increase in the variability in the treated population for 100 seed weight has also been observed (**Sarkar and Sharma, 1988**). Similarly, enough of genetic diversity for 100 seed weight in lentil seed following gamma ray irradiation has also been found (**Kalia and Gupta, 1989**)

Exposure to gamma rays with the doses ranging from 100-600 Gy from a Co-60 gamma rays source in lentil varieties lead to the screening of some mutant lines and the mutant lines viz., AEL-15/30, AEL-49/20, AEL-2/20, AEL-12/30, AEL-20/30, AEL-9/20, AEL-13/30 and AEL-23/40 gave higher grain yield than the mother variety viz., namely M-85 and ICARDA-8. Of these mutant lines significantly AEL-49/20 did flower sixteen days earlier than its mother variety M-85 and the grain yield of the mutant line (1274kg/ha) was significantly higher than its mother variety (889kg/ha) M-85 (**Ali and Shaikh, 2007**).

A clear relation between EMS dose and seedling height decrease has been observed by **Sharma (1969)** with the greatest reduction in height happening at the highest dose. **Kumar and Lal (2001)** similarly reported that with the increase in concentration of EMS, the height of the plants also increased. It has been reported that shoot length is generally reduced with increase in concentration of mutagen. (**Sharma and Sharma, 1986; Manju et al., 1983; Singh, 1987**). Similar such reports on reduction in shoot length with increase in concentration of mutagen have also been reported by **El-Mouhamady et al. (2020)**.

The frequency of chlorophyll mutation is considered to be a standard measure of the rates of the induced mutation and thus helps in the determination of effectiveness and efficiency of mutagens. Occurrence of different types of chlorophyll mutants has been reported in different crop plants such as lentil (**Wani, 2018**), chickpea (**Kharakwal, 1998**), Mungbean (**Khan et al., 1998**), urd bean (**Singh et al., 1998**), Cowpea (**Ahmed John, 1999**) and grass pea (**Kumar and Dubey, 1998; Das and Kundagrami, 2000; Waghmare and Mehra, 2001**).

In an experiment to study induced morphological mutations in green gram by Gamma irradiation **Singh and Rao (2007)** observed a wide spectrum of morphological mutations into two green gram variety viz., Sujata and TARM-1. The frequency of such mutations increased with increase in the dose of radiation.

Khan and Wani (2006) induced mutation by EMS in green gram and studied four quantitative traits in M2 and M3 generations namely viz., fertile branches per plant, pods per plant, 100 seed weight (g) and yield per plant (g). A significant shift in the mean values in positive direction was observed for all the traits studied and in all the cases high values of heritability and genetic advance over the control was indicative of the possibility of induction of desirable mutation for polygenic traits accompanied by effective selection in M2 generation. The increased heritability in M3 in comparison to M2 generation may be due to increased homozygosity of the genes involved. Similar such results have been reported by **Singh et al., (2001); Khan et al., (2001)** and **Raut et al., (2004)**.

In their study **Sandhu et al. (1979)** observed that seed protein content of the mutants did not have significant alteration in any of the EMS treatment. However, in mutagenic treatment the coefficient of variation was higher compared to the control indicating the

possibility of improvement in the seed protein content. Yield per plant did not show any association with seed protein thereby indicating the independent genetic control of the two characters and the lack of association can be easily combined in a single genotype.

Jency *et al.* (2017) while estimating the lethal dose of EMS in Kodo millet variety CO-3 observed growth reduction of seedlings after EMS treatment and there was a reduction in germination, survival rate of seedlings, root length, shoot length, seedling height, vigour index under *invitro* condition as the EMS concentration increased while emergence and survival under field (*in vivo*) conditions in M1 generation decreased compared to the control as the concentration of EMS increased.

Tyagi and Gupta (1991) reported spontaneous mutation with fasciation in lentil. They reported that the increase in yield of fasciated plant was associated with increase in the flower bearing zone, branches per plant, internode length and pods per bunch.

Bhatia and Swaminathan (1962) reported that at higher concentration of EMS the shift in the mean values of various quantitative traits was in a direction opposite to that of selection and was found to be very common at higher concentration of mutagen.

A positive association between germination, survival and seed fertility was found in peas which got reduced following treatment with X-rays, gamma rays and EMS. (**Wellensiek, 1965**) However, in Lathyrus **Waghmare and Mehra (1998)** observed no such mutagenic effect on germination.

When subjected to the three doses of ethyl methane sulfonate (EMS), the three lentil cultivars Giza 9 (G1), Giza 29 (G2), and Giza 51 (G3) displayed unmatched superiority for all growth, germination, and other attributes under research when compared to the control. Furthermore, the control experiment was outperformed by all the three dosages of this mutagen, with the third dose showing the greatest superiority. In all examined features, particularly at the third dose of EMS, the greater limit of positive percentages values of induced mutagenesis in M2 lentil generation (the second mutant generation) was observed (0.3). For the three doses of EMS, the three lentil cultivars (Giza 9, Giza 29, and Giza 51) reported the highest mean values of all those examined. In this regard, the third dose of mutagen (0.3) came in first, followed by the second dose (0.2), and finally the first dose. (**El-Mouhamady, 2020**).

Induced mutagenesis with EMS, HZ, and SA, was used to establish genetic diversity for several quantitative features, including fertile branches per plant, pods per plant, and grain production per plant in the M2 and M3 generations of lentil (*Lens culinaris Medik*). In the M3 generation, the mean number of fertile branches, number of pods, and grain production per plant dramatically increased. Significant inter-population heterogeneity for the observations was discovered by analysis of variance. Phenotypic features were all quantifiable in both the M2 and M3 generations (**Wani and Laskar, 2021**).

On the twenty-four (24) lentil genotypes produced at three rainfed sites in Syria and Lebanon, a test of the cooking quality of lentil seed was created and applied. A standard check that was repeatedly sampled and cooked had a coefficient of variation of 4.1%, demonstrating the repeatability of the test with 33 minutes being the average cooking time (**Erskine, 1985**).

Following treatment with different doses of ethyl methane sulphonate, hydrazine hydrate, and sodium azide on two lentil types, phenotypic selection was used for three generations. Six high-yielding mutant lines with consistent characteristics were isolated in the M2 generation. The results showed a significant rise in genotypic coefficient of variation (GCV), heritability (h^2), and genetic progress along with a notable increase in mean values for quantitative and physiological variables (GA). According to the correlation study plant yield was found to be significantly and positively influenced ($P < 0.001$) by the number of fertile branches per plant, number of pods per plant, and the seed weight (**Raina, 2022**).

MATERIALS & METHODS

Materials and Methods

The present investigation named "Genetic Studies in Lentil (*Lens culinaris* Medik.) through Induced Mutagenesis" was conducted during the rabi season of 2021-22, with seeds of M2 generation in five (5) varieties of lentil to evaluate the performance of the varieties treated with different concentrations of Ethyl Methane Sulphonate (EMS) in M3 generation. The main objective of the experiment was to determine the spectrum of mutations for various traits in the M3 generation and thereby to examine the extent of variability inherited from the preceding generation (M2) under induction of mutation.

3.1 Experimentation site

The experiment was undertaken at the instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal being located at an elevation of 43 metres above mean sea level and at 26°24'09.1" N latitude and 89°23'08.3" E longitude. The soil of the experimental site was sandy loam with a high-water retention capacity. The laboratory analysis was carried out at the Department of Genetics and Plant Breeding, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar.

3.2 Experimentation materials

The present experiment was undertaken with M2 seeds of five (5) varieties of lentil and the list of varieties used have been presented in the Table 3.1

Table 3.1: Genotypes (varieties) of lentil used in the experiment

Sl.No.	Variety	Source of Original Collection
1.	HUL 57	Seed hub, UBKV
2.	IPL 506	Seed hub, UBKV
3.	WBL 77	Seed hub, UBKV
4.	Pusa Ageti	PORS, Behrampore
5.	Subrata	PORS, Behrampore

The genotypes (varieties) were chosen on the basis of their adaptability in the study area encompassing the northern districts of West Bengal.

3.3. Mutagen

For induced mutagenesis, the chemical mutagen Ethyl Methane Sulphonate (EMS) – also known as ($\text{CH}_3\text{SO}_2\text{C}_2\text{H}_5$) – was utilised. EMS is a highly mutagenic and volatile chemical substance that works primarily by alkylating nitrogenous bases of DNA molecule.

3.4. Design of experiment and layout

For studying the mutagenic effect, the M2 seeds (earlier treated with three concentrations of EMS, viz., 0.1%, 0.2% and 0.3% respectively) and that of untreated seeds as control were sown in a Factorial Randomized Block Design (FRBD) with three replications. In each plot, measuring 3m x 2m, the seeds were planted with a 30 cm interval between rows.

3.5. Treatments of the experiment

The details of the four (4) treatments utilised are listed in Table 3.2. The treatment combinations were chosen based on the literature studied in the crop under research and the experiment's goal.

Table 3.2 Treatments (mutagen concentration) used in experiment

Sl.No.	Treatment
1	Control (without mutagen)
2	0.1% EMS
3	0.2% EMS
4	0.3% EMS

3.6. Recording observations

Five plants were chosen at random in each of the replications in the M3 generation for recording the observations on morphological characters. For the characters enumerated below, the variant found in each of the replications for each of the treatments in each genotype was noted separately. According to the Descriptors (IBPGR) for Lentil and Guidelines for the Conduct of Tests for Distinctness, Uniformity, and Stability, the following morphological characters were recorded.

3.6.1. Days to 50% flowering:

It was recorded as the number of days required, in which flowers appeared in 50% of the plants.

3.6.2. Number of Primary branches/plant:

The number of primary branches on the plant was counted without considering the main stem as the primary branch from five (5) randomly selected plants in each plot in M3 generation.

3.6.3. Number of secondary branches/plant:

The number of secondary branches developed on the primary branches in each plant was counted from five (5) randomly selected plants in each plot in M3 generation.

3.6.4. Number of pods per plant:

The number of pods in each plant was counted as the average of the same from five randomly selected plants in each plot in each replication.

3.6.5. Pod weight per plant:

The weight of pods per plant was taken as the average of the weights of pods from five randomly selected plants in each plot in each replication

3.6.6. Number of seeds per pod

The numbers of seeds per pod were counted as the average of the same from five randomly selected pods for each of the varieties in each treatment and replication.

3.6.7. Plant height at maturity:

The height of the stem was taken from five random plants in M3 generations at the late pod filling stage and average of the same was considered.

3.6.8. Days to 90% maturity:

This was recorded as the number of days required, in which 90% of the plants mature.



Plate 1: Field view of Lentil varieties in M3 generation

3.6.9. 1000- seed weight/test weight (gm):

A weight of 1000 randomly chosen seeds were weighed for each treatment as well as in each replication for each of the varieties and it was expressed in grams as the average of the same.

3.6.10. Seed yield per plant:

Seed yield was recorded as the weight of fully dried seeds per plant in each replication.

3.6.11. Cooking time:

Cooking time was calculated for the seeds of each plot in each replication as the time taken to become the seeds soft upon cooking.

3.6.12. Seed Protein:

Seed protein content was expressed as the percentage of mg/100 gram of lentil and it was estimated by following the protocol of **Lowry *et al.* (1951)**.

3.6.13. Flower colour:

The Colour of the petal was recorded by visual assessment. There were five types of colour observed, viz., white, and white with blue veins and blue.

3.6.14. Plant growth habit:

Ten (10) plants were randomly selected from each plot and visual observation was made at the time when 50% of the plants came to flowering to determine the growth habit which was classified as, erect type ($<30^0$), semi erect type ($30-60^0$) and horizontal type ($>60^0$) and the same was scored as 1, 3 and 5 respectively.

3.6.15. Leaf Shape:

Leaflet size was classified into three groups and scoring was given on the basis of leaflet size in length viz; small (3), medium (5) and large (7).

3.6.16. Seed size:

Weight of the 100 seeds were taken and then classified into four categories, viz.,

Small: < 2 gm

Medium: 2-2.5 gram

Large: 2.6-3.0 gram

Very large: > 3.0-gram

3.7. Methods of Statistical Analysis

The data collected on different parameters were subjected to statistical analysis based on the sample means of various characters spread over four treatments and three replications. The following statistical analyses were done to ascertain the performance of the genotypes, treatments and also to study the genotype $\times$ treatment interaction. Computer analysis of data was carried out in R-project version Rv.4.1.1(<https://cran.r-project.org/>).

3.7.1. Analysis of Variance:

ANOVA is a statistical method that separates observed variance into different components; it had been done separately for each of the characters studied in lentils according to the factorial randomized block design with two factors viz., genotype and treatment (concentration of mutagen). The partitioning of the total phenotypic variation into its components was done according to the below stated model-

$$Y_{ij} = \mu + g_i + e_i \text{ where,}$$

Y_{ij}	Phenotypic observation in the i^{th} entry (genotype)
μ	Overall mean
g_i	Effect of the i^{th} genotype
e_i	Random error associated with i^{th} entry

In our mutagenic treatment, a factorial experiment design was used, and for the above design, two factors, A and B, were taken into consideration for an analysis. As a result, the degrees of freedom for the interaction effect were (a-1) and (b-1) respectively,

where a and b were the levels of each of the two factors, and "ab" treatment combinations were used. The factorial components of the treatment sums of squares in this instance relate to the primary impacts of the various factors and their interactions. Data was subjected to analysis of variance after **Gomez and Gomez (1983)**

Table: ANOVA for two factorial Randomized Block Design

Source of variation	Degrees of freedom	MS	Expected MS
Replication	(r-1)	M_G	$\sigma^2 r$
Genotype	(g-1)	M_G	$\sigma^2 g + g\sigma^2 r$
Treatment	(n-1)	M_T	$\sigma^2 e + n\sigma^2 r$
Genotype x treatment	(g-1) x (n-1)	M_{GT}	$n\sigma^2 r + g\sigma^2$
Error	(r-1) x (gn-1)	M_{GTE}	$\sigma^2 e$
total	(rgn-1)		

3.7.2. Test of significance

The significance test was used in the ANOVA to determine whether differences between the treatments were meaningful. At the specified degrees of freedom and the acceptable level of significance (5% and 1%), the observed F value and the tabulated value were compared. The differences between the treatments were deemed to be significant if the observed value of F was found to be higher than the calculated value, and vice versa. In the ANOVA, significant values were indicated with a * or ** depending on how significant they were. In the present study, ANOVA was used to analyze the following genetic parameters.

3.7.3. Mean

The arithmetic mean was calculated by dividing the total of all the individual observations (Y_i) by the number of observations and was calculated as follows

$$Y = \frac{1}{n} \sum_{i=1}^n Y_i$$

3.7.4. Variance:

The variance was calculated as the average of the squared deviation from the mean to measure the variability.

3.7.5. Standard Error and Critical Difference:

The standard error was assessed by dividing the estimates of standard deviation by the square root of the number of observations in a sample and was denoted by SE.

$$SE_{(m)} = (EMS/r)^{1/2}$$

Where, EMS= Error mean square

r= number of replications

In order to compare the mean values of various treatments, the critical difference at the appropriate level of significance was calculated by the following formula.

$$C.D = S.E.(d) * t = (2EMS/2)^{1/2} * t \text{ at 5\% or 1\% level of significance at error d. f.}$$

Where, S.E. (d) is the standard error of the difference of the treatment means to be compared. If the mean difference between any two varieties was greater than the calculated CD value, the difference was taken to be significant.

3.7.6. Genotypic correlation

Genotypic correlation is the inherent association between two characters; it may be either due to pleiotropic action of genes, linkage or more likely due to both. Genotypic correlation co-efficient is estimated according to the following formula:

$$r_g = \frac{G \text{ Cov}(x, y)}{\sqrt{GV(x) \times GV(y)}}$$

Simple correlations are calculated using the following formula suggested by **Johnson et al.** (1955)

$$r(x, y) = \frac{\text{Cov}(x, y)}{\sqrt{v(x) \times v(y)}}$$

Where,

r (x, y) = Correlation coefficient between variables x and y

cov (x, y) = Covariance between variables x and y

v(x) = Variance for the variable x

v(y) = Variance for the variable y

Simple correlation is of three types –

i) Phenotypic ii) Genotypic and iii) Environmental

Correlation co-efficient is tested by the following formula

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

with (n – 2) degrees of freedom at 5% and 1% level of significance.

Where,

r = Correlation coefficient

n = Number of treatments

3.7.7. Coefficient of Variation (CV):

The coefficient of variation (CV) being a unit less measurement, is a good basis for comparing the extent of variation between different characters with different scales.

$$C.V. = \frac{S.D}{\bar{X}} \times 100$$

Where, S.D. = Standard deviation = $\sqrt{\text{variance}}$

$$\text{i.e., S.D.,} = \sqrt{E.MS.}$$

$\bar{X}$ = Grand mean

3.7.8. Components of Variance:

Considering that all the genotypes tested here, were genetically uniform, the expected mean sum of square for error (EMS), i.e., σ^2_e will be purely a random environmental variance. The mean squares between genotypes will consist of two variances, i.e.,

- i) Attributing to varietal difference (i.e., genotypic difference) and
- ii) Due to environmental variation among individuals of each genotype.

Thus, the expected mean sum of squares is as follows –

$$GMS = \sigma^2_e + r\sigma^2_g$$

$$EMS = \sigma^2_e$$

Therefore,
$$\sigma^2_g = \frac{G.MS - EMS}{r}$$

Thus, the genotypic variance being σ^2_g and environmental variance as σ^2_e thus phenotypic variances i.e., σ^2_p will be equal to $\sigma^2_g + \sigma^2_e$

$$\text{Therefore, } \sigma^2_p = \sigma^2_g + \sigma^2_e$$

3.7.8.1. Genotypic Coefficient of Variation (GCV)

GCV was calculated by the formula given by **Burton** (1952) as

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100 = \frac{\text{Genotypic standard deviation}}{\text{Grand mean}} \times 100$$

3.7.8.2. Phenotypic Coefficient of Variation (PCV)

PCV was calculated by the formula given by **Burton** (1952) as

$$PCV = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100 = \frac{\text{Phenotypic standard deviation}}{\text{Grand mean}} \times 100$$

The estimates of PCV and GCV were classified as low (<15%), moderate (15-30%) and high (>30%).

3.7.9. Heritability (Broad sense):

Heritability was estimated by the formula suggested by **Hanson et al.**(1956).

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p} = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}}$$

The estimates of heritability (broad sense) were classified as low (<50%), moderate (50-80%) and high (>80%) as suggested by **Robinson (1966)**.

3.7.10. Genetic advance

The expected genetic advance (GA) was calculated by using the formula as suggested by **Lush (1949)** and **Robinson and Comstock (1955)**.

$$\text{Genetic advance (GA)} = h^2_b \times \sigma_p \times K$$

Where,

K= Selection differential at 5% selection intensity (2.06 in the present investigation)

σ_p = phenotypic standard deviation and

h^2_b = heritability in broad sense

3.7.11. Genetic Advance as Percentage of Mean (GA %):

Genetic advance as percentage of mean is expressed as the percent ratio of genetic advance to population mean and was calculated by the method given by **Johnson *et al.* (1955)**.

$$\text{Genetic advance as percentage of mean} = \frac{GA}{\bar{X}} \times 100$$

Where, GA = Genetic advance and

$\bar{X}$ = Grand mean

The magnitude of genetic advance as percentage of mean was categorized as low (<10%), moderate (10-20%) and high (>20%).

3.7.12. Path coefficient analysis

Path coefficient analysis was carried out according to **Dewey and Lu (1959)**, by dividing the genotypic correlation coefficient to show the direct effects of each attribute

on the dependent trait and their indirect effects through the other component traits on the same in addition to the residual effect of the factors hitherto not taken into consideration for analysis including environment.

For example, considering only the first three yield component traits *i.e.*, x_1 , x_2 and x_3 , the simultaneous equation is presented in a matrix form as below.

$$\begin{bmatrix} r_{x_1y} \\ r_{x_2y} \\ r_{x_3y} \end{bmatrix} = \begin{bmatrix} r_{x_1x_1} & r_{x_1x_2} & r_{x_1x_3} \\ r_{x_2x_1} & r_{x_2x_2} & r_{x_2x_3} \\ r_{x_3x_1} & r_{x_3x_2} & r_{x_3x_3} \end{bmatrix} \times \begin{bmatrix} a_a \\ b_{x_2} \\ c_{x_3} \end{bmatrix}$$

The path coefficient of a, b, c was calculated by solving the above matrix.

Where,

y = grain yield

x = component traits

a = path coefficient of x_1 to y

b = path coefficient of x_2 to y

c = path coefficient of x_3 to y

The path coefficients were ranked as very high (above 1.00), high (0.30-0.99), moderate (0.20-0.29), low (0.10-0.19) and negligible (below 0.10) as proposed by **Lenka and Misra (1973)**.

: -

3.7.13. Seed protein content: (Lowry *et al.*, 1951)

The biuret method was extended by the Lowry reaction of protein estimation being ten times more sensitive than the biuret method. The protein content of enzyme extracts was therefore predominantly determined using this method.

Principle: -

A blue-coloured complex results from a protein's reaction with the Folin-Ciocalteu reagent (FCR). The resulting colour is the result of the alkaline copper reacting with the protein in the biuret test and the reduction of the phosphomolybdic-phosphotungstic

components in the FCR by the tyrosine and tryptophan found in the protein. In a spectrophotometer with the model number UV-1900I from Shimadzu, Japan, the intensity of the blue colour was measured at 660 nm colorimetrically. The number of these aromatic amino acids present determines the colour's intensity, which varies for various proteins.

Materials:

1. Complex-forming reagent: It was prepared immediately before use by mixing the following stock solutions in the proportion 100:1:1 (by volume), respectively:

Solution A: 2% (w/v) Na_2CO_3 in distilled water.

Solution B: 1% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in distilled water.

Solution C: 2% (w/v) sodium potassium tartrate in distilled water.

2. 2 (N) NaOH solution.

3. Folin reagent (commercially available): Used at 1 (N) concentration.

4. Standards: A stock solution of standard protein (e.g., Bovine serum albumin fraction V) containing 2 mg/mL protein in distilled water was prepared and stored frozen at -20°C . The standards were prepared by diluting the stock solution with distilled water.

5. Reagent preparation

1. Solution A 2% Na_2CO_3 in 0.1 N NaOH

2. Solution B 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1% $\text{KNaC}_4\text{H}_4\text{O}_6$

3. Solution C: 50 ml of solution A was mixed with 1 ml of solution B to prepare the resultant solution.

Sample preparation:

1. 0.2 g of sample was weighed and ground with 4 ml of sodium phosphate buffer in a mortar pestle.
2. It was then centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for protein estimation.
3. 0.1 ml of solution was pipetted out and then extracted in a test tube.
4. Volume was made up to 1 ml with distilled water in test tube.

5. 5 ml of solution C was added and after that with the help of shaker it was mixed well and kept in the room temperature for 10 minutes.
6. 0.5 ml of FCR was added in the test tube and mixed well. After that it was kept in the dark for 30 minutes.
7. Then the contents of test tube were transferred in cuvette for taking the reading in spectrophotometer. First, absorbance for blank was recorded at 590 nm and then the reading of the samples was taken.
8. The amount of protein present in seed sample was calculated with the help of standard graph using different concentrations of bovine serum albumin and result was expressed as $\text{gm } 100\text{g}^{-1}$ of seed sample.

RESULTS & DISCUSSIONS

Results and discussions

The results of the present investigation have been presented under the following heads:

1. Performance of Lentil varieties in respect of different morphological characters under different concentrations of EMS in M3 generation.
2. Analysis of variance for different characters in lentil variety in M3 generation.
3. Studies on genetic parameter in respect of different morphological characters in lentil varieties under different concentration of EMS in M3 generation.
4. Genotypic correlation between various quantitative characters in lentil varieties under different concentrations of EMS in M3 generation.
5. Genotypic path for various quantitative characters in lentil varieties under different concentrations of EMS in M3 generation.
6. Studies on the shift in mean values of different characters in lentil varieties in M3 generation.

4.1 Performance of lentil varieties in respect of different morphological characters under different concentrations of EMS in M3 generation

4.1.1. Days to 50 % flowering

Significant variation was observed among the varieties in M3 generation with IPL 406 taking the longest duration for 50% flowering in plants with the maximum duration (72.00) being recorded in the plants of the variety raised from seeds of M2 generation treated earlier at 0.1% concentration of EMS. No significant variation was observed among the treatments (**Table 4.1a**).

4.1.2. Primary branches per plant

Primary branches per plant is considered to be an important yield attribute in lentil. In the present investigation no significant variation was observed neither among the varieties treated with different concentration of EMS nor among the treatments. The variety IPL 406 registered the highest number (2.44) of primary branches per plant (**Table 4.1b**).

Table 4.1a: Days to 50% flowering in lentil varieties under different concentrations of EMS in M3 generation

Days to 50% flowering					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	66.00	62.00	62.33	67.00	64.33 ^{ab}
V2	68.66	72.00	69.33	69.33	69.83 ^a
V3	63.00	74.00	62.33	53.00	63.08 ^b
V4	54.66	57.33	53.00	54.33	54.83 ^c
V5	53.33	56.00	53.66	56.66	54.91 ^c
Mean	61.13 ^a	64.26 ^a	60.13 ^a	60.06 ^a	
SE(m)	3.44				
C.V. (%)	8.72				
C.D	9.77				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

Table 4.1b: Primary branches per plant in lentil varieties under different concentrations of EMS in M3 generation

Primary branches/ plant					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	2.53	2.06	2.06	2.33	2.25 ^a
V2	2.80	2.34	2.13	2.50	2.44 ^a
V3	1.86	2.50	1.95	2.00	2.08 ^a
V4	2.06	2.37	2.00	2.06	2.12 ^a
V5	2.06	2.80	2.39	2.22	2.36 ^a
Mean	2.26 ^a	2.41 ^a	2.11 ^a	2.22 ^a	
SE(m)	0.23				
C.V. (%)	17.71				
C.D	0.67				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

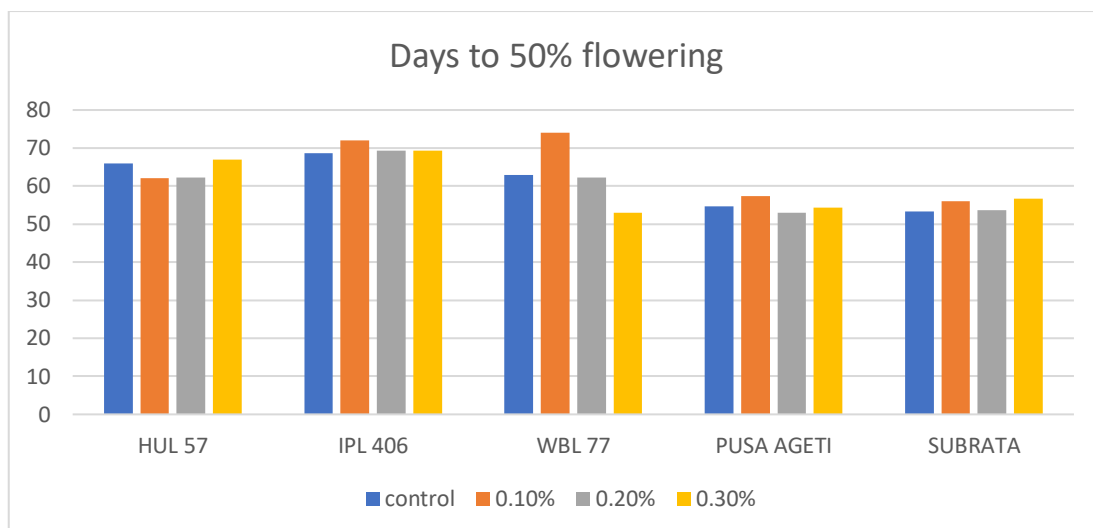


Fig 4.1: Days to 50% flowering in lentil varieties under different concentrations of EMS in M3 generation

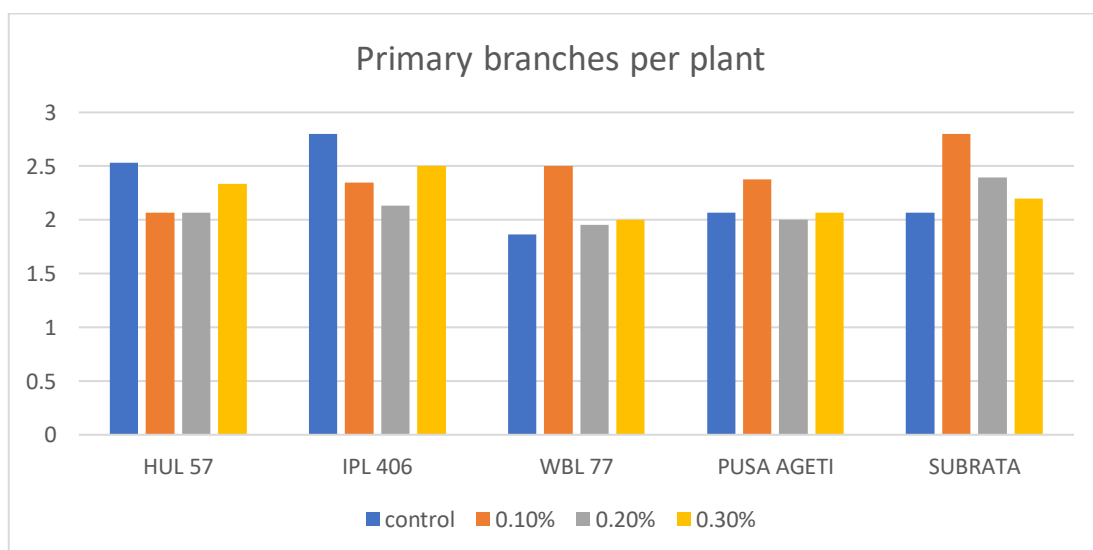


Fig 4.2: Primary branches per plant in lentil varieties under different concentrations of EMS in M3 generation

4.1.3. Secondary branches per plant

Like primary branches, secondary branches per plant are also an important yield attributing trait in lentil. Though no significant variation was observed among the treatments but significant variation was observed between the varieties Pusa Ageti and Subrata and also between Subrata and IPL 406 with the variety Subrata having recorded the maximum number of secondary branches per plant over the treatments, the highest (19.20) being recorded in the plants raised from untreated seeds. The variety IPL 406 in this case had the minimum (11.35) number of secondary branches per plant (**Table 4.1c**).

4.1.4. Number of pods/plants

When number of pods per plant was recorded in M3 generation significant variation was observed among the varieties WBL 77 and PusaAgeti , WBL 77 and Subrata, HUL 57 and IPL 406 and also between IPL 406 and WBL 77. The variety IPL 406 had the highest number (38.79) of pods per plant over the treatments while WBL 77 had the lowest number (31.17) of pods per plant. Unlike the other characters, significant variation was observed among the treatments with maximum number of pods per plant being recorded at 0.3% concentration of EMS (38.22) while the minimum (30.94) was recorded at 0.2% EMS (**Table 4.1d**).

4.1.5. Pod weight /plant

The pod weight was taken at maturity and the analysis revealed significant variation among the genotypes. At the level of the treatment, significant variation was observed between the treatment control and 0.1% EMS and also between control and 0.3% concentration of EMS. The maximum pod weight was observed in the variety Subrata (2.49) whereas the minimum value was observed in the variety IPL 406 (1.73); among the treatments the maximum (2.38) value was observed at 0.3% concentration of EMS (**Table 4.1e**).

4.1.6. Number of seeds/pods

Number of seeds per pod like others is also considered to be an important yield attributing trait. Significant variation was observed among the varieties HUL 57, IPL 406 and WBL 77 while no such significant variation was observed among the

treatments. The highest number of seeds per pod (1.85) in M3 generation was found in the variety WBL 77 followed by IPL 406 while HUL 57 had the lowest number (1.59) of seeds per pod over the treatments (**Table 4.1 f**).

4.1.7. Plant height at maturity

When the height of the plant at maturity was measured no significant variation was observed among the treatments whereas the variety PusaAgeti differed significantly from remaining varieties with the variety WBL 77 registering the maximum plant height (41.81) at 90% maturity and the minimum height was registered in the variety PusaAgeti (29.68) over the treatments (**Table 4.1g**).

4.1.8 Days to 90% maturity

As far as days to maturity (90%) in lentil varieties was concerned, significant variation was observed among the variety IPL 406 and PusaAgeti and also among IPL 406 and Subrata with HUL 57 taking the longest duration (118.16) while Subrata took the minimum (110.58) number of days to attain 90% maturity over the treatments. When computed over the varieties no such significant variation was observed among the treatments as far as the character was concerned (**Table 4.1h**).

4.1.9. 1000 seed weight

The 1000 seed weight in different lentil varieties in M3 generation exhibited no significant variation among the treatments while the varieties IPL 406 and PusaAgeti had significant variation among themselves with the former registering the maximum 1000 seed weight (21.58) and the later having the minimum (17.02) of the character over the treatments. In IPL 406, the maximum 1000 seed weight (23.33) was recorded at 0.3% concentration of EMS (**Table 4.1i**).

4.1.10 Yield per plant

When the yield was calculated at the level of individual plant in M3 generation significant variation was observed among the varieties HUL 57, WBL 77, PusaAgeti and Subrata with the later having registered the highest yield per plant (2.18g) while the minimum (1.53g) was recorded in the variety HUL 57 when averaged over the treatments. Significant variation was observed between the control and 0.1% EMS

Table 4.1c: Secondary branches per plant in lentil varieties under different concentrations of EMS in M3 generation

Secondary branches / plant					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	13.40	15.17	16.51	16.78	15.46 ^{ab}
V2	12.86	14.76	12.97	11.35	12.99 ^b
V3	13.46	13.82	16.26	17.26	15.20 ^{ab}
V4	17.46	15.57	12.02	9.98	13.76 ^b
V5	19.20	18.97	17.62	17.30	18.27 ^a
Mean	15.28 ^a	15.66 ^a	15.07 ^a	14.53 ^a	
SE(m)	2.07				
C.V. (%)	23.09				
C.D	5.87				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

Table 4.1d: No. of pods/plant in different lentil varieties under different concentrations of EMS in M3 generation

Number of pods/plants					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	25.63	35.53	27.40	38.88	31.86 ^{bc}
V2	35.53	42.53	42.16	39.92	38.79 ^a
V3	26.90	34.44	22.66	40.73	31.17 ^c
V4	38.76	38.46	34.50	38.73	37.62 ^a
V5	41.40	28.66	28.03	37.86	33.99 ^b
Mean	33.65 ^c	35.92 ^b	30.94 ^d	38.22 ^a	
SE(m)	3.27				
C.V. (%)	6.04				
C.D	9.28				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

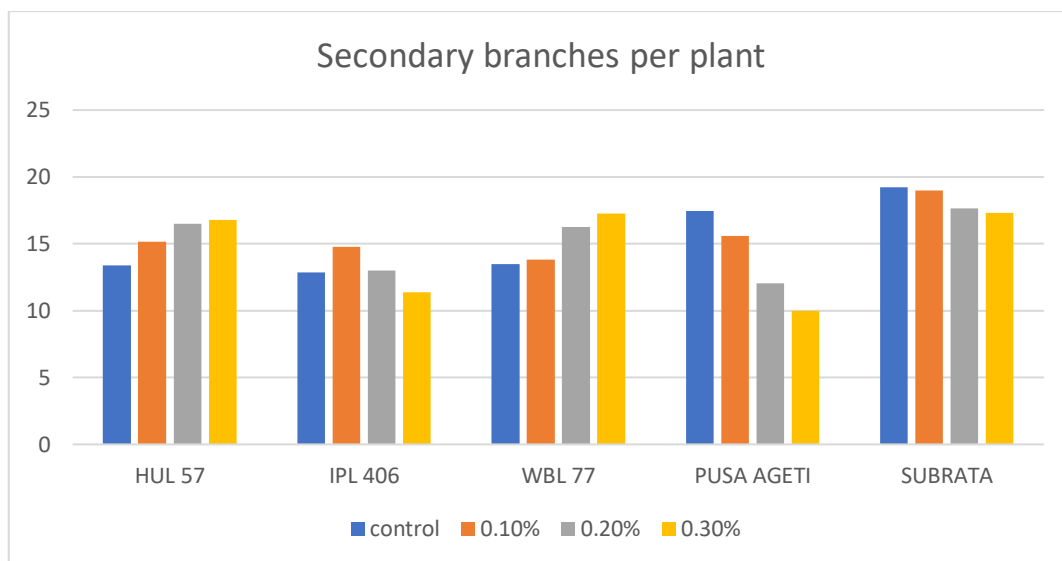


Fig 4.3: Secondary branches per plant in lentil varieties under different concentrations of EMS in M3 generation

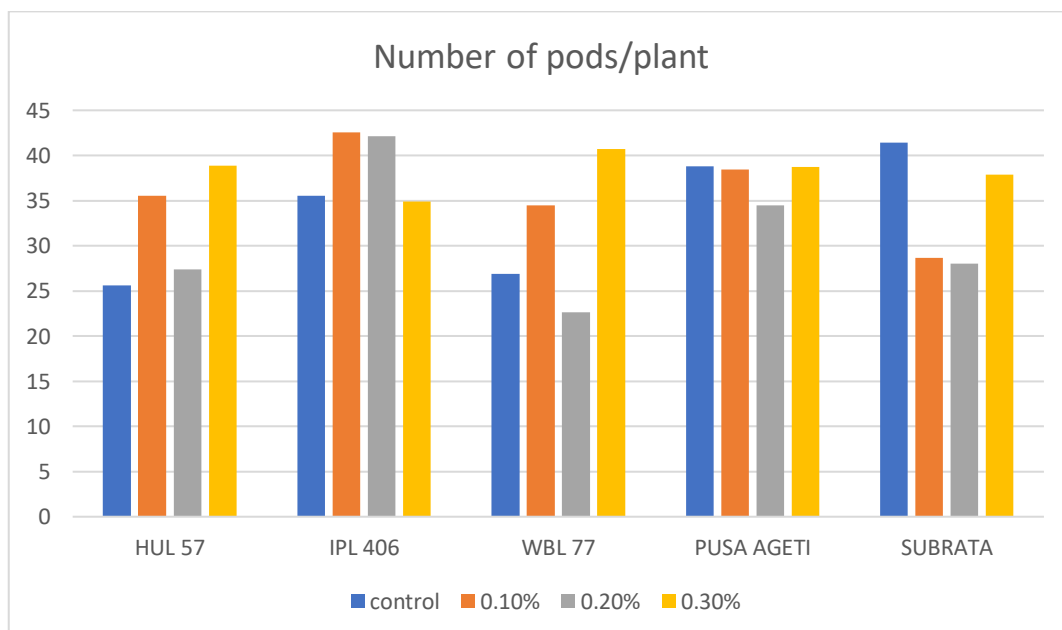


Fig 4.4: Number of pods per plant in lentil varieties under different concentrations of EMS in M3 generation.

Table 4.1e: Pod weight per plant in different lentil varieties under different concentrations of EMS in M3 generation

Pod weight/ plant (gm)					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	1.49	2.69	1.62	1.43	1.81 ^c
V2	1.69	1.53	1.13	2.56	1.73 ^c
V3	2.70	1.07	1.13	2.62	1.88 ^c
V4	1.51	2.35	2.56	2.31	2.18 ^b
V5	1.81	2.37	2.80	3.00	2.49 ^a
Mean	1.84 ^b	2.00 ^a	1.85 ^b	2.38 ^a	
SE(m)	0.35				
C.V. (%)	12.32				
C.D	1.02				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

Table 4.1f: Number of seeds per pod in different lentil varieties under different concentrations of EMS in M3 generation

Number of seeds/pods					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	1.50	1.50	1.73	1.63	1.59 ^b
V2	1.86	1.73	1.86	1.73	1.80 ^a
V3	1.73	1.86	1.93	1.86	1.85 ^a
V4	1.63	1.86	1.66	1.73	1.72 ^{ab}
V5	1.93	1.86	1.66	1.86	1.83 ^a
Mean	1.73 ^a	1.76 ^a	1.77 ^a	1.76 ^a	
SE(m)	0.07				
C.V. (%)	6.89				
C.D	0.22				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

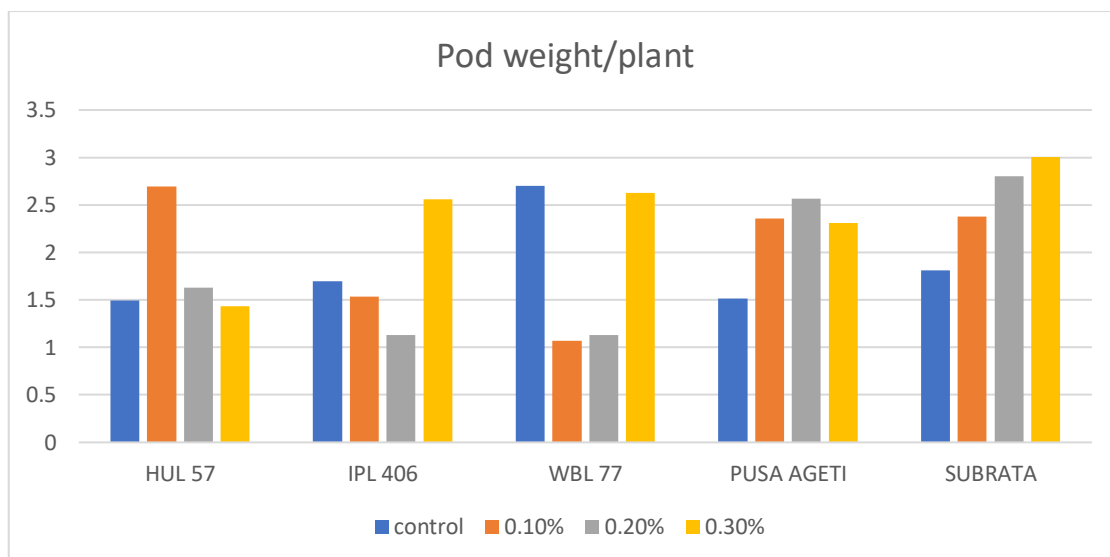


Fig 4.5 Pod weight per plant in lentil varieties under different concentrations of EMS in M3 generation.

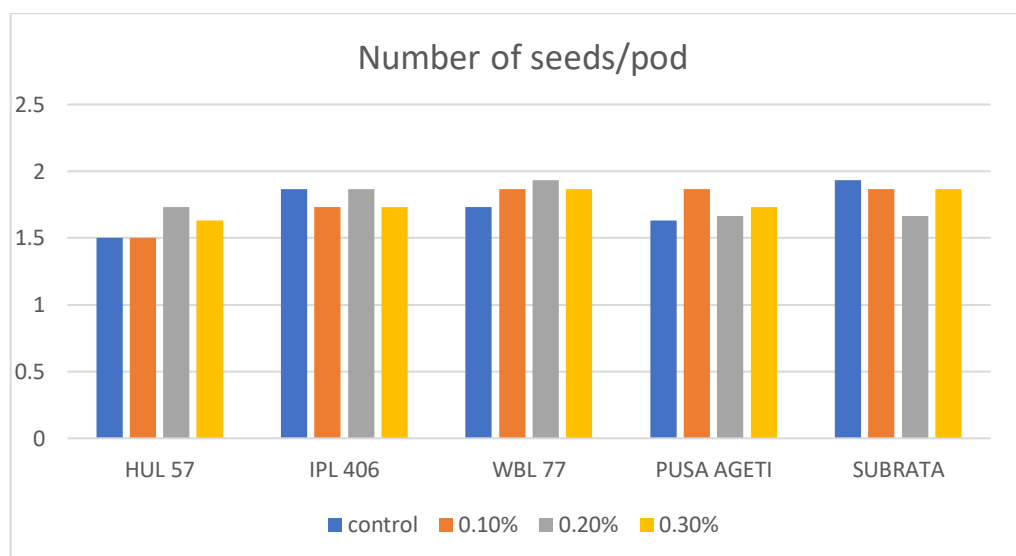


Fig 4.6 Number of seeds per pod in lentil varieties under different concentrations of EMS in M3 generation

Table 4.1g: Plant height at 90% maturity in different lentil varieties under different concentrations of EMS in M3 generation

Plant height at 90% maturity(cm)					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	40.73	42.33	40.90	39.23	40.80 ^a
V2	42.86	32.60	37.20	41.26	38.45 ^a
V3	40.33	38.46	42.20	46.26	41.81 ^a
V4	31.33	29.60	28.26	29.53	29.68 ^b
V5	42.26	39.06	40.20	42.53	41.03 ^a
Mean	39.50 ^a	36.41 ^a	37.76 ^a	39.74 ^a	
SE(m)	2.76				
C.V. (%)	14.48				
C.D	7.84				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

Table 4.1h: Days to 90% maturity in lentil varieties under different concentrations of EMS in M3 generation

Days to 90% maturity					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	120.66	112.66	116.66	122.66	118.16 ^{ab}
V2	121.00	118.66	124.00	128.33	123.00 ^a
V3	114.00	127.00	119.00	110.33	117.58 ^{ab}
V4	106.66	117.66	116.00	111.58	113.00 ^{bc}
V5	113.33	109.33	110.33	109.33	110.58 ^c
Mean	115.13 ^a	117.06 ^a	117.20 ^a	116.46 ^a	
SE(m)	3.63				
C.V. (%)	4.52				
C.D	10.32				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

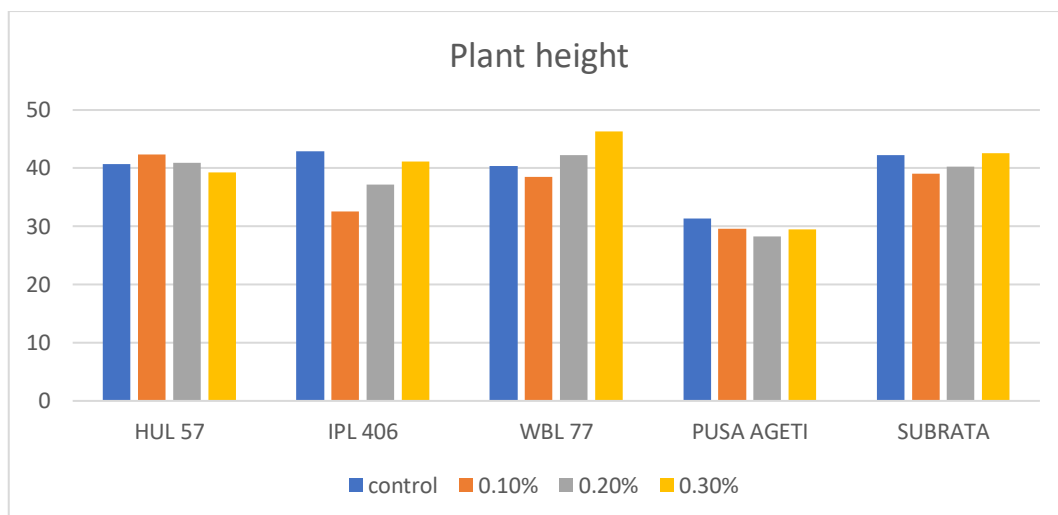


Fig 4.7 Plant height in lentil varieties under different concentrations of EMS in M3 generation.

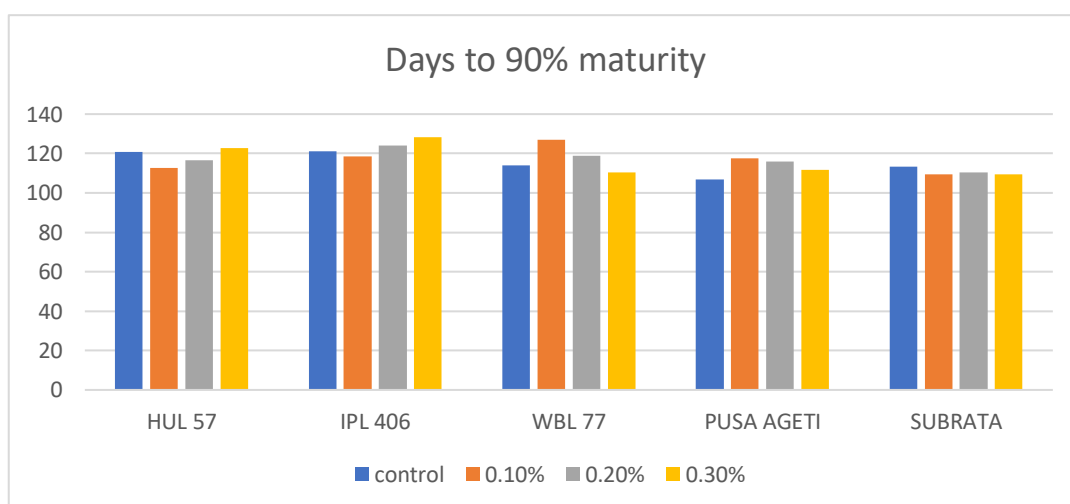


Fig 4.8 Days to 90% maturity in lentil varieties under different concentrations of EMS in M3 generation

Table 4.1i: 1000 Seed weight in different lentil varieties under different concentrations of EMS in M3 generation

1000 Seed weight(gm)					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	11.66	15.63	19.33	21.60	18.51 ^{ab}
V2	19.66	20.33	23.00	23.33	21.58 ^a
V3	13.33	14.66	16.63	17.60	18.75 ^{ab}
V4	12.11	11.33	11.33	10.60	17.02 ^b
V5	18.10	16.33	19.50	18.33	18.06 ^{ab}
Mean	18.46 ^a	18.12 ^a	19.16 ^a	19.40 ^a	
SE(m)	1.77				
C.V. (%)	16.15				
C.D	5.02				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

Table 4.1j: Yield per plant in different lentil varieties under different concentrations of EMS in lentil varieties

Yield /plant (gm)					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	0.99	2.44	1.20	1.49	1.53 ^c
V2	1.59	0.90	2.37	2.34	1.80 ^{bc}
V3	1.01	0.59	0.74	2.25	1.15 ^d
V4	1.43	1.65	2.51	2.56	2.04 ^{ab}
V5	2.05	1.58	2.60	2.48	2.18 ^a
Mean	1.41 ^c	1.43 ^c	1.88 ^b	2.22 ^a	
SE(m)	0.36				
C.V. (%)	15.02				
C.D	1.02				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

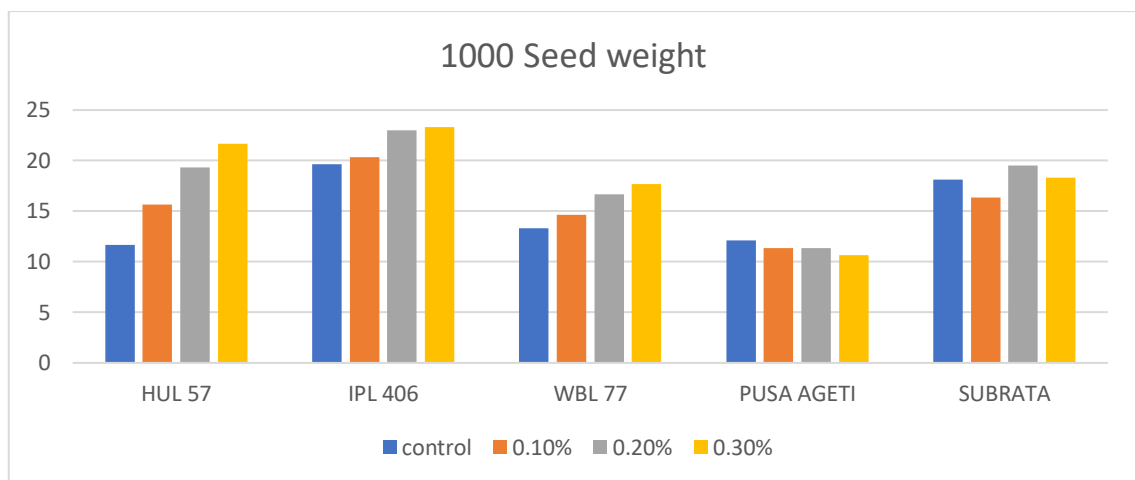


Fig 4.9 1000 seed weight in lentil varieties under different concentrations of EMS in M3 generation.

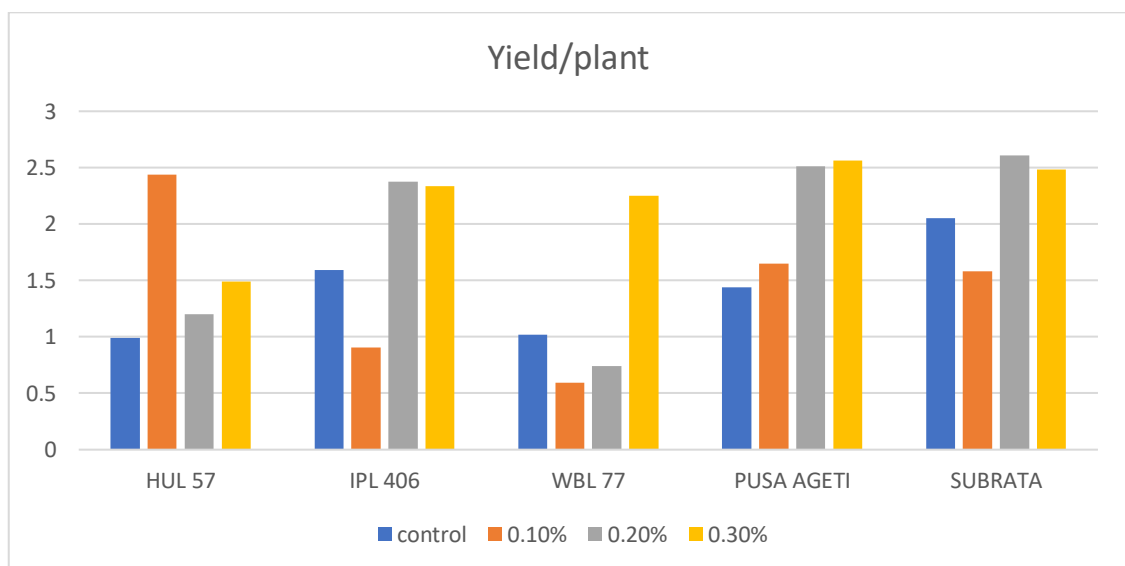


Fig 4.10 Yield per plant in lentil varieties under different concentrations of EMS in M3 generation

concentration while the remaining treatments also had significant variation over the control (**Table 4.1j**).

4.1.11. Cooking time

In lentil, cooking time is considered to be an important quality parameter and the seeds raised in M3 generation did not differ significantly in terms of cooking time except between the variety HUL 57 and IPL 406, Subrata and IPL 406 and IPL 406 and WBL 77. In case of IPL 406, the seeds took the maximum time (32.50 min) while the minimum time was taken by the variety WBL 77. Significant variation was observed among the treatments viz., 0.2% and 0.3%, 0.1% and 0.2% and between control and 0.3% with the maximum cooking time being recorded in the seeds treated with 0.3% concentration of EMS (**Table 4.1k**).

4.1.12. Seed Protein content

Protein content in seeds was measured after Lowry *et al.* (1951) following harvest in M3 generation and the analysis revealed that the seeds of the variety PusaAgeti had the maximum seed protein content (26.37) while the seeds of the variety Subrata had the minimum seed protein content (24.96). No such significant variation was observed among the varieties in M3 generation but significant variation was observed among the same in untreated seeds and that between the seeds treated with 0.3% concentration of EMS (**Table 4.1l**).

4.2. Analysis of Variance for different characters in lentil varieties in M3 generation

The analysis of variance revealed significant to highly significant variation among the varieties for most characters in M3 generation, the exception being the primary branches per plant. For all of the aforesaid characters the coefficient of variation was found to be within the statistically permitted range. Only four characters, viz., number of pods per plant, pod weight per plant, yield per plant, cooking time and seed protein content showed highly significant differences among the treatments. At the level of the genotype (variety) versus treatment interaction the number of pods per plant, pod weight per plant, number of seeds per pod, days to 90% maturity, yield per plant, cooking time and seed protein content were found to exhibit highly significant variation

Table 4.1k: Cooking time in different lentil varieties under different concentrations of EMS in M3 generation

Cooking time(min.)					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	25.00	32.66	21.66	31.66	27.75 ^b
V2	36.66	31.66	28.33	33.33	32.50 ^a
V3	28.33	33.33	26.66	21.66	27.50 ^b
V4	26.66	26.66	28.33	38.33	30.00 ^{ab}
V5	26.66	28.33	30.00	31.66	28.75 ^b
Mean	28.33 ^{bc}	30.53 ^{ab}	27.00 ^c	31.33 ^a	
S.E(m)	2.73				
C.V. (%)	9.02				
C.D	7.76				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

Table 4.1l: Seed protein content in different lentil varieties under different concentrations of EMS in M3 generation

Protein content(mg/gram)					
Treatment					
Variety	Control	0.1%	0.2%	0.3%	Mean
V1	27.35	26.18	28.12	23.80	26.36 ^a
V2	27.62	28.19	27.02	27.62	27.61 ^a
V3	30.13	26.65	26.33	19.96	25.77 ^a
V4	26.73	28.15	24.63	25.96	26.37 ^a
V5	25.15	24.34	25.57	24.80	24.96 ^a
Mean	27.39 ^a	26.70 ^{ab}	26.33 ^{ab}	24.43 ^b	
S.E(m)	1.41				
C.V. (%)	2.63				
C.D	4.02				

V1: HUL 57 V2: IPL 406 V3: WBL 77 V4: PusaAgeti V5: Subrata

*The values with same alphabet (a, b, c) do not differ significantly,

S.E(m) = Standard Error of mean, C.V. %= Coefficient of variation, C.D = Critical Difference

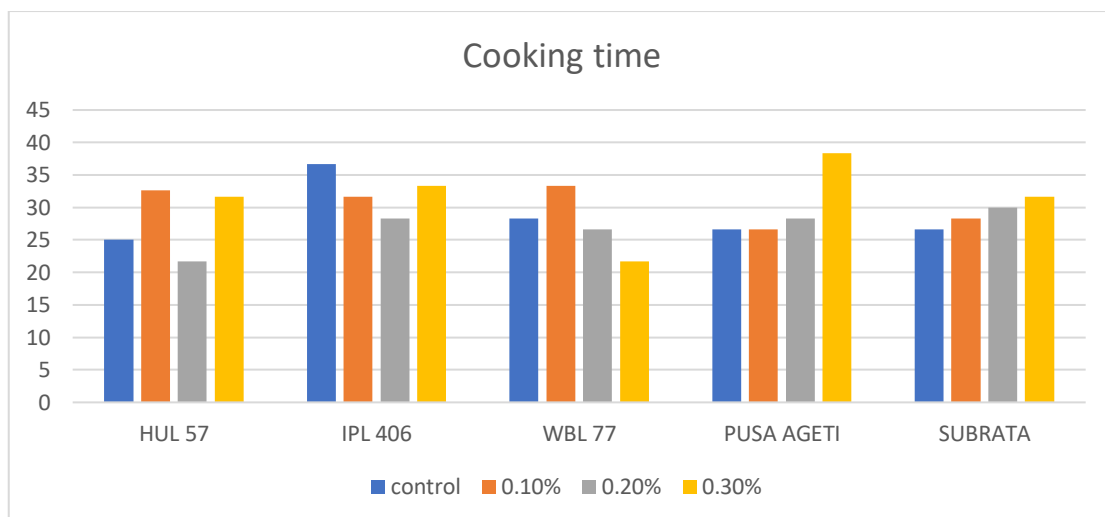


Fig 4.11 Cooking time in lentil varieties under different concentrations of EMS in M3 generation.

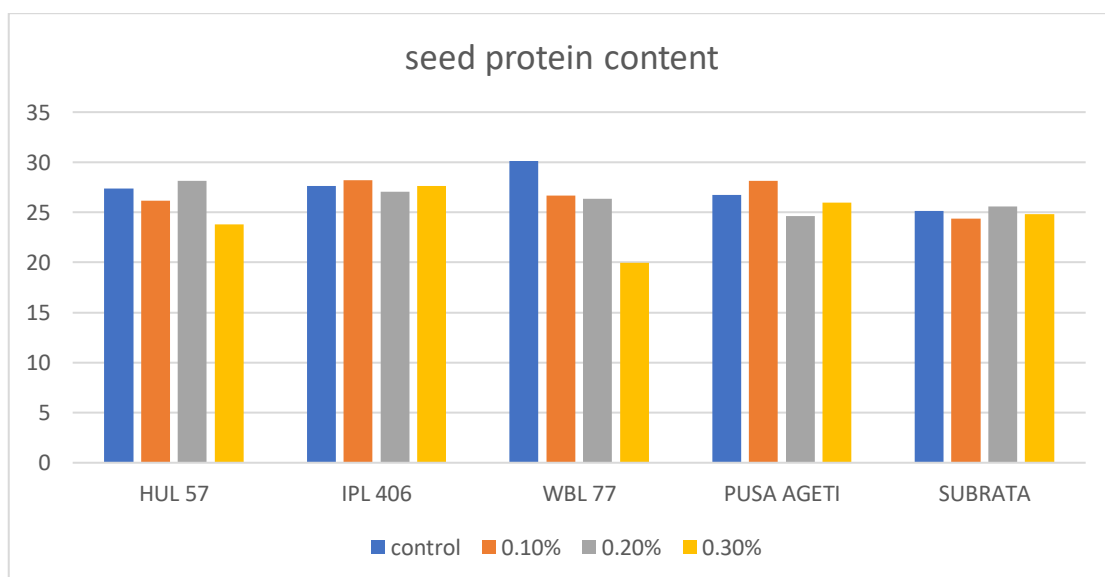


Fig 4.12 Seed protein content in lentil varieties under different concentrations of EMS in M3 generation

Table 4.2 Analysis of variance (ANOVA) for different characters in lentil varieties under different concentrations of EMS in M3 generation

		Days to 50% flowering	Primary Branches/plant	Secondary Branches/plant	No. of pods /plant	Pod weight /plant	No. of seeds /pod	Plant height	Days to 90% maturity	1000 Seed weight	Yield per plant	Cooking time	Protein content
Source	df	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
Replication	2	40.20	0.31	398.10	2.28	0.09	0.001	138.86	66.52	14.58	0.04	6.65	0.47
Treatment	3	58.40	0.24	3.30	145.75**	0.97**	0.004	36.80	13.38	5.31	2.26**	59.40**	32.99 **
Genotype	4	503.10**	0.28	49.40*	138.86**	1.21**	0.13**	301.02**	280.36**	34.55*	2.03**	50.03**	18.78**
Genotype x Treatment	12	51.80	0.19	14.30	91.68**	1.27**	0.03*	19.22	84.34**	11.11	0.94**	62.2**	16.73**
Error	38	51.80	0.15	13.20	4.40	0.06	0.01	23.04	27.74	9.21	0.06	7.00	0.48
C.V.%		8.72	17.71	23.99	6.04	12.32	6.89	14.48	4.52	16.15	15.02	9.02	2.63

thereby highlighting the impact of different concentrations of mutagen in inducing the variability in the varieties studied (**Table 4.2**).

4.3. Studies of different genetic parameters in respect of different characters in lentil varieties treated with different concentration of EMS in M3 generation

The values for phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense (h^2), genetic advance (GA) and genetic advance as percentage of mean (GAM) are presented in **Table 4.3**

The difference between the PCV and GCV was less for days to 50% flowering and plant height at maturity. The same exhibited high heritability along with high genetic advance as percentage of mean, thereby indicating the high genotypic influence on the characters in the generation itself. High heritability and moderate genetic advance as percentage of mean was observed seeds per pod and days to 90% maturity; the influence of the environment on the resultant variability for the same character for the characters was supported by high difference between PCV and GCV. Moderate heritability with low GAM was observed for secondary branches per plant, pods per plant, 1000 seed weight, yield per plant and seed protein content thus indicating the fact that the Impact of mutagen in the characters need to studied further in coming generations. The characters days to 50% flowering, plant height and days to 90% maturity showed high genetic advance whereas the characters yield per plant, plant height, secondary branches per plant, pod weight per plant, days to 50% flowering showed high genetic advance as percentage of mean. The high heritability estimates for quantitative characters could be beneficial for choosing the relevant genotypes.

4.4. Genotypic correlation between yield and yield attributing characters in lentil varieties under different concentrations of EMS in M3 generation

The analysis revealed a positive correlation between yield and number of pods per plant and that between yield and pod weight per plant. But only pod weight per plant had shown a significant and positive correlation (0.669) with yield. Other traits such as days to 50 % flowering, secondary branches per plant, number of seeds per pod, plant height at maturity, days to maturity and seed weight had shown negative correlation with yield and among them days to 50 % flowering had a significant but negative correlation (-0.578) with yield (**Table4.4**).

Table 4.3: Estimates of genetic parameters for different characters in lentil varieties in M3 generation

Character	Mean	Range	GCV	PCV	h²(bs) (%)	GA	GAM
Days to 50% flowering	61.40	27.00	20.33	22.53	81.40	23.20	37.78
Primary branches/plant	2.25	0.92	8.59	20.32	17.87	0.16	7.48
Secondary branches/plant	15.13	23.40	23.03	33.04	48.59	5.00	33.08
No. of pods /plant	34.68	23.60	17.19	23.72	52.52	8.90	25.66
Pod weight/plant	2.02	2.39	25.89	40.26	41.36	0.69	34.30
No. of seeds/pod	1.76	0.60	11.11	13.60	66.84	0.32	18.72
Plant height	38.35	29.60	25.10	28.03	80.15	17.75	46.29
Days to 90% maturity	116.46	27.00	7.68	9.40	66.87	15.08	12.95
1000 Seed weight	18.78	15.00	15.40	22.45	47.07	4.09	21.77
Yield/plant	1.74	2.54	42.56	55.71	58.37	1.16	66.99
Cooking time	29.30	20.00	10.34	19.20	29.01	3.36	11.47
Protein content	26.28	10.00	7.85	12.18	41.49	2.73	10.41

GCV = Genotypic coefficient of variation, PCV= Phenotypic coefficient of variance, h²(bs) (%) = Heritability in broad sense, GA= Genetic advance, GAM= Genetic advance as percentage of mean

Inter correlation among traits:

The trait days to 50% flowering had a significant negative correlation with secondary branches per plant as well as pod weight per plant. Days to 50% flowering had a significant positive correlation with days to maturity and 1000 seed weight. Secondary branches per plant had a significantly positive correlation with plant height and significantly negative correlation with days to maturity and 1000 seed weight. Number of pods per plant and number of seeds per pod did not show any significant correlation with other traits. Pod weight per plant showed significantly negative correlation with days to 50% flowering and days to maturity. Plant height showed significantly positive correlation with secondary branches per plant while days to maturity exhibited significant and positive correlation days to 50 % flowering and 1000 seed weight. 1000 Seed weight was found to have a significant and positive correlation with days to 50 % flowering and days to maturity whereas the same showed significant negative correlation with secondary branches per plant (**Table 4.4**). **Sharma and Sharma (1984)** similarly observed a substantial rise in the negative correlation of days to flowering with pods and seed yield per plant in all the treatments used.

4.5. Genotypic path for various characters in lentil varieties under different concentrations of EMS in M3 generation.

There were six traits which had positive direct effect on yield viz., secondary branches per plant, number of pods per plant, pod weight per plant, plant height, days to maturity and 1000 seed weight. The maximum positive direct effect was recorded by days to maturity (3.134) followed by 1000 seed weight (0.668). The residual effect of 0.0247 was indicative of the fact that the resultant variability under induction of mutation was primarily accounted for the by the genotypic differences and the traits which were studied had contributed greatly towards variability in grain yield of lentil. (**Table 4.5**)

Table 4.4. Genotypic correlation between yield and yield attributing characters in lentil varieties under different concentrations of EMS in M3 generation

	Days to 50% flowering		Secondary branches/plant		No. of pods/plant		Pod weight /plant		Number of seeds/pods		Plant height		Days to maturity		1000 Seed weight		Yield/plant	
Days to 50% flowering	1.000		-0.789	**	0.001		-0.654	**	-0.148		0.222		0.929	**	0.753	**	-0.578	**
Secondary branches/plant	-0.789	**	1.000		-0.050		-0.002		0.423		0.693	**	-0.971	**	-0.532	*	-0.091	
No. of pods/plant	0.001		-0.050		1.000		-0.022		0.173		-0.326		0.008		0.284		0.414	
Pod weight/plant	-0.654	**	-0.002		-0.022		1.000		-0.173		0.085		-0.626	**	-0.307		0.669	**
No. of seeds/pod	-0.148		0.423		0.173		-0.173		1.000		0.229		0.011		0.052		-0.074	
Plant height	0.222		0.693	**	-0.326		0.085		0.229		1.000		0.141		-0.031		-0.050	
Days to maturity	0.929	**	-0.971	**	0.008		-0.626	**	0.011		0.141		1.000		0.730	**	-0.390	
1000 Seed weight	0.753	**	-0.532	*	0.284		-0.307		0.052		-0.031		0.730	**	1.000		-0.106	
Yield/plant	-0.578	**	-0.091		0.414		0.669	**	-0.074		-0.050		-0.390		-0.106		1.000	

Table 4.5: Genotypic path for different characters in lentil varieties under different concentrations of EMS in M3 generation

	Days to 50 % flowering	Secondary branches/plant	No. of pods/plant	Pod weight/plant	No. of seeds/pod	Plant height	Days to maturity	1000 Seed weight	Yield/plant
Days to 50% flowering	-3.808	-0.365	0.001	-0.087	0.160	0.106	2.913	0.503	-0.578**
Secondary branches/plant	3.004	0.462	-0.029	-0.001	-0.458	0.331	-3.045	-0.356	-0.091
No. of pods/plant	-0.003	-0.023	0.573	-0.003	-0.188	-0.155	0.024	-0.189	0.414
Pod weight/plant	2.489	-0.001	-0.012	0.133	0.188	0.040	-1.963	-0.205	0.669**
No. of seeds/pod	0.562	0.195	0.099	-0.023	-1.085	0.109	0.034	0.035	-0.074
Plant height	-0.845	0.320	-0.187	0.011	-0.249	0.477	0.443	-0.021	-0.050
Days to maturity	-3.539	-0.449	0.004	-0.084	-0.012	0.067	3.134	0.487	-0.390
1000 Seed weight	-2.865	-0.246	-0.015	2.287	0.162	-0.041	-0.057	0.668	-0.106

Residual effect = 0.0247

4.6 Studies on the shift in mean values of different characters in lentil varieties in M3 generation.

The observations on various morphological characters in lentil varieties revealed positive and significant shift in mean values in M3 generation to a differential extent as compared to the same in M2 generation. For days to 50% flowering, a significant shift in the positive direction was observed in WBL 77 at 0.1% EMS and the same was found to be much higher when compared to the same in M2 generation. In the variety IPL 406 positive shift was observed in all the concentrations of EMS as in the variety Subrata, however, they were not significant. Thus, the character has been significantly affected because of mutagenesis in WBL 77 at 0.1% EMS concentration particularly. The high heritability value for the character in both the generation substantiated the observation. **Sharma and Sharma (1984)**, working with lentil genotypes, reported that mean values for days to flowering dropped in M3 generation and early flowering plants identified in M2 appeared to be the greatest producing plant and bred true in M3 generation

Positive shift in mean values was observed in all the concentrations in the variety WBL 77 when primary branches per plant was recorded and analysed. However, the magnitude of shift was greater only at 0.1% EMS in M3 compared to M2. In the variety PusaAgeti similarly a positive shift was observed at 0.1% EMS in M3 generation. In the variety Subrata a positive and significant shift in the mean values in primary branches was observed at 0.1% EMS when compared to the untreated plants while in rest of the concentration though a shift in the positive direction was observed similar to M2 generation but it was not significant. Further the heritability value as recorded in M2 generation was found to decline in M3 generation which warranted further validation of the shift as observed in one such situation for the character (**Table 4.6**).

Secondary branches per plant is also considered to be an important yield attributing character and in variety HUL 57, a positive shift in mean values was observed in all the concentrations in M3 generation though it was not significant. It may be mentioned that a negative shift was observed in M2 generation in all the concentration in the varieties concerned for the same character. A similar positive shift in mean values was observed in WBL 77 though the shift was not significant. At 0.2% EMS, secondary branches per

plant registered a positive shift in M3 generation which was found to negative in M2 generation. For the rest of the varieties negative shift in mean values was noted for the said character in M3 generation. The shift in the heritability from high to moderate in M3 generation indicated that the heritable nature of variation as noted through significant positive shift in mean value needed to be studied further in coming generations to exploit the effect mutagen, if any on the character.

When number of pods per plant was recorded and analysed, the character registered a positive and significant shift in mean values in the variety HUL 57 both at 0.1% and 0.3% EMS respectively though the magnitude of shift was less at 0.1% concentration of EMS and more at 0.3% EMS concentration when compared to the same in M2 generation. A similar trend was observed in the variety WBL 77 in 0.3% EMS where a significant positive shift was observed in M3 generation and was found to be higher than that in M2 generation. At 0.1% EMS, the variety registered a positive shift in mean for number of pods per plant though the shift was not significant. For rest of the varieties negative shift in mean values was observed in all the concentrations. Like secondary branches per plant, the character exhibited a similar shift in heritability estimate which need to be studied further in coming generation (**Table 4.6**). **Sharma and Sharma (1984)** also observed that pods per plant and seed yield per plant in M3 generation were higher compared to the corresponding controls.

As far as pod weight per plant was concerned, significant shift in the positive direction in mean values was observed in the variety HUL 57 at 0.1% EMS, in Pusa Ageti at 0.2% EMS and at 0.3% EMS in the variety Subrata respectively. In Pusa Ageti though positive shift in mean was observed in rest of the concentrations but it was not significant similar to that in the variety Subrata i.e., at 0.1% and 0.2% EMS. The character had a moderate heritability in the generation which need to be studied further in coming generation to establish its relationship to the effect of mutagen as far as the generation of variability was concerned.

The number of seeds per pod registered a positive and significant shift in mean values in the variety HUL 57 at 0.2% EMS and in variety Pusa Ageti at 0.1% EMS in M3 generation, while a negative shift was observed for the said character in M2 generation in respective varieties at aforesaid concentrations. In the variety WBL 77 positive shift was observed in all the concentrations though it was not significant, similar to that in

Pusa Ageti at 0.2% and 0.3% EMS respectively. For the remaining varieties non-significant and negative shift was observed in the said character in the generation itself. The high heritability of the character in M3 generation similar to that in M2 generation substantiated the observation in respect of significant and positive shift in mean values and thus could be made to use in next generation.

No such significant shift in mean values was observed in any of the varieties and in any of the concentration when plant height at maturity was considered for assessing the impact of mutagenesis. Only positive shift in mean was observed in HUL 57 at 0.1% and 0.2% EMS, in WBL 77 at 0.2% and 0.3% EMS and in variety Subrata at 0.3% EMS respectively (**Table 4.6**). However, a clear relation between EMS dose and seedling height decrease has been observed by **Sharma (1969)** with the greatest reduction in height happening at the highest dose though **Kumar and Lal (2001)** reported that with the increase in concentration of EMS, the height of the plants also increased.

When days to 90% maturity was taken into consideration for ascertaining the effect of mutagen on the character a positive and significant shift in mean was observed only in case of WBL 77 and Pusa Ageti at the same concentration, i.e., 0.1% EMS while in Pusa Ageti though positive shift in days to maturity in the remaining concentrations as in IPL 406 and HUL 57 at 0.3% EMS was observed but shift was not significant. A significant and negative shift in mean value in respect of the character in the variety HUL 57 could well be utilised in having the character of early maturity, considered to be important in many cropping situations. The stability of the high value of heritability in successive generations (66.87 in M3 and 99.12 in M2 generation) for the character is an indicator in this direction.

The test weight of harvested seeds upon analysis revealed that the mutagenesis had an effect on inducing mutation in positive direction in variety HUL 57 as significant and positive shift was observed for the character at 0.2% and 0.3% EMS in M3 generation as against negative shift observed in M2 generation at the same concentration. A similar trend was observed in variety IPL 406 and WBL 77 in all the concentrations where a positive shift though not significant, in mean values was observed in M3 generation though the same recorded a negative shift in M2 generation. In Pusa Ageti, on the contrary a reverse trend was noted as a negative shift was observed in all the

concentration in M3 as against a positive shift in M2 generation thereby indicating that the variation observed in earlier generation might not be due to mutagenic effect. In Subrata test weight of the seed registered a positive shift in mean at 0.2% and 0.3% EMS in M3 generation as against negative shift noted in M2 generation which further need to be studied in subsequent generations. The estimate of heritability in M3 generation showed a shift from very high in M2 generation to moderate heritability which warranted further study on the character in coming generation as far as the effect of mutagen on the character was concerned. Similar intervarietal variation in test weight was observed in lentil varieties K-75 and K-303 when the seeds were treated with mutagen. (**Tripathi and Dubey, 1992**)

The mutagen had significant effect in positive direction in the varieties HUL 57 at 0.1 % EMS, in WBL 77 at 0.3% EMS and in Pusa Ageti at 0.2 % and 0.3% EMS respectively. As in IPL 406, a positive shift in mean values was also observed in the variety Subrata for yield per plant at both the concentrations of 0.2% and 0.3% EMS similar to that in Pusa Ageti at 0.1% EMS but the said shift was found not be significant. Similarly in variety HUL 57 though a positive shift in mean was recorded in rest of the concentrations but the shift was not found to be significant enough. The heritability value for the character in the generation was found to be moderate (58.37%). **Khan and Wani (2006)** induced mutation by EMS in green gram and studied four quantitative traits in M2 and M3 generations namely viz., fertile branches per plant, pods per plant, 100 seed weight (g) and yield per plant (g). A significant shift in the mean values in positive direction was observed for all the traits studied and in all the cases high values of heritability and genetic advance over the control was indicative of the possibility of induction of desirable mutation for polygenic traits accompanied by effective selection in M2 generation. The increased heritability in M3 in comparison to M2 generation may be due to increased homozygosity of the genes involved. Similar such results have been reported by **Singh et al., (2001)**; **Khan et al., (2001)** and **Raut et al., (2004)**. **Alka (2008)** similarly reported differential response to mutagenic treatment for the quantitative characters viz., plant height, days to flowering, days to maturity, number of fertile branches, number of pods etc. Of these characters, plant height, days to flowering, days to maturity, seeds per pod and 100 seed weight were found to decline with the effect of the mutagen used.

Cooking time in lentil is considered to be an important attribute and when measured a positive and significant shift was observed only in Pusa Ageti in generation itself at 0.3% EMS while positive and non-significant shift was found in variety Subrata in all the concentrations, in Pusa Ageti at 0.2% EMS, in WBL 77 at 0.1% EMS and in HUL 57 at 0.1% and 0.3% EMS concentration. While for the remaining varieties and remaining concentrations a negative shift in mean values was observed in M3 generation. Though in terms of quality of the grain a lesser cooking time would be favoured, but in none of the varieties and in none of the concentrations such character of significance (negative and significant shift) could be identified.

Seed protein content is also considered to be an important quality parameter in lentil. In the present investigation the mutagenesis has no such significant impact on seed protein content. Only in variety HUL 57 and Subrata and in variety IPL 406 and Pusa Ageti seed protein content registered a positive shift in mean at 0.2% and 0.1% EMS respectively while for the remaining concentration and in rest of the varieties only negative shift in mean values was observed when character was considered for studying the effect of mutagenesis. (**Table 4.6**). The results are in conformity with the findings of **Sandhu *et al.* (1979)** who also observed that seed protein content of the mutants did not have significant alteration in any of the EMS treatment. However, in mutagenic treatment the coefficient of variation was higher compared to the control indicating the possibility of improvement in the seed protein content.

Table 4.6: Estimates of shift in mean values for different characters in lentil under different concentrations of EMS in M3 generation

Variety	Treatment	Mean±S. E	Shift in $\bar{X}$ in M3	h ² (bs) in M3	Shift in $\bar{X}$ in M2	h ² (bs) in M2
Days to 50% flowering						
HUL 57	Control	66.00 ± 3.44	--	81.40	---	99.50
	0.1% EMS	62.00 ± 3.44	-2.00		+2.99*	
	0.2% EMS	62.33 ± 3.44	-3.67		+2.66*	
	0.3% EMS	67.00 ± 3.44	-1.00		-0.01	
IPL 406	Control	68.66 ± 3.44	--		--	
	0.1% EMS	72.00 ± 3.44	+ 3.34		+4.33*	
	0.2% EMS	69.33 ± 3.44	+ 0.67		+4.33*	
	0.3%EMS	69.33 ± 3.44	+ 0.67		+1.33	
WBL 77	Control	63.00 ± 3.44	--		--	
	0.1% EMS	74.00 ± 3.44	+ 11.00*		+1.00	
	0.2% EMS	62.33 ± 3.44	-0.67		+1.67	
	0.3% EMS	53.00 ± 3.44	-10.00		+2.33*	
PusaAgeti	Control	54.66 ± 3.44	--		--	
	0.1% EMS	57.33 ± 3.44	+ 2.67		+0.66	
	0.2% EMS	53.00 ± 3.44	- 1.66		+2.66*	
	0.3% EMS	54.33 ± 3.44	- 0.33		+0.66	
Subrata	Control	53.33 ± 3.44	--		--	
	0.1% EMS	56.00 ± 3.44	+ 2.67		+1.00	
	0.2% EMS	53.66 ± 3.44	+ 0.33		+5.00*	
	0.3% EMS	56.66 ± 3.44	+ 3.33		+5.33*	
Primary branches per plant						
HUL 57	Control	2.53 ± 0.23	--	17.87	--	56.82
	0.1% EMS	2.06 ± 0.23	- 0.47		-0.17	

	0.2% EMS	2.06 ± 0.23	-0.47		+0.06		
	0.3% EMS	2.33 ± 0.23	- 0.20		+0.13		
IPL 406	Control	2.80 ± 0.23	- -		--		
	0.1% EMS	2.34 ± 0.23	- 0.46		-0.33		
	0.2% EMS	2.13 ± 0.23	-0.67		-0.40		
	0.3%EMS	2.50 ± 0.23	-0.30		-0.33		
WBL 77	Control	1.86 ± 0.23	--		--		
	0.1% EMS	2.50 ± 0.23	+ 0.64		+0.16		
	0.2% EMS	1.95 ± 0.23	+ 0.09		-0.04		
	0.3% EMS	2.00 ± 0.23	+ 0.14		+0.33		
PusaAgeti	Control	2.06 ± 0.23	--		--		
	0.1% EMS	2.37 ± 0.23	+ 0.31		-0.06		
	0.2% EMS	2.00 ± 0.23	- 0.06		+0.40		
	0.3% EMS	2.06 ± 0.23	0.00		+0.14		
Subrata	Control	2.06 ± 0.23	--		--		
	0.1% EMS	2.80 ± 0.23	+ 0.74*		-0.26		
	0.2% EMS	2.39 ± 0.23	+ 0.33	+0.27			
	0.3% EMS	2.22 ± 0.23	+ 0.16	+0.47			
Secondary branches per plant							
HUL 57	Control	13.40 ± 2.07	--	48.59	--	81.37	
	0.1% EMS	15.17 ± 2.07	+ 1.77		-0.63		
	0.2% EMS	16.51 ± 2.07	+ 3.11		-0.87		
	0.3% EMS	16.78 ± 2.07	+ 3.38		-0.13		
	Control	12.86 ± 2.07	--		--		

IPL 406	0.1% EMS	14.76 ± 2.07	+ 1.90	52.52	+ 1.87	85.49
	0.2% EMS	12.97 ± 2.07	+ 0.11		+ 1.07	
	0.3%EMS	11.35 ± 2.07	- 1.51		+ 0.20	
WBL 77	Control	13.46 ± 2.07	--		--	
	0.1% EMS	13.82 ± 2.07	+ 0.36		+ 1.66	
	0.2% EMS	16.26 ± 2.07	+ 2.80		-0.77	
	0.3% EMS	17.26 ± 2.07	+ 3.80		1.36	
PusaAgeti	Control	17.46 ± 2.07	--		--	
	0.1% EMS	15.57 ± 2.07	- 1.89		-1.07	
	0.2% EMS	12.02 ± 2.07	- 5.44		+ 1.73	
	0.3% EMS	9.98 ± 2.07	- 7.48		+ 1.40	
Subrata	Control	19.20 ± 2.07	--		--	
	0.1% EMS	18.97 ± 2.07	- 0.23		+0.80	
	0.2% EMS	17.62 ± 2.07	- 1.58		+ 2.07*	
	0.3% EMS	17.30 ± 2.07	- 1.90		+ 3.07*	
Number of pods per plant						
HUL 57	Control	25.63 ± 3.27	--	52.52	--	85.49
	0.1% EMS	35.53 ± 3.27	+ 9.30*		+ 34.12*	
	0.2% EMS	27.40 ± 3.27	+ 1.77		+ 6.34*	
	0.3% EMS	38.88 ± 3.27	+ 13.25*		+1.07	
IPL 406	Control	35.53 ± 3.27	--		-	
	0.1% EMS	42.53 ± 3.27	+ 7.00		+ 37.95*	
	0.2% EMS	42.16 ± 3.27	+ 6.63		+ 32.67*	
	0.3%EMS	39.92 ± 3.27	+ 4.39		+ 15.42*	
WBL 77	Control	26.90 ± 3.27	--		--	
	0.1% EMS	34.44 ± 3.27	+ 7.54		+ 18.5*	
	0.2% EMS	22.66 ± 3.27	- 4.24		-0.9	

	0.3% EMS	40.73 ± 3.27	+ 13.83*		+2.76*	
PusaAgeti	Control	38.76 ± 3.27	--		--	
	0.1% EMS	38.46 ± 3.27	- 0.30		-11.58	
	0.2% EMS	34.50 ± 3.27	- 4.26		+7.21*	
	0.3% EMS	38.73 ± 3.27	- 0.03		-4.44	
Subrata	Control	41.40 ± 3.27	--		--	
	0.1% EMS	28.66 ± 3.27	- 12.74		+32.28*	
	0.2% EMS	28.03 ± 3.27	- 13.37		+51.44*	
	0.3% EMS	37.86 ± 3.27	- 3.54		+16.09*	
Pod weight per plant						
HUL 57	Control	1.49 ± 0.35	--		--	
	0.1% EMS	2.69 ± 0.35	+ 1.20*			
	0.2% EMS	1.62 ± 0.35	+ 0.13			
	0.3% EMS	1.43 ± 0.35	- 0.06			
IPL 406	Control	1.69 ± 0.35	--			
	0.1% EMS	1.53 ± 0.35	- 0.16			
	0.2% EMS	1.13 ± 0.35	- 0.56			
	0.3%EMS	2.56 ± 0.35	+ 0.87			
WBL 77	Control	2.70 ± 0.35	--			
	0.1% EMS	1.07 ± 0.35	- 1.63			
	0.2% EMS	1.13 ± 0.35	- 1.57			
	0.3% EMS	2.62 ± 0.35	- 0.08			
PusaAgeti	Control	1.51 ± 0.35	--			
	0.1% EMS	2.35 ± 0.35	+ 0.84			
	0.2% EMS	2.56 ± 0.35	+ 1.05*			
	0.3% EMS	2.31 ± 0.35	+ 0.80			
	Control	1.81 ± 0.35	--			

Subrata	0.1% EMS	2.37 ± 0.35	+ 0.56			
	0.2% EMS	2.80 ± 0.35	+ 0.99			
	0.3% EMS	3.00 ± 0.35	+ 1.19*			
Number of seeds per pod						
HUL 57	Control	1.50 ± 0.07	--	66.84	--	73.63
	0.1% EMS	1.50 ± 0.07	0.00		-0.1	
	0.2% EMS	1.73 ± 0.07	+ 0.23*		-0.22	
	0.3% EMS	1.63 ± 0.07	+ 0.13		-0.33	
IPL 406	Control	1.86 ± 0.07	--		--	
	0.1% EMS	1.73 ± 0.07	- 0.13		-0.33	
	0.2% EMS	1.86 ± 0.07	0.00		-0.36	
	0.3%EMS	1.73 ± 0.07	- 0.13		-0.60	
WBL 77	Control	1.73 ± 0.07	--		--	
	0.1% EMS	1.86 ± 0.07	+ 0.13		-0.37	
	0.2% EMS	1.93 ± 0.07	+ 0.20		-0.47	
	0.3% EMS	1.86 ± 0.07	+ 0.13		-0.60	
PusaAgeti	Control	1.63 ± 0.07	--		--	
	0.1% EMS	1.86 ± 0.07	+ 0.23*		-0.17	
	0.2% EMS	1.66 ± 0.07	+ 0.03		-0.23	
	0.3% EMS	1.73 ± 0.07	+ 0.10		-0.30	
Subrata	Control	1.93 ± 0.07	--	--		
	0.1% EMS	1.86 ± 0.07	- 0.07	-0.03		
	0.2% EMS	1.66 ± 0.07	- 0.27	-0.10		
	0.3% EMS	1.86 ± 0.07	- 0.07	-0.30		
Plant height						
HUL 57	Control	40.73 ± 2.76	--	80.15	--	95.35
	0.1% EMS	42.33 ± 2.76	+ 1.60		-2.42	

	0.2% EMS	40.90 ± 2.76	+ 0.17		-0.41	
	0.3% EMS	39.23 ± 2.76	- 1.50		-1.87	
IPL 406	Control	42.86 ± 2.76	--	66.87	--	99.12
	0.1% EMS	32.60 ± 2.76	- 10.26		-1.32	
	0.2% EMS	37.20 ± 2.76	- 5.66		-1.57	
	0.3%EMS	41.26 ± 2.76	- 1.60		-3.17	
WBL 77	Control	40.33 ± 2.76	--		--	
	0.1% EMS	38.46 ± 2.76	- 1.87		-1.97	
	0.2% EMS	42.20 ± 2.76	+ 1.87		-2.51	
	0.3% EMS	46.26 ± 2.76	+ 5.93		-6.74	
PusaAgeti	Control	31.33 ± 2.76	--		--	
	0.1% EMS	29.60 ± 2.76	- 1.73		-0.7	
	0.2% EMS	28.26 ± 2.76	- 3.07		-1.44	
	0.3% EMS	29.53 ± 2.76	- 1.80		+1.16	
Subrata	Control	42.26 ± 2.76	--		--	
	0.1% EMS	39.06 ± 2.76	- 3.20		-2.89	
	0.2% EMS	40.20 ± 2.76	- 2.06		-4.67	
	0.3% EMS	42.53 ± 2.76	+ 0.07		-2.65	
Days to 90% maturity						
HUL 57	Control	120.66 ± 3.63	--	66.87	--	99.12
	0.1% EMS	112.66 ± 3.63	- 8.00*		+2.00	
	0.2% EMS	116.66 ± 3.63	- 4.00		+ 5.00*	
	0.3% EMS	122.66 ± 3.63	+ 2.00		+ 4.34*	
IPL 406	Control	121.00 ± 3.63	--		--	
	0.1% EMS	118.66 ± 3.63	- 2.34		+2.00	
	0.2% EMS	124.00 ± 3.63	+ 3.00		+1.33	
	0.3%EMS	128.33 ± 3.63	+ 7.33		+ 3.33*	

WBL 77	Control	114.00 ± 3.63	--		--	
	0.1% EMS	127.00 ± 3.63	+ 13.00*		+0.33	
	0.2% EMS	119.00 ± 3.63	+ 5.00		+ 4.00*	
	0.3% EMS	110.33 ± 3.63	- 3.67		+ 2.33*	
PusaAgeti	Control	106.66 ± 3.63	--		--	
	0.1% EMS	117.66 ± 3.63	+ 11.00*		+1.67	
	0.2% EMS	116.00 ± 3.63	+ 9.34		+ 5.00*	
	0.3% EMS	111.58 ± 3.63	+ 4.92		+0.67	
Subrata	Control	113.33 ± 3.63	--		--	
	0.1% EMS	109.33 ± 3.63	- 4.00		+ 4.00*	
	0.2% EMS	110.33 ± 3.63	- 3.00		+ 8.67*	
	0.3% EMS	109.33 ± 3.63	- 4.00		+ 6.00*	
1000 seed weight						
HUL 57	Control	11.66 ± 1.77	--	47.07	--	81.89
	0.1% EMS	15.63 ± 1.77	+ 3.97		-3.74	
	0.2% EMS	19.33 ± 1.77	+ 7.67*		-4.84	
	0.3% EMS	21.60 ± 1.77	+ 9.94*		-5.47	
IPL 406	Control	19.66 ± 1.77	--		--	
	0.1% EMS	20.33 ± 1.77	+ 0.67		-2.74	
	0.2% EMS	23.00 ± 1.77	+ 3.34		-3.2	
	0.3%EMS	23.33 ± 1.77	+ 3.67		-4.4	
WBL 77	Control	13.33 ± 1.77	--		--	
	0.1% EMS	14.66 ± 1.77	+ 1.33		-0.77	
	0.2% EMS	16.63 ± 1.77	+ 3.30		-0.8	
	0.3% EMS	17.60 ± 1.77	+ 4.27		-2.4	
PusaAgeti	Control	12.11 ± 1.77	--		--	
	0.1% EMS	11.33 ± 1.77	- 0.78		+ 2.3*	

	0.2% EMS	11.33 ± 1.77	- 0.78		+4.07*	
	0.3% EMS	10.60 ± 1.77	- 1.51		+3.3*	
Subrata	Control	18.10 ± 1.77	--		--	
	0.1% EMS	16.33 ± 1.77	- 1.77	-1.84		
	0.2% EMS	19.50 ± 1.77	+ 1.40	-1.97		
	0.3% EMS	18.33 ± 1.77	+ 0.23	-2.47		
Yield per plant						
HUL 57	Control	0.99 ± 0.36	--	58.37	--	
	0.1% EMS	2.44 ± 0.36	+ 1.45*			
	0.2% EMS	1.20 ± 0.36	+ 0.21			
	0.3% EMS	1.49 ± 0.36	+ 0.50			
IPL 406	Control	1.59 ± 0.36	--			
	0.1% EMS	0.90 ± 0.36	- 0.69			
	0.2% EMS	2.37 ± 0.36	+ 0.78			
	0.3%EMS	2.34 ± 0.36	+ 0.75			
WBL 77	Control	1.01 ± 0.36	--			
	0.1% EMS	0.59 ± 0.36	- 0.42			
	0.2% EMS	0.74 ± 0.36	- 0.27			
	0.3% EMS	2.25 ± 0.36	+ 1.24*			
PusaAgeti	Control	1.43 ± 0.36	--			
	0.1% EMS	1.65 ± 0.36	+ 0.22			
	0.2% EMS	2.51 ± 0.36	+ 1.08*			
	0.3% EMS	2.56 ± 0.36	+ 1.13*			
Subrata	Control	2.05 ± 0.36	--			
	0.1% EMS	1.58 ± 0.36	- 0.47			
	0.2% EMS	2.60± 0.36	+ 0.55			
	0.3% EMS	2.48 ± 0.36	+ 0.43			

Cooking time						
HUL 57	Control	25.00 ± 2.73	--	29.01	--	
	0.1% EMS	32.66 ± 2.73	+ 7.66			
	0.2% EMS	21.66 ± 2.73	- 3.34			
	0.3% EMS	31.66 ± 2.73	+ 6.66			
IPL 406	Control	36.66 ± 2.73	--			
	0.1% EMS	31.66 ± 2.73	- 5.00			
	0.2% EMS	28.33 ± 2.73	- 8.33			
	0.3%EMS	33.33 ± 2.73	- 3.33			
WBL 77	Control	28.33 ± 2.73	--			
	0.1% EMS	33.33 ± 2.73	+ 5.00			
	0.2% EMS	26.66 ± 2.73	- 1.67			
	0.3% EMS	21.66 ± 2.73	- 6.67			
PusaAgeti	Control	26.66 ± 2.73	--			
	0.1% EMS	26.66 ± 2.73	0.00			
	0.2% EMS	28.33 ± 2.73	+ 1.67			
	0.3% EMS	38.33 ± 2.73	+ 11.67*			
Subrata	Control	26.66 ± 2.73	--			
	0.1% EMS	28.33 ± 2.73	+ 1.67			
	0.2% EMS	30.00 ± 2.73	+ 3.34			
	0.3% EMS	31.66 ± 2.73	+ 5.00			
Seed Protein content						
HUL 57	Control	27.35 ± 1.41	--	41.49	--	
	0.1% EMS	26.18 ±1.41	- 1.17		+0.79	
	0.2% EMS	28.12 ±1.41	+ 0.83		+1.15*	
	0.3% EMS	23.80 ±1.41	- 3.55		+1.48*	
	Control	27.62±1.41	--		--	

IPL 406	0.1% EMS	28.19 ±1.41	+ 0.57		-0.53	
	0.2% EMS	27.02 ±1.41	- 0.60		-0.94	
	0.3%EMS	27.62 ±1.41	0.00		+0.40	
WBL 77	Control	30.13±1.41	--		--	
	0.1% EMS	26.65 ±1.41	- 3.48		+0.33	
	0.2% EMS	26.33 ±1.41	- 3.80		+0.57	
	0.3% EMS	19.96 ±1.41	- 10.17		+0.22	
PusaAgeti	Control	26.73 ±1.41	--		--	
	0.1% EMS	28.15±1.41	+ 1.42		+1.27*	
	0.2% EMS	24.63 ±1.41	- 2.10		+1.09	
	0.3% EMS	25.96 ±1.41	- 0.78		-0.24	
Subrata	Control	25.15 ±1.41	--		--	
	0.1% EMS	24.34 ±1.41	- 0.81		+0.41	
	0.2% EMS	25.57 ±1.41	+ 0.42		+0.63	
	0.3% EMS	24.80 ±1.41	- 0.35		+0.05	

SUMMARY & CONCLUSION

Summary and Conclusion

Pulses are considered to be a good source of protein, dietary fibre and essential minerals and thus can be used as a crop for eradicating the world's most serious malnutrition problem of protein deficiency, particularly among the children in the developing countries.

Lentil (*Lens culinaris Medikus subsp. culinaris*) is one of the first crops domesticated can be a good source being rich in protein, minerals, and vitamins. Moreover, its capacity to store carbon and nitrogen in turn enhances the nutrient status of the soil thereby ensuring the sustainability of the production systems. Despite considerable improvement so far made in lentil through conventional breeding approaches, productivity of the crop has been far from satisfactory. The narrow genetic base of the crop germplasm leading to repeated use of the same genotype in crop breeding program has been identified to be the major reason. Besides the incidence of diseases non-availability of short duration varieties in areas having shortage of water is also considered to be another major constraint in enhancing the productivity of the crop in the desired direction. Hence, need of the hour is to broaden the genetic base of the crop so that the diverse genotype can be effectively utilised in furthering the cause of crop improvement. Lentil being principally raised under poor conditions of growth with very little fertilizers and inputs liker water the genotypes might responding to inputs like fertilisers and irrigation might have been lost thereby making it difficult to develop improved genotypes using such limited germplasms available. Thus, artificial means of generating the desired variability need to be resorted to and mutation breeding in this regard is considered to be one of the most important possible approaches to broaden the genetic bases in lentil under such conditions. Artificial induction of mutation eliminates the necessity to wait for occasional mutation and thereby opens up new possibility like introduction of new characters to a species, breaking the undesirable linkage between the desirable and undesirable characters and finally increasing the variability present in the population for further selection to happen.

With this background information an experiment was undertaken in the instructional farm of Uttar Banga Krishi Vishwavidyalaya during 2021-22 in M3 generation with

five varieties of lentil, raised through the treatment with three different concentrations of EMS (Ethyl Methane Sulphonate) with the following objectives:

1. To study the genetic variability in different characters in lentil under induction of mutation in M3 generation.
2. To study the frequency and spectrum of mutations for different characters in lentil in M3 generation.

The present investigation was conducted during the rabi season of 2021-22, with seeds of M2 generation in five (5) varieties of lentil to evaluate the performance of the varieties treated with different concentrations of Ethyl Methane Sulphonate (EMS) in M3 generation. The genotypes (varieties) were chosen on the basis of their adaptability in the study area encompassing the northern districts of West Bengal. For studying the mutagenic effect, the M2 seeds (earlier treated with three concentrations of EMS, viz., 0.1%, 0.2% and 0.3% respectively) and that of untreated seeds as control were sown in a Factorial Randomized Block Design (FRBD) with three replications. Five plants were chosen at random in each of the replications in the M3 generation for recording the observations on morphological characters. Observations were recorded for days to 50% flowering, primary branches per plant, secondary branches per plant, number of pods per plant, pod weight per plant, number of seeds per pod, plant height at maturity, days to 90% maturity, 1000 seed weight, yield per plant, cooking time and seed protein content. The results of the experiment have been presented under the following heads, viz.,

1. Performance of Lentil varieties in respect of different morphological characters under different concentrations of EMS in M3 generation.
2. Analysis of variance for different characters in lentil variety in M3 generation.
3. Studies on genetic parameter in respect of different morphological characters in lentil varieties under different concentration of EMS in M3 generation.
4. Genotypic correlation between various quantitative characters in lentil varieties under different concentrations of EMS in M3 generation.
5. Genotypic path for various quantitative characters in lentil varieties under different concentrations of EMS in M3 generation.
6. Studies on the shift in mean values of different characters in lentil varieties in M3 generation.

The analysis of variance revealed significant to highly significant variation among the varieties for most characters in M3 generation except primary branches per plant. Among the treatments number of pods per plant, pod weight per plant, yield per plant, cooking time and seed protein content showed highly significant differences while number of pods per plant, pod weight per plant, number of seeds per pod, days to 90% maturity, yield per plant, cooking time and seed protein content were found to exhibit highly significant variation in genotype versus treatment interaction. Days to 50% flowering and plant height at maturity exhibited high heritability along with high genetic advance as percentage of mean, thereby indicating the high genotypic influence on the characters in the generation itself. Pod weight per plant had shown a significant and positive correlation (0.669) with yield while days to 50 % flowering had a significant but negative correlation (-0.578) with yield. A low residual effect of 0.0247 in the genotypic path analysis was indicative of the fact that the resultant variability under induction of mutation was primarily accounted for by the genotypic differences and the traits under study had contributed greatly towards variability in grain yield of lentil.

A significant shift in mean value in the positive direction was observed in WBL 77 at 0.1% EMS for days to 50% flowering and the same was found to be much higher when compared to the same in M2 generation. Secondary branches per plant registered a positive shift in mean values in variety HUL 57 in all the concentration, though not significant. Pods per plant had recorded a positive and significant shift in mean values in the variety HUL 57 both at 0.1% and 0.3% EMS respectively though the magnitude of shift was less at 0.1% concentration of EMS and more at 0.3% EMS concentration when compared to the same in M2 generation. A similar trend was observed in the variety WBL 77 in 0.3% EMS where a significant positive shift was observed in M3 generation and was found to be higher than that in M2 generation. As far as pod weight per plant was concerned, significant shift in mean values in the positive direction was observed in the variety HUL 57 at 0.1% EMS, in PusaAgeti at 0.2% EMS and at 0.3% EMS in the variety Subrata respectively. The moderate heritability of the character in the generation warranted further studies in coming generation to establish its relationship to the effect of mutagen as far as the generation of variability was concerned. Though the number of seeds per pod registered a positive and significant shift in mean values in the variety HUL 57 at 0.2% EMS and in variety PusaAgeti at

0.1% EMS in M3 generation, no such significant shift in mean values was observed in any of the varieties and in any of the concentration for plant height at maturity. A positive and significant shift in mean was observed for days to maturity only in case of WBL 77 and Pusa Ageti at the same concentration, i.e., 0.1% EMS while in Pusa Ageti though positive shift in the remaining concentrations as in IPL 406 and HUL 57 at 0.3% EMS was observed but the shift was not significant. In case of 1000 seed weight significant and positive shift was observed for the character at 0.2% and 0.3% EMS in M3 generation in the variety HUL 57 as against negative shift observed in M2 generation at the same concentration. A similar trend was observed in variety IPL 406 and WBL 77 in all the concentrations where a positive shift, though not significant, in mean values was observed in M3 generation though the same recorded a negative shift in M2 generation. The mutagen had significant effect in positive direction in yield per plant as was observed in the varieties HUL 57 at 0.1 % EMS, in WBL 77 at 0.3% EMS and in Pusa Ageti at 0.2 % and 0.3% EMS respectively. As in IPL 406, a positive shift in mean values was also observed in the variety Subrata for yield per plant at both the concentrations of 0.2% and 0.3% EMS similar to that in Pusa Ageti at 0.1% EMS but the said shift was found not significant. Similarly in variety HUL 57 though a positive shift in mean was recorded in rest of the concentrations but the shift was not found to be significant enough. The heritability value for the character in the generation was found to be moderate (58.37%). The mutagen had no such significant impact on seed protein content.

Hence, the characters showing moderate to high heritability with moderate to high genetic advance and with a significant shift in the positive direction could well be considered for studying the variability generated through mutagenesis.



Plate 2: Plants of lentil variety HUL 57 treated with different concentrations of EMS in M3 generation

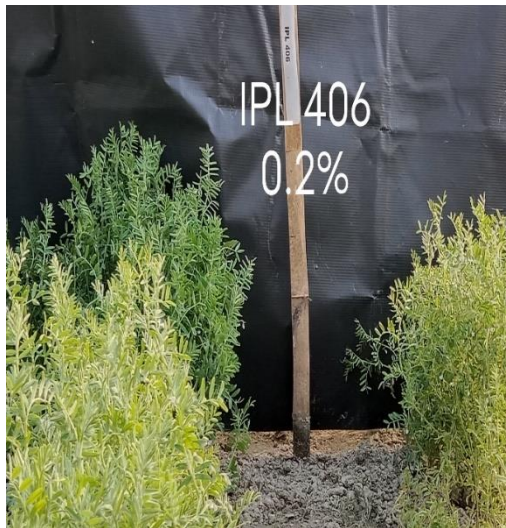
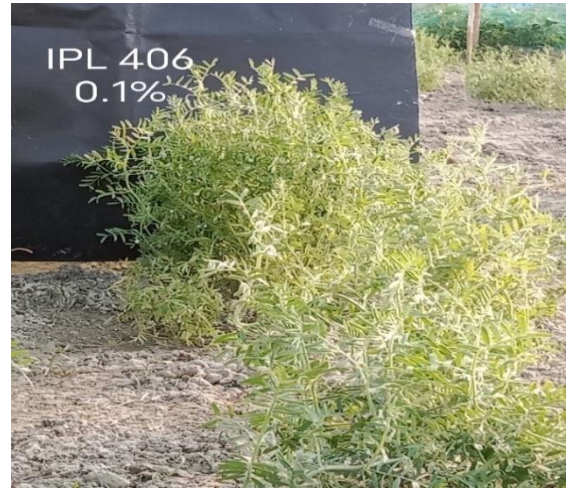


Plate 3: Plants of lentil variety IPL 406 treated with different concentrations of EMS in M3 generation



Plate 4: Plants of lentil variety WBL 77 treated with different concentrations of EMS in M3 generation

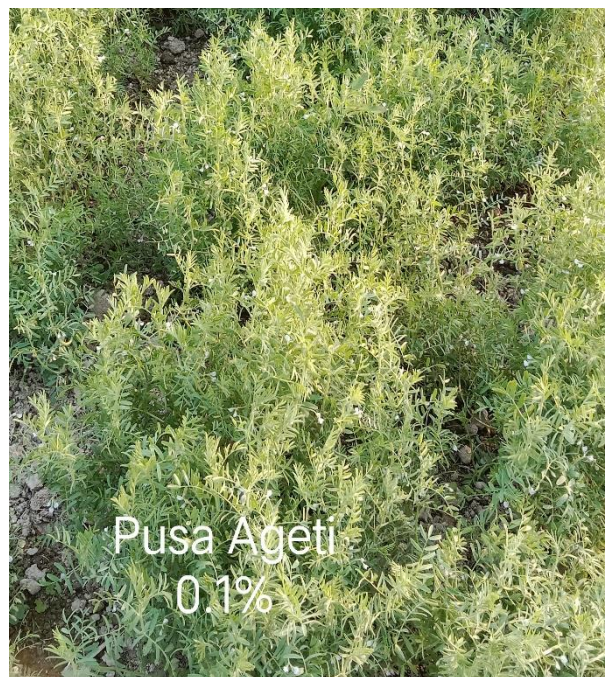


Plate 5: Plants of lentil variety Pusa Ageti treated with different concentrations of EMS in M3 generation

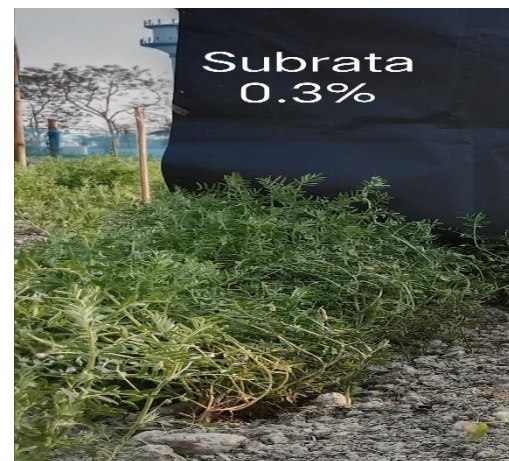


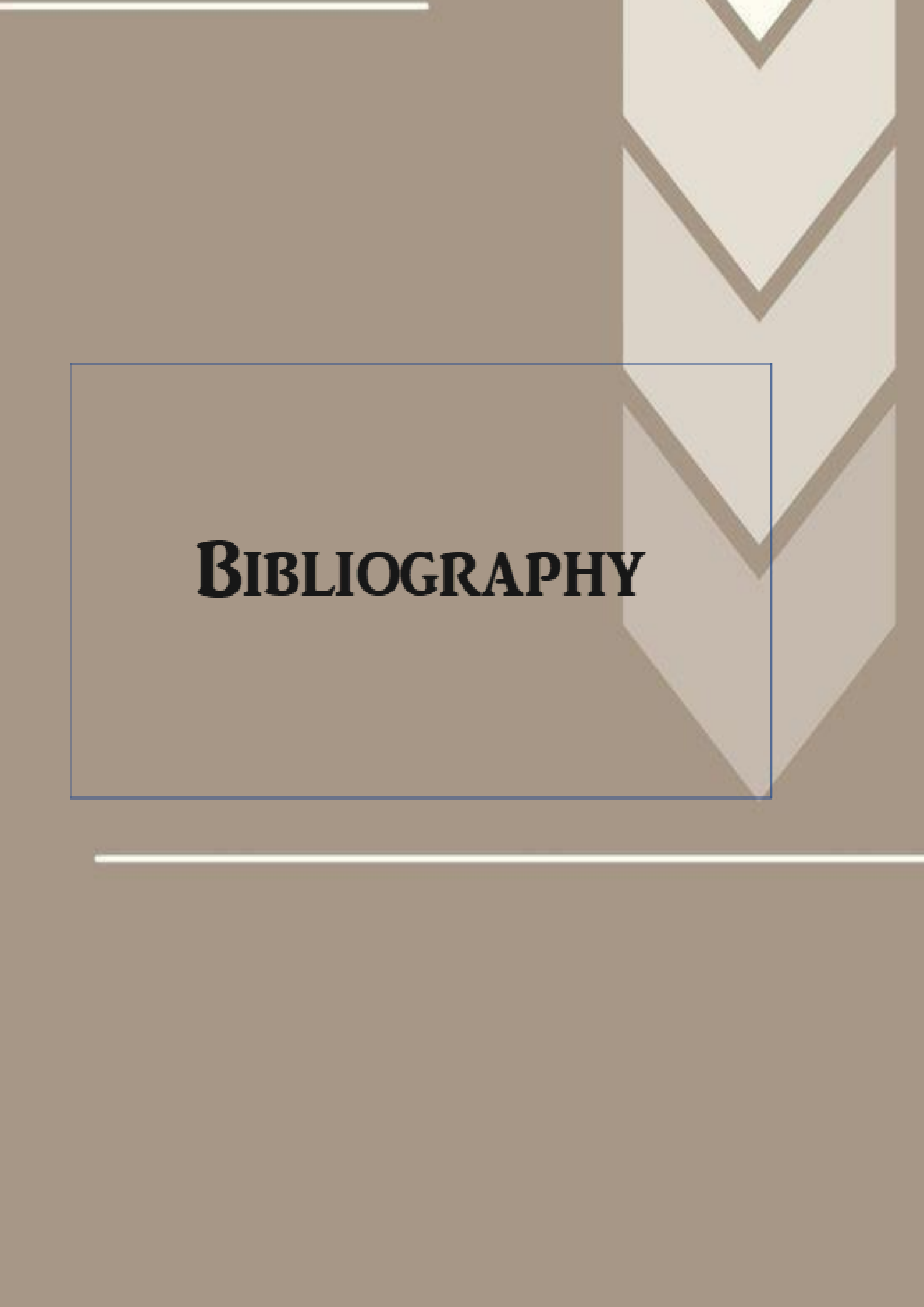
Plate 6: Plants of lentil variety Subrata treated with different concentrations of EMS in M3 generation



FUTURE SCOPE OF RESEARCH

Future Scope of Research

Variability in the crops is the prerequisite for improvement in the desired direction. Mutagenesis has always been a potential source for generating variability and thereby increasing the genetic base of the crop. The present investigation was aimed at generating variability in the positive direction so that the same may be utilized in subsequent crop improvement programme. The significant shift in mean values in the positive direction for the characters could be effectively utilised for forwarding the variability in the coming generation (M4 onwards) and can be profitably harnessed for further improvement of not only the crop concerned but also in related species.



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Annexure 1

Table: Agrometeorological data during the growing season of 2021-22

Months	Temperature		Relative Humidity		Total rainfall	Bright Sunshine hours	Average evaporation
	Max. Avg.	Min. Avg.	Max. Avg.	Min. Avg.			
November	29.00	12.00	74.00	51.00	0.00	7.00	1.99
December	27.00	12.00	79.00	51.00	1.20	6.00	1.00
January	24.00	11.00	90.00	55.00	4.00	4.00	1.00
February	24.00	11.00	82.00	51.00	47.00	5.00	2.00
March	32.00	18.00	71.00	45.00	28.00	6.00	3.00

(**Source:** Gramin Krishi Museum Seva, UBKV, Pundibari, Cooch Behar)

Annexure II

Description of Qualitative Characters in M3 generation

Character: Flower colour

Variety	Treatment			
	Control	0.1%	0.2%	0.3%
HUL 57	3	3	3	3
IPL 406	3	3	3	3
WBL 77	3	3	1	1
PusaAgeti	3	3	3	3
Subrata	3	3	3	3

White = 1 Blue = 3

Character: Plant Growth Habit

Variety	Treatment			
	Control	0.1%	0.2%	0.3%
HUL 57	3	3	3	3
IPL 406	3	3	1	3
WBL 77	1	3	3	3
PusaAgeti	3	3	3	3
Subrata	3	3	3	3

Erect = 1 Semi erect = 3 Horizontal = 5

Annexure II (Contd.)

Character: Leaflet shape

Variety	Treatment			
	Control	0.1%	0.2%	0.3%
HUL 57	5	5	5	5
IPL 406	5	5	3	5
WBL 77	3	7	5	5
PusaAgeti	5	5	5	5
Subrata	5	5	3	5

Small = 3

Medium = 5

Large= 7

Character: Seed size

Variety	Treatment			
	Control	0.1%	0.2 %	0.3 %
HUL 57	3	3	3	3
IPL 406	5	5	7	5
WBL 77	3	3	3	3
PusaAgeti	3	3	3	3
Subrata	3	3	3	3

Small = 3 Medium = 5 Large = 7 Very large =9

VITA

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For Masters' Degree

Bachelors' degree

Name of the university	Banaras Hindu University
Year of Award	2020
OGPA	8.65

Masters' degree

OGPA	8.57
Title of Master' s Thesis	Genetic studies in lentil (<i>Lens culinaris</i> Medik.) through induced mutagenesis.
Awards/ Distinctions/ Scholarships	University Merit Scholarship