

**Genetic improvement of linseed for
inducing earliness in maturity and reducing
the undesirable compounds through
mutation**

THESIS

By

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CERTIFICATE – I

This is to certify that the thesis entitled, “**Genetic improvement of linseed for inducing earliness in maturity and reducing the undesirable compounds through mutation**” submitted in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Genetics and Plant Breeding** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Arjun Kumar** son of Sh. Ganga Ram, under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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CERTIFICATE- II

This is to certify that the thesis entitled, “**Genetic improvement of linseed for inducing earliness in maturity and reducing the undesirable compounds through mutation**” submitted by **Arjun Kumar (Admission No. A-2016-40-011)** son of Sh. Ganga Ram to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Genetics and Plant Breeding** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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LIST OF ABBREVIATIONS USED

S. No.	Abbreviation	Meaning
1.	%	per cent
2.	&	And
3.	/	Per
4.	@	at the rate of
5.	a.m.s.l.	above mean sea level
6.	°C	degree Celsius
7.	conc.	Concentration
8.	Mm	milli meter
9.	Cm	centi meter
10.	DMS	dimethyl sulfate
11.	Cv	Cultivar
12.	DAS	days after sowing
13.	EMS	ethyl methane sulphonate
14.	et al.	et alii (and others)
15.	Etc.	et cetera
16.	Fig.	figure(s)
17.	G	Gram
18.	Gy	Gray
19.	H	hour(s)
20.	Ha	Hectare
21.	HZ	hydrazine hydrate
22.	<i>i.e.</i>	id est (that is)
23.	Kg	kilo gram
24.	kR	Kiloroentgen
25.	L.	Linnaeus
26.	LD	lethal dose
27.	M	meter(s)
28.	Mm	Milimetre
29.	MMS	methyl methane sulphonate
30.	NEU	n-nitroso ethyl urea
31.	NMU	n-nitroso-n-methyl urea
32.	P	page(s)
33.	pH	power of hydrogen
34.	Q	Quintal
35.	SA	sodium azide
36.	T	Tones
37.	Var.	Variety
38.	<i>viz.</i>	Namely
39.	GAM	Genetic Advance as percentage of Mean

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ABSTRACT

The present investigation entitled, “Genetic improvement of linseed for inducing earliness in maturity and reducing the undesirable compounds through mutation” was carried out in the experimental farm of Department of Genetics and Plant Breeding, COA, CSK HP KV, Palampur to assess the nature and magnitude of induced variability, determine the inheritance pattern of earliness in induced lines; determine the relative proportion of oil content of linseed mutants and isolate lines/mutants with reduced linolenic content in addition to earliness. Experimental material comprised of three linseed varieties viz. Baner, Him Alsi-2 and Surbhi, seeds of which were treated with five doses of gamma rays (20, 30, 40, 50, 60 kR) and EMS (0.2 %, 0.4 %, 0.5 %, 0.6 %, 0.8 %) to generate M₁ generation. On the basis of survival percentage in M₁ generation under lab conditions the LD₅₀ dose was estimated i.e. 96.63 kR (Baner), 72.94 kR (Him Alsi-2) and 53.22 kR (Surbhi) for gamma rays and 0.86 % (Baner), 0.72 % (Him Alsi-2) and 0.35 % (Surbhi) for EMS. Plants harvested from M₁ were carried forward to M₂ generation. Both the mutagens were found effective and efficient in producing chlorophyll mutations and viable macromutations in M₂ generation. Gamma rays and EMS also found effective in producing variability in range and mean values for different quantitative characters for which significant differences were obtained in both positive and negative directions. 113 mutants were carried forwarded to M₃ generation based on their performance in M₂ generation. Mean performance of M₃ mutants showed significant shifts from their respective control. Analysis of variance was found significant for all the characters studied. Low GCV and PCV was found for number of days to 75 per cent maturity but high GCV (21.92 %) and PCV (27.05 %) was present in mutants for seed yield per plant. High heritability (96.52) coupled with low GAM (7.00 %) was found for number of days to 75 per cent maturity showing limited scope of its improvement, while high heritability (65.68 %) coupled with high GAM (36.60 %) was found for seed yield per plant showing presence of additive type of gene action. Line M₃₋₃₋₅ showed highest oil percentage i.e. 58.44 per cent. The maximum reduction in linolenic acid percentage was found in M₃₋₁₉₋₈ (36.11 %) followed by M₃₋₁₉₋₄ (38.59 %) as compared to all the controls. Early maturing lines i.e. M₃₋₁₄₋₂ (196.20 days) and M₃₋₁₁₋₂ (196.20 days), high yielding line i.e. M₃₋₁₃₋₂ (4.58 g) along with high oil content and low linolenic acid can be further evaluated to achieve the higher edible oil production of linseed.

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1. INTRODUCTION

Linseed (*Linum usitatissimum* L., $2n=30$) is an annual *Rabi* season crop and belongs to self-pollinated and homozygous species of Linaceae family and is a sole species with agricultural importance. It is an oldest crop cultivated by man having planting history of 6,000-7,000 years (Gill 1897). Numerous wild species are present in nature but most of the investigators are of the opinion that the wild flax (*Linum angustifolium*) which is a native of Mediterranean region may be the ancestor of the cultivated species *Linum usitatissimum*. The morphological aspect shows, two geographical primary centre of origin of linseed (i) South-western Asia (comprising India, Afghanistan and Turkey) for the seed type, and (ii) Mediterranean countries (extending from Spain and Algeria to Greece and Egypt) for the fibre type with Transcaucasian regions (with Asia Minor, Caucasus, Black sea and Caspian sea areas) for the dual purpose forms (Vavilov 1926). Linseed is extensively cultivated throughout the tropical and temperate regions of the world for oil as well as fibre (Diederichsen et al. 2008). India ranks second in terms of area after Canada and third in production in the world. Globally linseed is grown in an area of 27.29 lakh hectare having yield of about 25.20 lakh tones with productivity of 923.00 kg per ha. In India, linseed is grown in an area of 3.38 lakh hectare having production and productivity of 1.52 lakh tones and 473.00 kg per hectare, respectively (Anonymous 2019). In Himachal Pradesh, it is grown in an area of 0.81 thousand hectare with production of 0.29 thousand tones having an average productivity of 350.00 kg per hectare (Anonymous 2018).

Every aerial part of the linseed plant i.e. seed and stem are utilized commercially either directly or after processing. Linseed contains good percentage of oil varying from 33 to 45 percent in different varieties and its oil is the richest plant source of omega-3 (36-57 %) and omega-6 (18-24 %) fatty acids (Choo et al. 2007; Singh et al. 2011), which are not synthesized in organisms and must be ingested in food to influence blood platelet aggregation, lower the blood cholesterol concentration and prevent coronary heart disease and cancer (Singh and Marker 2006). High level of linolenic acid imparts the property of drying oil, suitable for

manufacturing paints, varnishes, printer's ink, linoleum etc (Gill 1987). In spite of the vast area and varied utility of the crop, India has very low productivity of 473.00 kg per hectare against world average of 923.00 kg per hectare. The main reasons for low productivity of linseed in India are non availability of seed of improved varieties, cultivation on marginal and sub-marginal land under rain fed condition, coupled with biotic and abiotic stresses and poor crop management.

The linseed oil is not edible due to high percentage of linolenic acid which makes oil highly sensitive to oxidation, as a result of which drying property increases and gives a pungent flavour and rancidity. Further, the laxative properties of the mucilage in seed coat of the seed make the oil unsuitable for edible purpose. Oil has lipid peroxidation property which leads to quality deterioration in oil. Use of linseed oil in food applications/table purposes, stability is essential (Green and Marshall 1984). This stability can be achieved by isolating mutants with as low as 1 to 3 percent α -linolenic acid, a level which is considered suitable/desirable with self-stability for traditional edible oil applications (Rowland 1990).

Considering the benefits of linseed as a functional food, there is need of development of varieties which contain fatty acid profile, so that linseed can be used in daily life of modern trendy society. Conventional breeding of linseed has focused on stabilizing yield across diverse environments, enhancing oil production and quality, discovering desirable resistance to major diseases like rust, powdery mildew and wilt (Kenaschuk and Rowland 1993; Mpofu and Rashid 2001), improving lodging resistance and adopting crop phenology for regional climatic limitations. But over the generations, there has been a continuous reduction in genetic diversity as breeders have increasingly focused on so-called “elite” cultivars which limit the genetic stock of linseed that have high economic value. As a result, genetic diversity is lost through fixation, thereby increasing dependency of breeders on smaller sets of superior genotypes that has created successive bottlenecks.

Green and Marshall (1984) concluded that hybridization and selection within the available germplasm would be unlikely to achieve the required reduction in linolenic acid content and further suggested that induced mutation in plants has substantial potential to widen the spectrum of the available traits in linseed, including

further modification of fatty acid profiles and to reduce its anti nutritional components. Therefore to overcome such bottlenecks, mutation breeding can be adopted because usefulness and effective potential of the induced mutation played a significant role in meeting challenges related to world's food and nutritional security. Mutation breeding is a successful tool in bringing amelioration in various fields of studies i.e. morphology, cytogenetics, biotechnology and molecular biology. It is an efficient mean of supplementing existing germplasm enhancement and their utilization for the development of new mutant varieties by recovering superior mutant plant types having high seed yield, earliness besides high oil content.

Mutations are sudden and heritable changes in the genetic sequence and are a main cause of diversity among organisms. These changes occur at many numerous levels and can have widely differing consequences. Physical and chemical mutagens have been used to accelerate mutation rate (Muller 1927) besides inducing useful phenotypic variations in plants (Vasline et al. 2005). Mutagens may cause genetic changes, break the linkages and can produce many new promising traits for the genetic improvement of crops (Shah et al. 2008). The choice of mutagen and its different doses is one of the important steps for creating new variability in the population which is the need of time for crop improvement. In plants, EMS usually causes point mutations whereas gamma rays are known to influence plant growth and development by bringing amelioration in cytological, genetical, biochemical, physiological and morphogenetic characters of cells and tissue (Gunckel and Sparrow 1961). The physical and chemical mutagens have been employed successfully in genetic improvement of many crops i.e. Linseed (Bachyais 1978; Green 1986; Green and Marshall 1984; Deka 2016; Jahan et al. 2019), Sesame (Kumari et al. 2016), Mustard (Julia et al. 2018), Cowpea (Giriraja and Dhanavel 2013; Bind et al. 2016), Peas (Dhulgande et al. 2011). These mutagens provides powerful tool for creation of new variation in crop plants for both qualitative and quantitative traits (Das and Misra 2005; Baisakh et al. 2011).

Considering the importance of linseed (*Linum usitatissimum* L.) as well as proven mutagenicity of chemical and physical mutagen, the present research work entitled “**Genetic improvement of linseed for inducing earliness in maturity and**

reducing the undesirable compounds through mutation” was initiated in linseed with the following objectives:

To,

- assess the nature and magnitude of induced variability,
- determine the inheritance pattern of earliness in induced lines,
- determine the relative proportion of oil content of linseed mutants,
- isolate lines/mutants with reduced linolenic acid content in addition to earliness

2. REVIEW OF LITERATURE

Essence of genetic variability present in the germplasm/base population becomes the main source of success of breeding programme. Since the essence lies within the genotypes, deciphering such potential needs remarkable genetic tools. Hence, in crop improvement program, mutation breeding provides new genetic variability in a shortest possible period of time as compared to hybridization and selection. Keeping in view, the relevant literature pertaining to the different aspects of the present research work entitled **“Genetic improvement of linseed for inducing earliness in maturity and reducing the undesirable compounds through mutation”** has been reviewed under the following heads:

2.1 Induced mutation for improvement of linseed

2.2 Induced chlorophyll mutations

2.3 Induced morphological mutations

2.4 Induced mutagenic effectiveness and efficiency

2.5 Induced variation for high yield and its related components

2.6 Induced mutation for oil content and fatty acid profile

2.1 Induced mutation for improvement of linseed

A clear understanding on the causes of mutations was limited till 1927. Despite many abortive and failed attempts, induced artificial mutations could not be assessed successfully due to non availability of sensitive detection techniques as well as incomplete knowledge of various effective mutagens. X-rays induced genetic variants in *Drosophila melanogaster* are indistinguishable from naturally occurring ones were demonstrated by Muller (1927). Later the attempts in plants were made by Stadler (1928) in barley and maize, and Goodspeed (1929) in *Nicotiana*.

Stadler (1928) expressed his doubt about the usefulness of genetic changes induced by X-rays and performed a large scale, planned, and carefully executed experiment with cereals and found some disappointing results, therefore he expressed

his doubt on this new field of plant breeding. Brock (1970) considered that induced mutation is an alternative to naturally occurring variations as the source of germplasm for plant improvement and as an alternative to hybridization and recombination in plant breeding. Induced mutation is one of the best methods to evolve new cultivars by producing variability at gene level. Kaul and Nirmala (1999) reported 2832 cultivars, improved through induced mutagenesis in different crops.

Currently, large numbers of known chemical compounds are able to induce mutations in both prokaryotic and eukaryotic cells. Despite the large number of mutagenic compounds, only a small number has been tested in plants. Among them, there is only a very restricted group of alkylating agents viz. sodium azide (SA) and ethyl methane sulfonate (EMS) which produce different side effects on the genetic structure of treated populations. Among different chemical mutagens, ethyl methane sulphonate is reported as the most efficient, effective and powerful mutagen for generating variations (Minocha and Arnason 1962). In plants, EMS usually causes point mutations but loss of a chromosome segment or deletion can also occur (Okagaki et al. 1991).

The first known case of linseed mutations were reported as early as 1922 by Tammes. Since then, many attempts have been made by many investigators to induce mutations in linseed using both physical mutagens (Petrey 1928; Zankevich and Brunst 1937; Levan 1944; Sparrow and Gunckel 1956; Klockov 1966; Srinivasachar et al. 1972; George and Nayar 1973; Rai and Das 1975a and 1976b; Sinha et al. 1980; Green 1986) and chemical mutagens (Marki and Bianu 1969; Bachyalis 1979; Green and Marshall 1984; Green 1986; Nichterlein 1987; Rowland and Batty 1990; Rowland 1991; Dribnenki and Green 1995; Rowland et al. 2003). These investigators have successfully developed new mutant types in many crops having economic desired characters. Different types of mutations have been observed in linseed which included chlorophyll mutations, mutation for superior height, seed weight, seed oil content, iodine value, desired composition of seed oil, disease resistance, early flowering and maturity etc. Seetharam and Srinivasachar (1970) developed mutants of phylogenetic interest in peninsular type of Indian linseed.

2.2 Induced chlorophyll mutations

Levan (1944) treated three diploid and tetraploid varieties of flax i.e. Herkules, Concurrent and Palermo with three different doses of X-rays and obtained three different types of chlorophyll mutants i.e. virescens, chlorina and xantha. Among 294 mutants, 17 mutants showed virescens, 21 showed chlorina, and three mutants showed xantha.

Marki and Bianu (1969) treated dry seeds of two homozygous flax varieties viz. fibre variety Concurrent and oil variety Raja with EMS, NMU and TEM and recorded total of 296 progenies showing different chlorophyll mutations, mostly viridis, followed by xantha and albina. The treatment with EMS and NMU induced maximum mutation frequency, whereas that treatment of TEM showed extremely low frequency, no different from the spontaneous frequency was observed in Raja.

Bianu and Marki (1970) observed that radio sensitivity was more in oil variety Raja than the fibre variety Concurrent. Further observed that both varieties were more sensitive to ethyl methane sulphonate and N-nitrosomethylurea as compared to gamma rays. More albina type, fewer xantha and viridis type chlorophyll mutants were observed in the gamma rays treated mutant populations than among those treated with EMS and NMU. Segregation ratios in the M_2 characteristic of recessive monogenic mutations were observed in 12.5 per cent of the albina mutants but only 5.1 per cent was observed in the viridis. Five out of 56 viridis mutants showed a 3: 1 segregation indicating semi-dominance mode of inheritance.

Beard (1971) irradiated seven varieties of flax with 30 kR of high energy X-rays. The chlorophyll frequency obtained on the basis of basal branch progenies were found 0.32×10^{-2} and 0.02×10^{-2} for irradiated and control population respectively, with six mutations in 24,816 branch progenies and 151 mutations in 47,614 branch progenies respectively. The spectrum of chlorophyll mutations consisted of six classes i.e. light-green, yellow-green, yellow virescent, yellow, albino virescent and albino.

The Sagnard et al. (1996) treated seeds of linseed variety 'Antares' with ethyl methane sulphonate (EMS) at the concentration of 0.5 per cent to obtain nuclear recessive albina mutants. Albina mutants were observed in M_2 generation and

observed progeny from albina mutants showed that among four of these mutants, albinism character were found to be encoded by recessive nuclear gene.

Sheeba et al. (2004) studied effectiveness and efficiency of gamma rays and EMS in relation to chlorophyll mutations on two varieties of sesame viz. SVPR 1 and CO 1. Observation of four types of chlorophyll mutants was recorded namely xantha, chlorina, striata and xantha viridis. Among the four chlorophyll mutants, xantha viridis was found to be the maximum followed by chlorina and striata in gamma ray irradiated population in both the varieties. Efficiency and effectiveness of gamma rays was found more than EMS in both the varieties whereas the effectiveness and efficiency of both the mutagens was more in SVPR 1 than CO 1.

Shah et al. (2006) conducted an experiment to study the frequency and spectrum of chlorophyll mutations induced by physical (gamma rays) and chemical (EMS) mutagens on two desi (Pb2000, C44), one each of kabuli (Pb1) and desi x kabuli introgression line (CH 40/91) of chickpea (*Cicer arietinum* L.). The treatments included two doses each of gamma irradiation and concentrations of EMS. The overall frequencies and spectrum of four types of induced chlorophyll mutants were viridis (9.01 %), followed by xantha (8.61 %), chlorina (5.82 %), albina (0.5 %) and others (0.26 %). EMS treatments were found to be more efficient and effective than gamma rays in all the varieties.

Girija and Dhanewal (2013) observed spectrum and frequency of chlorophyll mutations in M_2 generation of cowpea variety CO7 by treating seeds with gamma rays, EMS and their combinations. The individual doses of EMS were found more efficient than gamma rays to induce chlorophyll mutants. A progressive increase in mutation frequency of chlorophyll mutations was observed with increased doses of EMS and gamma rays. Different types of chlorophyll mutants such as albina, xantha and viridis were found at different doses of both the mutagens. The highest frequency (2.41 %) of chlorophyll mutations was reported in EMS.

Gandhi et al. (2014) studied phenotypic responses in M_1 and M_2 generations of treated seeds of mungbean variety Vamban at different doses of gamma rays and ethyl methane. The spectrum of chlorophyll mutations were worked out which

revealed four types of chlorophyll mutations i.e. albino, xantha, chlorine and viridis. The mutation frequency increased with increase in dose/concentration of mutagen.

Patil and Rane (2015) studied spectrum and frequency of chlorophyll mutations in cluster bean var. NCB-12 at different doses i.e. 10, 20, 30, 40 and 50 kR of gamma rays. The highest chlorophyll frequency 4.22 per cent was found at 50 kR treatment while lowest frequency of 1.87 per cent was found at 10 kR dose of gamma rays. The spectrum of chlorophyll mutations showed four types of chlorophyll mutants namely chlorina, xantha, albino and striata. Out of these mutants, chlorina mutant (5.72 %) showed the maximum frequency whereas albina mutant (3.06 %) showed minimum frequency.

Bind et al. (2016) conducted an experiment using combination of gamma rays and EMS, and obtained spectrum and frequency of chlorophyll mutations in M₂ generation of EMS treated cowpea variety Pusa Komal. Four different types of induced chlorophyll mutants such as albino, xantha, chlorina and viridis were observed. A progressive increase in mutation frequency of chlorophyll mutations was observed with increased doses of mutagens. The highest frequency of chlorophyll mutants (2.00 %) was found at dose of 200 Gye + 0.2 per cent EMS. The individual treatment of EMS was found more efficient than gamma rays to induce chlorophyll mutants.

2.3 Induced morphological mutation

Sarov (1968) studied mutagenesis in flax varieties Svetoc, T10 and P255 treated with different mutagens i.e. *N*-nitrosoethylurea (NEU), *N*-nitro-somethylurea (NMU) and dimethyl sulfate (DMS), NEU and NMU which produced dominant and recessive mutations in various plant characters such as plant height, leaf colour, seed colour, pod size, seed size and duration of the vegetative period. DMS gave a narrower spectrum of mutations.

George and Nayar (1973) treated seeds of a high yielding variety of linseed, Neelum with four doses of gamma-rays and obtained comparatively short height plants along with sparsely arranged narrow leaves as compared to control. Early dwarf mutants which matured 30 days earlier as compared to the parents were also found in treated population.

Rai and Das (1975) studied mutants in different varieties of linseed after irradiation with different doses of gamma rays. An overall increase in mutation frequency was observed with an increase in dose of irradiation. In all the mutants, days to flowering, days to maturity, number of non-bearing tillers per plant and number of leaves on the main tiller increased considerably, while plant height, number of capsules per plant and number of seeds per capsule were reduced drastically.

Bacelis (2001) irradiated the seeds of flax variety “Svetoch” and “Vaizgantas” with physical (gamma rays) and chemical mutagens (NEU, NMU, EMS). The seeds were soaked in water solution of nitrosoethyl urea (NEU), nitrosomethyl urea (NMU), ethyl methane sulphonate (EMS), ethylene imine (EI), dimethyl sulphonate (DMS). He observed that with increased irradiation doses (from 20 kR), number of chlorophyllic mutations in both varieties changed differently. Morphological and physiological mutations were most frequent in the flax variety “Svetoch” when the seed was treated with 50 to 150 kR doses, while in “Vaizgantas” when the seeds were treated with 80 to 125 kR. Mutants such as large seeded, small seeded, early, late, long, with increased fibre output, lodging resistance were also observed.

Tejkllová (2002) studied two varieties of flax lines i.e. Te 93/13 and Su 45/85 treated with ethyl methane sulphonate (EMS) which resulted in curly stem mutations in both the lines, besides of other mutations. On further genetic analysis of one CS mutant line confirmed a monogenic inheritance of the changed stem shape. During ontogenesis, lethal mutations (e.g. yellow and white chlorophyll mutations), plant sterility and increased sensitivity of some plants to diseases appeared in the treated material.

Patil and Wakode (2011) studied variability parameters of two varieties of *Glycine max* viz. PKV-1 and JS-335 treated with gamma rays and EMS. The maximum variability was noted in cv. PKV-1 as against JS-335 for plant height, number of branches, number of clusters, number of pods and yield per plant. A positive shift was observed in number of pods per plant and yield per plant in all doses/concentrations. However negative shift was recorded in number of branches, plant height and 100 grain weight.

Alka and Ansari (2013) studied the effect of chemical mutagen (sodium azide), physical mutagen (gamma rays) and their combinations on cyto morphological

behaviour of linseed. Different types of morphological variants were observed in cotyledonary and vegetative leaves in M_1 generation. They also observed that the average height of mature plants was reduced with increased doses of all mutagens and mature plants showed variations in habit, stem, branches, morphology, yield and changes in colour, shape and size of leaves, flowers and seeds. Various micro mutational characters, such as average number of branches, capsules, yield per plant, seeds per capsule and 1000 seeds weight were studied in M_1 , M_2 and M_3 generations. All these characters decreased with the increasing concentrations/doses of mutagens

Burghate et al. (2013) observed treated seeds of the groundnut variety TAG-24 with physical mutagen (gamma rays) and chemical mutagen (ethyl methane sulphonate) individually and in their combinations and showed maximum reduction in germination at higher doses of the mutagens. There was progressive decreased in seedling growth viz. root and shoot length with increase in doses of gamma rays, ethyl methane sulphonate and their combinations in M_1 generation. The pollen sterility was also estimated during M_1 generation and was found increased significantly with an increase in doses of mutagens.

RajaRamadoss et al. (2014) studied mutagenic effectiveness and efficiency of gamma rays in two genotypes of sesame i.e. TTVS 51 and TTVS 19. A large number of viable mutants were isolated from all the mutagenic treatments. This included mutants with alteration in branching habit, plant height, phyllotaxy, nodal distance of the first capsule, flower, nodal length and seed characters etc. The frequency of viable mutants was high in 450 Gye and on seedling basis and the mutagenic effectiveness and efficiency increased with the decrease in dose or concentration in sesame.

Kumari et al. (2016) evaluated mutagenised seeds of sesame local variety 'LTK-4 for chlorophyll and vital mutations. Mutants for plant height, number of primary as well as secondary branches per plant, leaf shape such as round and narrow leaves were found. Mutants with white flowers and purple lip as well as tall, dwarf and bushy mutants were also observed in different mutagenic treatments. The maximum variation in plant height was observed in the lower dose (150Gy) which also exhibited highest number of dwarf mutants with <100 centimeter height (28) among all the doses. The lower doses of gamma rays as well as EMS concentration appeared to be more effective in the induction of variability than higher doses.

However, gamma rays proved to be more effective in producing viable mutants as compared to EMS treated mutagens.

Mogali et al. (2016) studied crop seeds of linseed variety NL 115 which were treated with 0.3 and 0.4 per cent EMS for developing non-lodging and early maturing linseed genotypes. Promising mutants for traits like early maturity (Mutant No. 66) and ideal plant type (Mutant No. 78) with non-lodging ideotype were isolated. The mutant line 66 derived from 0.3 per cent EMS treatment recorded early days to flower initiation and maturity at 35 days and 95 days respectively. The mutant 78-2 derived from 0.3 per cent EMS treatment exhibited an erect growth habit and thicker stem thus avoiding the lodging of the plant. 0.4 per cent EMS concentration was ideal with highest mutation frequency.

More and Malode (2016) studied the treated seeds of *Brassica napus* L. cv. Excel with chemical mutagens (Ethyl methane sulphonate and Sodium Azide) and physical mutagen (gamma rays) for mutagenic effects on different morphological characteristics of the variety. They observed different types of alterations in shape and number of cotyledons such as single cotyledon with fused cup shape, two cotyledons one with fused cup shape and another normal, three cotyledons, bilopedleaf, abnormal bud and siliqua with three replum were recorded in EMS, SA and Gamma radiations.

Julia et al. (2018) studied mutagenic effectiveness and efficiency of gamma rays in three genotypes of Indian mustard (two local cultivars and one improved variety). Mutants for chlorophyll, early maturity, dwarfness, bold seediness and oppressed siliqua were obtained. From the results obtained mutagenic effectiveness was found to be higher at lower doses.

Jahan et al. (2019) studied effect of sodium azide on qualitative and quantitative traits of *Linum usitatissimum* L (var. Padmini) in M₁ generation with different concentrations (0.1%, 0.2%, 0.3% and 0.4% SA) for several qualitative and quantitative traits. On evaluation it was found that induced mutations affected almost all plant parts i.e. plant height, number of fertile branches per plant, number of capsules per plant, number of seeds/capsule, weight of 1000 seeds and yield per plant. Increase in qualitative and quantitative traits were found with decrease in doses of mutagens. They further found that dose 0.2 per cent of SA was the optimum dose for

morphological mutation in linseed and concluded that lower doses of sodium azide would be helpful for plant breeders to induce desirable mutations to enhance useful traits in crop plants.

2.4 Induced mutagenic effectiveness and efficiency

Pathania and Sood (2007) studied the mutagenic effectiveness and efficiency of gamma radiation and EMS in two chickpea cultivars and found that 30 kR dose of gamma rays was most effective and efficient on family and plant basis. Among others, 0.15 per cent of EMS was found more effective on family basis while dose 0.30 per cent on plant basis. EMS was also found to be more effective and efficient. The physical mutagen gamma-rays had high mutation rate in small seeded variety than in bold seeded variety.

Kumar and Yadav (2010) studied seeds of sesame mutagenised at 0.5 per cent solution of ethyl methane sulphonate and found that the mutagen was efficient in causing meiotic abnormalities in PMCs which was exponential with increase in dose. Morphological parameters such as plant height and number of capsules were also showed changed behavior at different doses of mutagens. It was also observed that mutagenic effectiveness increased with increase in dose.

Dhulgande et al. (2011) evaluated mutagenic effectiveness and mutagenic efficiency of physical mutagen (gamma rays) and chemical mutagen (EMS) on two varieties of pea i.e. DDR-53 and DMR-55. On evaluation of treated population, EMS was found to be the most effective and the highest values of effectiveness was shown by EMS followed by gamma rays doses in both the varieties. It was further observed that the lower dose of the two mutagens proved to be most effective than the higher ones in all the mutagenic treatments.

Nair et al. (2014) investigated mutagenic effectiveness and efficiency which was calculated on the basis of pollen sterility (%) and lethality in two morphologically divergent cultivars of cowpea viz. Pusa Komal and Arka Garima. On evaluation, the mutagen effectiveness and efficiency increased with increased dosage and then declined. Dose dependent linear decline in the effectiveness was observed in Arka Garima while mutagenic efficiency decreased at higher dose of gamma rays in Pusa Komal.

Deka (2016) studied seeds of linseed treated with EMS and dES solutions and obtained a wide spectrum of chlorophyll mutations and viable mutations in M_2 generation. Higher doses of EMS and dES were found to be more injurious resulting in low recovery of both chlorophyll and viable mutations. Results obtained showed that both the mutagens were equally effective in inducing mutations in linseed whereas higher doses of dES were found to be more injurious to EMS.

2.5 Induced variation for high yield and its related components

Bari (1971) studied the effects of chronic and acute irradiation on morphological characters, yield and yield related characters. On evaluation, it was found that the seed yield per plant in irradiated plants was significantly higher than that of the non-irradiated plants, and this increase was due to an increase in the number of capsules per plant, although the number of seeds per capsule decreased constantly with increasing radiation exposures. There was also significant increase in plant height as well as in the number of primary branches.

Rai and Das (1976) conducted the experiment to study the effect of gamma rays in inducing mutations in three linseed varieties viz. Hira, Mukta and Neelum for yield contributing traits viz. number of effective branches, number of capsules per plant, average number of seeds per capsule and seed index. The different types of variation was observed in seed and yield related characters.

Rai et al. (2013) studied two diverse and improved varieties of linseed exposed at different doses of gamma rays. Results showed the presence of induced polygenic variability in the two varieties studied. It was observed that the concentrations showed negative effect on mean but had positive effect on the variability for grain yield per plant.

Kulmi et al. (2016) studied mutagens induced polygenic variability in linseed (*Linum usitatissimum* L.) variety Indira Alsi and NL-115 with the help of estimating data for various quantitative traits and found wide variation for all the quantitative characters except for days to maturity in segregating M_2 progenies. The highest GCV and PCV were observed in 0.1 per cent EMS treated Indira Alsi for number of secondary branches per plant in M_2 progenies. High GCV and PCV were exhibited

for seed yield per plant under all EMS treated M₂ progenies of Indira Alsi and NL-115 genotype.

Kulmi et al. (2017) studied the high yielding mutants from the M₃ progeny of linseed varieties Indira Alsi and NL-115 which were treated with 0.10, 0.20, 0.30, 0.40 and 0.05 per cent EMS. Superior mutants were isolated based on performance for seed yield. Mutants were isolated from 0.20 per cent EMS treated NL-115 which recorded highest yield of 15.99 gram per plant as compared to 3.16 g in control (NL-115), this considerable increase in yield was due to increase in number of secondary branches (75), number of capsules per plant (230) and enhanced test weight of 10.25 gram.

2.6 Induced mutations for oil content and fatty acid profile

Srinivasachar et al. (1972) isolated yellow seeded mutations possessing combination of high oil content and high iodine value of oil of yellow seeded varieties of linseed with high seed yield of brown seeded ones which was not found feasible by ordinary method of hybridization. High seed yield, high oil percentage and high iodine value of oil were induced in three brown seeded varieties i.e. NPRR 9, EB 3 and M 10 by different doses of gamma rays and ethyl methane sulphonate.

Sinha et al. (1981) conducted a comparison experiment of various doses of X-rays in two varieties of linseed (*Linum usitatissimum*) i.e. NP 5 and T 39 which indicated that variants with high yield and oil content could be obtained with 40, 50 and 60 kR doses in NP 5 and with 30 kR in T 39 varieties of linseed

Green and Marshall (1984) isolated two mutant lines in the linseed cultivar Glenelg, in which linolenic acid constituted approximately 29 per cent of the total fatty acid content as compared with 43 per cent from untreated Glenelg plants. The reduced level of linolenic acid in the mutants was accompanied by an increase in the level of linoleic acid to 30 per cent as compared with 18 per cent in Glenelg but there was no change in the proportion of other fatty acids.

Green (1986) studied the inheritance of two mutants of flax (*Linum usitatissimum*), having altered proportions of the C18 polyunsaturated fatty acids i.e. linoleic and linolenic. Both lines, 'M1589' and 'M1722', were homozygous for a single

gene mutation which reduces linolenic acid content from 34 to 22 per cent and raises linoleic acid from 15 to 27 per cent. Upon further evaluations, it was found that recombinant homozygous genotypes for the mutant alleles at both loci were very low in linolenic acid (2%) and high in linoleic acid (48%), with unaltered proportions of other fatty acids.

Nichterlein et al. (1988) studied the seeds of linseed cultivars Raulinus and Bionda which were treated with different doses of EMS in order to broaden the variation of fatty acids composition in the seed oil. Upon screening, the reduced linolenic acid (C18:3) content was found in some mutants and were isolated. In M₅ generation, reduced linolenic acid content was observed in Raulinus mutants, along with and correspondingly increased linoleic or oleic contents. Based on these, the low linolenic acid content lines were selected. These mutants proved to be genetically stable.

Rowland (1990) screened EMS induced M₁, M₂, M₃ and M₄ progeny for low linolenic acid mutant in McGregor flax by using the half-seed technique. On evaluation, a stable low linolenic acid (2 %) mutant was found in the M₄ generation which was controlled by recessive alleles at two independent loci apparently the result of a rare double mutation. These mutant lines were also screened for high oil content along with altered fatty acid profile by different doses of EMS.

Ntiamoah and Rowland (1997) studied the inheritance and characterization of two low linolenic acid EMS-induced McGregor flax mutants (E1929 and E1536) along with their Diallel crosses involving 1) E1929, E1536, and McGregor, and 2) E1929, E1536, and E1747. On biochemical analysis of fatty acids through gas chromatography from seed oil of F₁ and F₂ populations, results indicated that reduction in $\Delta 15$ desaturase activity in E1929 than that of control and presence of the a single recessive gene was observed for the trait. E1929 gene appeared to be allelic to one of the two genes possessed by E1747 and was characterized as a moderately low linolenic-high oleic acid mutant.

Badere and Chaudhary (2004) studied the two varieties of linseed i.e. NL-97 and RLC-6 which were treated with physical mutagen (gamma rays) and chemical mutagens (sodium azide and ethyl methane sulphonate). On evaluation of variants of

these varieties, some high yielding mutants were isolated. Also, the increase in oil percentage of the mutants was found moderate to as high as 66.90 per cent. The high yielding mutants were categorized for high seed number and high 1000 seed weight (test weight).

Yalcin et al. (2011) studied the effect of different doses of physical mutagens (2.5, 4.0, 5.5 and 7.0 kGy) on chemical properties and volatile contents of linseed and observed the presence of consistent decrease in both protein and oil content of the irradiated linseed samples with increased irradiation doses. The ash content of the irradiated linseed samples increased significantly with increased irradiation doses except for 5.5 kGy. Irradiation treatment caused irregular changes in palmitic and stearic acid content.

3. MATERIALS AND METHODS

Present investigation entitled “**Genetic improvement of linseed for inducing earliness in maturity and reducing the undesirable compounds through mutation**” was carried out in the experimental farm of Department of Genetics and Plant Breeding, COA, CSK HP KV, Palampur. This chapter contains different methods adopted and the materials used in the present experiment, climatic conditions prevalent in the locality, where the experiments were conducted, experimental details, procedure for pre harvest and post harvest observations recorded and statistical analysis which are given below:

3.1 Source of materials

3.2 Climate and weather condition

3.3 Methods of treatments

3.4 Handling of treated material

3.5 Statistical Analysis

3.6 Bio chemical analysis

3.1 Source of materials

The experimental material consists of three well adapted varieties of linseed namely, Baner, Him Alsi-2 and Surbhi obtained from Department of Genetics and Plant Breeding, CSKHPKV Palampur.

3.2 Climate and weather condition

Linseed research farm unit of Department of Genetics and Plant Breeding, COA, CSK HPKV, Palampur is located at 32°6' N latitude, 76°3' E longitude and at an altitude of 1290 meters above mean sea level. Agro-climatically, Palampur falls under sub-temperate humid zone of Himachal Pradesh, which is characterized by mild summers, severe winters and experiences occasional snowfall during winters. The region receives total rainfall of 2600 mm per annum, of which about 80 per cent is received during June to September. Winter rainfall is received during December to February. October, November, April and May are mostly dry months which usually receive very low or no rainfall. Mean weekly meteorological data recorded at the meteorological observatory of the Department of Agronomy, Forages and Grassland Management during the crop seasons have been presented in Fig. 3.1, 3.2 and 3.3 (Appendix I, II and III).

Fig. 3.1 Mean weekly meteorological data from November 2016 to May 2017

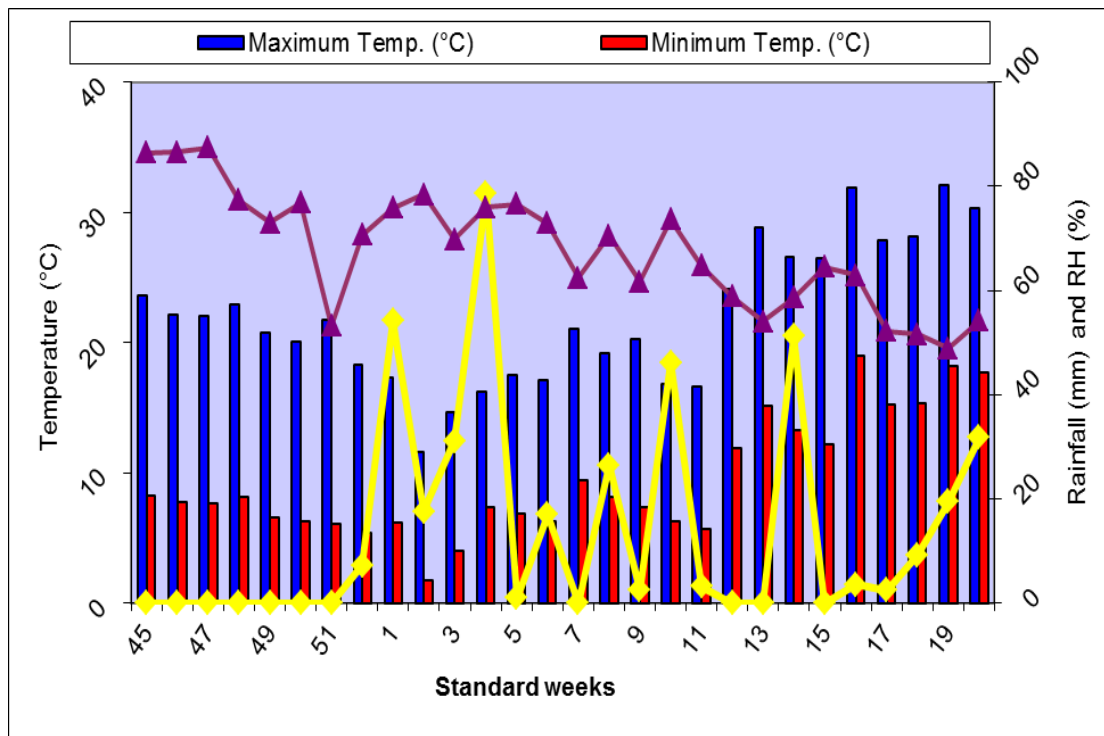


Fig. 3.2 Mean weekly meteorological data from October 2017 to April 2018

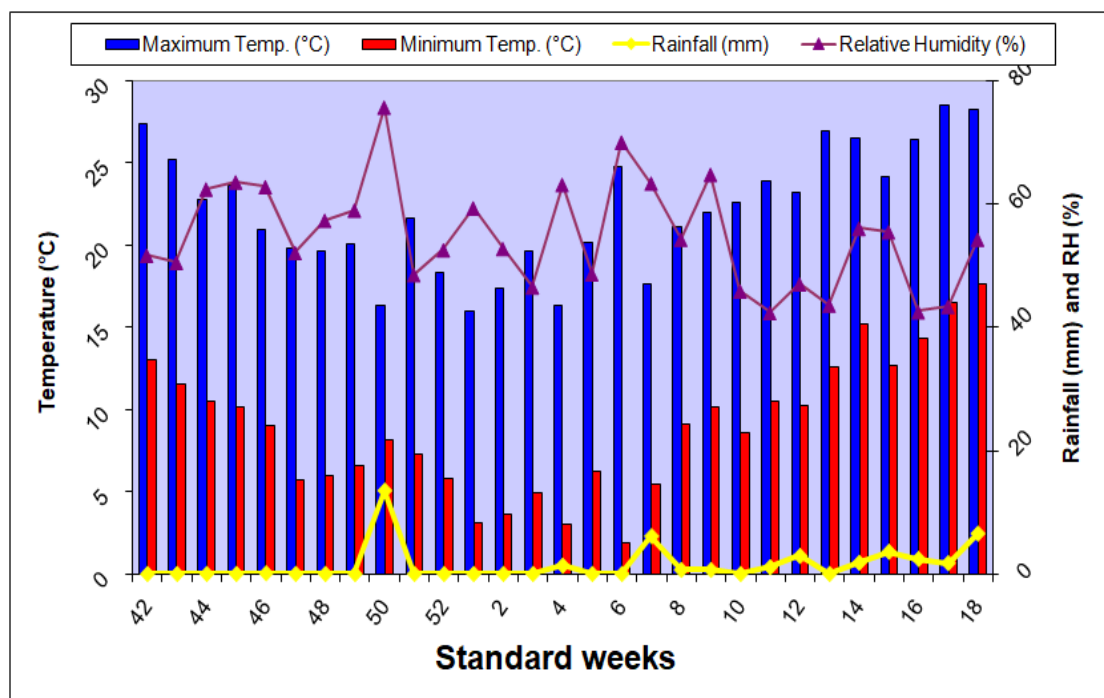
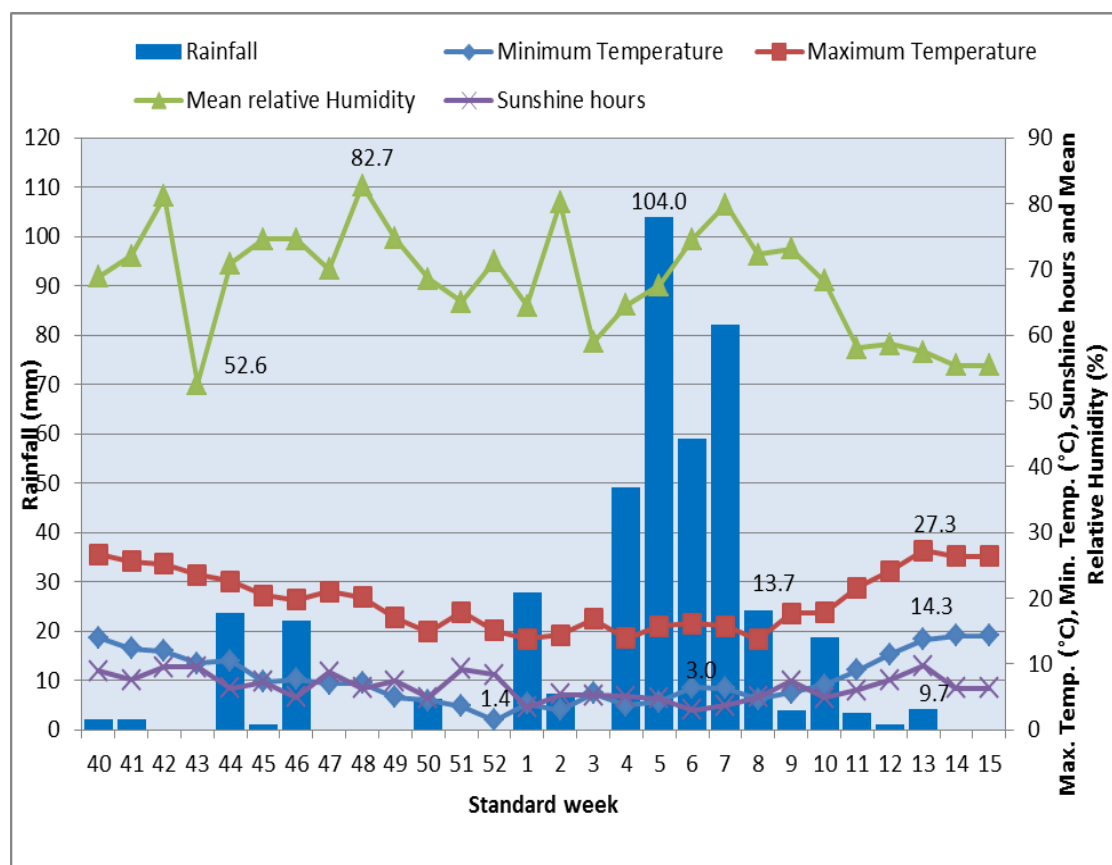


Fig. 3.3 Mean weekly meteorological data from October 2018 to April 2019



3.3 Methods of treatment:

3.3.1 Preparation of chemical mutagen solutions (EMS Solution)

Chemical mutagen treatments were prepared in the department Lab. EMS is an alkylating agent with micro functional ethyl ($-C_2H_5$) group as reactive component of molecule. Alkylating agents are very reactive. The process involves slow generation of a very reactive intermediate that alkylates DNA with only some discrimination for nitrogen atoms. Thus treatment of DNA or whole cells with EMS leads to many DNA products. Alkylating agents are very reactive, able to react also with water (hydrolysis) and give rise usually to compound that are no longer mutagenic. This means that solutions must be freshly prepared just before use and never restored. The following five different per cent concentrations (by volume) of EMS viz. 0.2, 0.4, 0.5, 0.6, 0.8 per cent were prepared from 1 per cent EMS concentration.

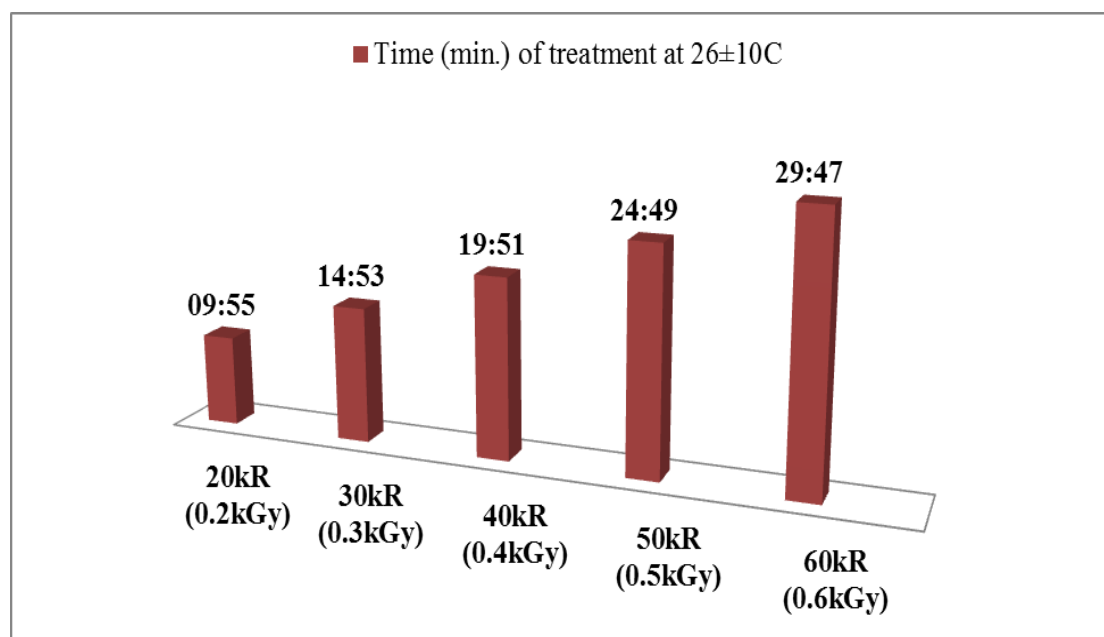
3.3.2 Treatment with EMS Mutagen

500 healthy and mature seeds of cultivars viz. Baner, Him Alsi-2 and Surbhi pre-soaked in water for 6 hours were treated with EMS with concentration of 0.2, 0.4, 0.5, 0.6 and 0.8 percent for 6 to 8 hours at room temperature. Solution of EMS was prepared in distilled water at pH 7.0 in different concentrations (0.2, 0.4, 0.5, 0.6 and 0.8 %). After the treatment, seeds were thoroughly washed with running tap water for 1-2 hours to eliminate the residual effects of the mutagen, if any, sticking to the seed surface. The volume of mutagen solution used was three time that of seeds so that EMS effect can be equally distributed. The control sample consisted of equal number of seeds soaked in distilled water alone under identical conditions.

3.3.3 Irradiation of seed with physical mutagen (Gamma rays)

Seeds of three linseed genotypes i.e. Baner, Him Alsi-2 and Surbhi were treated with gamma radiation of different dose rate at Nuclear Research lab. ICAR-IARI, New Delhi. Time of treatment of these three depends upon the concentration of radiation dose. Gamma chamber, a automated machine having gamma radiation source of ^{60}C with activity of 13000 curie and capacity of 5000 cc (volume). The seed were treated at 20, 30, 40, 50, 60 kR gamma rays shown in figure 3.4.

Fig. 3.4 The gamma rays irradiation relationships with time in gamma chamber.



3.4 Handling of treated material

3.4.1 M₁ generation

M₁ generation was raised during *Rabi* 2016-17. The mutagen treated seeds were germinated aseptically on moist filter paper in petriplates before transferring to the experimental field and were evaluated for germination percentage on the basis of which LD₅₀ doses for both varieties were estimated with the help of probit analysis. Petri dishes were placed in germination chamber for 7 days at 25 ± 2 °C. Then at 4th and 7th days after treatments, germination and survival percentage were recorded. The LD₅₀ was then determined based on the survival percentage after treatments with different doses of gamma rays and EMS. These petriplates seed were used for measuring the percentage of germination and survival at controlled conditions. From each treatment, 400 seeds were sown on dated 20.10.2016 in the experimental field with spacing of 25 x 5 cm. The field was made homogenous and divided into 36 equal sized plots (1.2 m²); each plot allotted to individual treatments and the untreated control (parent). The land was thoroughly prepared and only farm yard manure was applied in the field at recommended rate. The treated germinating seeds and the untreated one were sown in each plot such a manner that the seeds remain under about of 1.5 cm depth. Frequent irrigation was given to the plot to reduce the stress level.

At maturity, all survived M₁ plants from each treatment of both EMS and gamma rays were harvested and treatment wise threshed individually. In M₁ generation, biological effects of the different doses of EMS and gamma rays were measured from the observed data collected on the following parameters:

1. **Seed germination (both controlled and field condition):** Germination counts were taken from fourth day onwards both from controlled and field condition. The data were expressed in terms of percentage of control.

$$\frac{\text{treated seed germinated}}{\text{control seed germinated}} \times 100$$

2. **Survivability percentage:** Survivability percentage was recorded from mature plants grown in the field and the data was expressed in terms of percentage of control.

The formula used for the calculation of percentage reduction in seed germination and plant survival over control was as given below:

$$P = \left[1 - \frac{SG \%_t}{SG \%_{nt}} \right] \times 100$$

$$P = \left[1 - \frac{PS \%_t}{PS \%_{nt}} \right] \times 100$$

Where,

P = per cent population reduction over control

SG%_t = per cent seed germination in treatment

SG%_{nt} = per cent seed germination in control

PS%_t = per cent plant survival in treatment

PS%_{nt} = per cent plant survival in control

3. Pollen fertility: To measure the pollen fertility, flower buds were collected from random plants in each treatment of EMS and gamma rays and the control. Pollen fertility was studied by following the methods as suggested by Sharma and Sharma (1980). Mature pollen grains from the collected flower buds were stained with 2 per cent acetocarmine solution and scored for pollen fertility under microscope (10x x 10x). Under the microscope, 100 pollen grains were considered for scoring. 10 such counts were made in each treatment and the data is expressed in terms of percentage of control.

4. Ovule fertility: Ovule fertility in M₁ plants was measured in terms of seed setting per capsule from 25 random plants in each treatment and the control. The data were expressed in terms of average.

3.4.2 M₂ generation

M₂ generation was raised during the Rabi 2017-18 from the seeds harvested from individual M₁ plants in each dose/treatment. M₂ generation was raised in plant progeny rows. There were a total of 800 progeny rows. All the available seeds in a

single selected M_1 plant were sown on dated 18.10.2017 in plant progeny row. The field was prepared similarly as was prepared for the M_1 generation. Recommended agronomical procedures were followed during the crop period (Gill 1987). All the M_2 lines in each treatment were used for recording various quantitative traits and observations were recorded on individual plant of each progeny.

3.4.2.1 Qualitative traits

1. **Plant Growth habit:** The growth habit refers to the stature of linseed plant and its branching pattern. It is erect, semi-erect and bushy type.
2. **Chlorophyll deficient chimeras:** Chlorophyll mutations were screened through regular visual observations at seedling stage as well as during their entire period of growth. Plants showing chlorophyll mutations were harvested and threshed individually at maturity.
3. **Flower Shape:** It is the shape of flower corolla which differs from disc type to funnel, tubular and star shape.
4. **Flower Aestivation:** It is the arrangement of petals in the corolla. It is classified into twisted, semi-twisted or valvate types.
5. **Seed colour:** Seed colour of mutants was observed as dark brown, brown, yellow and fawn.

3.4.2.2 Quantitative traits

- a) **Daye to 50 per cent flowering:** For each genotype, number of days taken from the date of sowing to the day on which 50 per cent plants showed flowering was recorded and expressed as the number of days taken for 50 per cent flowering
- b) **Plant height (cm):** Plant height of each selected plants were recorded in centimeters with the help of meter rod at maturity as distance from ground level to the tip of the main shoot.
- c) **Number of primary branches per plant:** The number of branches emerging directly from the main stem were counted for each plant.

- d) Number of secondary branches plant :** The number of branches emerging from the primary branches were counted.
- e) Days to 75 per cent maturity:** Total number of days till 75 per cent maturity of each treatment was recorded.
- f) Number of capsules per plant:** Total number of fully formed capsules were counted at the time of maturity.
- g) Number of seed per capsules:** The number of seeds per capsule was taken from the randomly selected 10 capsules from each plant and average was recorded as number of seeds per capsule.
- h) Seed yield per plant:** The weight of cleaned seeds from five randomly selected plants was recorded separately and mean was expressed in gram as seed yield per plant

3.4.3. M₃ generation: Selected M₂ mutants after evaluation for particular characteristics were to carry forwarded to M₃ generation. These M₃ mutant lines were on dated 14.10.2018 and raised in compact family block design with two replication in *Rabi* 2018-19. The experimental field was well prepared and recommended doses of fertilizers were applied @ 50 kg (N), 40 kg (P₂O₅) and 20 kg (K₂O) per hectare. Half dose of N and full dose of P₂O₅ and K₂O were applied as basal and the remaining half nitrogen was top dressed after 45 days of sowing and regular weeding was done to keep the experimental field weed free. 10 plants from each mutant line were selected to study their breeding behavior of different qualitative and quantitative trait.

- 1. Days to 50 per cent flowering:** For each genotype, number of days taken from the date of sowing to the day on which 50 per cent plants showed flowering was recorded and expressed as the number of days taken for 50 per cent flowering.
- 2. Number of primary branches per plant:** The number of branches emerging from the base of the plant was noted at the time of maturity.

3. **Number of secondary branches per plant:** The total number of secondary branches emerged from primary branches was count and recorded in numbers.
4. **Number of capsules per plant:** The total number of seeds bearing capsules on the plant was counted.
5. **Number of seeds per capsule:** The number of seeds per capsule was taken from the randomly selected 10 capsules from each plant and average was recorded as number of seeds per capsule.
6. **Plant height (cm):** Plant height was measured in cm from the base of the plant to the tip of the plant at maturity.
7. **Seed colour:** Seed colour was recorded for plant at maturity. The classes were brown, golden yellow and dark brown colour.
8. **Days to 75 per cent maturity:** Days to maturity was recorded in term of number of days taken from the date of sowing to the physiological maturity of the crops.
9. **Seed yield per plant:** The weight of cleaned seeds from five randomly selected plants was recorded separately and mean was expressed in gram as seed yield per plant.
10. **1000-seed weight (g):** 1000 well-developed seeds were collected from the bulk of 10 selected plants and weight was recorded with the help of electronic balance and expressed in gram.
11. **Biological yield per plant (g):** The weight of each plant was taken at harvesting and recorded in grams.
12. **Harvest Index (%):** The ratio of economic yield to the biological yield of each plant was calculated and expressed as percentage.
13. **Oil Content (%):** Oil content in seed was estimated from bulk seed of individual entry having less than 0.9 per cent moisture content.



Plate 3.1 Layout of Compact family block design of experimental field of linseed M_3 mutants

14. Screening for disease resistance (powdery mildew as per AICRP scale 0-5):

Table 3.1 Description of disease scale for powdery mildew

Grade	Disease (% area of leaves)	Disease reaction
0	Free from disease	Highly resistant (HR)
1	1 to 10	Resistant (R)
2	10 to 25	Moderately Resistant (MR)
3	26 to 50	Moderately Susceptible (MS)
4	51 to 75	Susceptible (S)
5	Above 75	Highly Susceptible (HS)

Disease scoring for powdery mildew was done on 0-5 scale as per AICRP scale. Data on powdery mildew and rust were recorded on ten plants in each line when disease was at its peak.

3.5 Statistical Analysis

Mean values for every progeny as well as every treatment were calculated for all the characters studied and used for statistical analysis. From the per plant data, the range for a character in each treatment was recorded.

3.5.1. Coefficient of variation was calculated as per Sharma (1998). The formula used is as follows:

$$CV(\%) = \frac{100 \times SD}{\bar{X}}$$

Where,

CV(%)	=	Coefficient of variation in per cent
SD	=	Standard deviation
$\bar{X}$	=	Population mean for the treatment/dose

3.5.2 Relative coefficient of variation was calculated as per formula given by Montalvan and Andro (2005) which is as follows:

$$\text{Relative coefficient of variation} = \frac{CV_t}{CV_{nt}}$$

Where,

CV_t	=	Coefficient of variation of treated population
CV_{nt}	=	Coefficient of variation of the non treated population

Dose wise analysis based on individual plant was carried out for the polygenic traits studied to get the mean performance of individual line as per the method given by Sharma (1998).

3.5.3. Computation of standard error (mean)

The standard error of mean SE (m) was to be calculated for comparing the means of any two treatments.

SE(m) $\pm$ for treatment was computed as

$$\sqrt{\frac{\text{total within line variance in a dose}}{\text{total number of plants studied in that dose}}}$$

For comparing the population means 't' test was used as:

$$'t' = \frac{m_1 - m_2}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where, m_1 and m_2 are the means based on independent samples of size n_1 and n_2 and s is the pooled standard deviation given as:

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

where, s_1^2 and s_2^2 are the variances with $(n_1 - 1)$ and $(n_2 - 1)$ degree of freedom, respectively. The observed 't' values were compared with 't' values at $(n_1 + n_2 - 2)$ degree of freedom at 5% level.

3.5.4. Mutation effectiveness and efficiency

M_2 generation was screened for different morphological mutants during entire growth period. Mutation frequency in M_2 generation was calculated by the formula given below:

$$\text{Mutation frequency on } M_2 \text{ plant basis (M}_f\text{)} = \frac{\text{Number of mutant plants}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

Frequency of mutation was calculated based on formula suggested by Gaul (1960). The mutagenic effectiveness and efficiency were calculated based on formula proposed by Konzak et al. (1965).

$$\text{Effectiveness of physical mutagen} = \frac{M}{\text{Dose of mutagen in kR}}$$

$$\text{Effectiveness of the chemical mutagen} = \frac{M}{\text{conc.} * \text{time}}$$

$$\text{Efficiency of the chemical mutagen} = \frac{M_f}{\% \text{ lethality or } \% \text{ pollen sterility}}$$

where,

Mf = mutation frequency (plant basis)
 Conc. = concentration of EMS in per cent
 % BI = per cent biological damage

Per cent lethality / Biological injury =

$$\frac{\text{Plants germinated - Plants survived at the time of maturity}}{\text{Total number of M1 seeds sown in each treatment}} \times 100$$

3.5.5. Analysis of Variance (ANOVA) for CFBD

The means were subjected to analysis of variance by the standard procedure given by **Fisher (1936)**. Analysis of variance was done for partitioning the total variance into components of variation i.e. phenotypic, genotypic and environmental variance in the given data. It detects the existence of genetic variability. Analysis of variance was done for partitioning the total variance into variation due to treatments and replications according to procedure given by **Panse and Sukhatme (1967)**. Total variance was divided into three components viz. replication, genotypes, error and significance of 'F' was determined.

Analysis of variance was done under the fixed effect model given below:

$$Y_{ijk} = \mu + g_i + f_j + (gf)_{ij} + (pf)_{j(k)} + E_{ijk}$$

Where,

Y_{ijk} = phenotypic observation in the i^{th} (treatment), j^{th} (replication) and k^{th} family

μ = overall mean

g_i = effect of i^{th} (treatment)

e_{ijk} = random error associated with the i^{th} (treatment), j^{th} (replication), k^{th} family

Here,

i = 1, 2, 3, 4.....

j = 1, 2

H_0 = $g_1 - g_2$

Sum of square was arranged in the following manner to test the significance of difference between treatments.

Table 3.2 Skeleton of ANOVA (Analysis of Variance) of CFBD

Source of variation	Degree of freedom	Mean sum of square
Blocks/replication	(r-1)	MSS
Families/treatments	(t-1)	TSS
Error (a)	(r-1)(t-1)	ESSa
Mutant lines (main plots)	m-1	MSS _m
Treatment:mutants	p-1	MSS _p
Error (b)	(r-1)(p-1)	ESSb

Where,

- r = number of replications
 t = number of genotypes/ treatments
 MSS = Mean sum of squares
 TSS = Treatment sum of squares
 MSS_m = Mean Sum of square due to mutant lines
 EMS = Error mean sum of squares

Standard error of mean

Standard error of mean was calculated by following formula.

$$SEm = \sqrt{2EMS/r}$$

Critical difference

Critical difference was calculated by following formula.

$$C.D. = \sqrt{2EMS/r} \times t_{\text{edf at 5\%}}$$

Where,

- r = number of replications
 EMS = Error mean sum of squares
 t = table value of t at error degree of freedom at 0.05 level of significance.

Test of significance

If the variance ratio (calculated F) was greater than the tabulated F value at 1 and 5 per cent level of significance the difference between the different genotypes in

term of different character was considered to be significant and if it was lower than the tabulated value, it was considered to be non significant.

Mean: The mean value of each character was worked out by dividing the total by the corresponding number of observations.

$$\text{Mean} = \frac{\sum x}{N}$$

Where,

$\sum X$ = Sum of all the observations for each character in each replication

N = Corresponding number of observations

Range: Range is the difference between highest mean and the lowest values for each character were taken in the observation in a sample.

$$\text{Range} = X_n - X_t$$

Where,

X_n = Highest mean value of characters

X_t = Lowest mean value of characters

3.5.6. Component of variance: This was calculated by the formula suggested by **Burton (1952)**.

3.5.6.1. Genotypic variance: The genotypic variance (VG or σ_g^2) is the variance due to the genotypes present in the population. The formula used for calculation of genotypic variance was as follows

$$\text{Genotypic variance } (\sigma_g^2) = \frac{MST - EMS}{\text{number of replication } (r)}$$

Where,

MST = MSS due to treatment

EMS = error mean sum of squares

3.5.6.2 Environmental or Error variance

The environmental variance (VE or σ_e^2) is the variance due to environmental deviation:

$$\sigma_e^2 = \text{EMS}$$

3.5.6.3 Phenotypic variance

Phenotypic variance (VP or σ_p^2) denotes the total variance present in a population for particular character and is calculated by following formula

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

Where,

$$\sigma_g^2 = \text{Genotypic variance}$$

$$\sigma_e^2 = \text{Error variance}$$

3.6.6.4 Coefficient of Variation

It is the measure of variability evolved. Coefficient of variation is the ratio of deviation of a sample to its mean and expressed in percentage.

$$\text{CV}(\%) = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

In the present investigation three type of coefficient of variation were estimated viz. phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and error/environment coefficient of variation (ECV). The formulae used to calculate PCV, GCV and ECV given by **Burton (1952)**.

$$\text{PCV}(\%) = \frac{\text{phenotypic standard deviation}}{\text{grand mean}} \times 100$$

$$= \frac{\sqrt{VP}}{\bar{X}} \times 100$$

$$\text{GCV}(\%) = \frac{\text{Genotypic standard deviation}}{\text{grand mean}} \times 100$$

$$= \frac{\sqrt{VG}}{\bar{X}} \times 100$$

$$\begin{aligned} \text{ECV (\%)} &= \frac{\text{Error standard deviation}}{\text{grand mean}} \times 100 \\ &= \frac{\sqrt{VE}}{\bar{x}} \times 100 \end{aligned}$$

The PCV and GCV were classified as suggested by **Deshmukh et al. 1986**, **Sivasubramanian and Madhavamenon (1973)**

Low	: less than 10%
Moderate	: 10 -20%
High	: more than 20%

3.5.6.5. Heritability (broad sense)

Heritability (broad sense) is the ratio of genetic variance to the phenotypic variance. The heritability for the recorded data was estimated using the formula suggested by **Burton and Devane (1955)**.

$$h^2 (\text{bs}) = \frac{V_g}{V_p} \times 100$$

Where,

V_g	=	genotypic variance
V_p	=	phenotypic variance

Low	: less than 10%
Moderate	: 10 -20%
High	: more than 20%

3.5.6.6. Genetic advance (GA)

Genetic advance is the improvement in the mean genotypic value of selected plants over the parental population. Genetic advance was calculated by the following formulae given by **Johnson et al. (1958)**

$$\text{GA} = K. h^2. \sigma_p$$

Where,

GA	=	genetic advance.
----	---	------------------

- K = selection intensity, which assumes the value of 2.06 at 5%.
 h^2 = heritability co-efficient.
 σ_p = phenotypic standard deviation.

3.6 Biochemical analysis:

Oil extraction from the selected M₃ mutant lines along with control viz. Baner, Him Alsi-2 and Surbhi was done with the help of standard methods of AOAC (1990). After the oil extraction, the percentage of oil content has been determined and further subjected to Iodine Value and Acid Value. The fatty acid profile was determined from the oil by subjecting the oil through GC-MS (Gas Chromatography - Mass spectrometry) analysis. The Biochemical analysis of linseed mutant was done in Laboratory of CSIR-IHBT, Palampur, Kangra HP. The detailed procedure for biochemical analysis is given below:

3.6.1 Determination of Oil content: Oil was extracted by the sonication method. 5 gram of seed sample was grounded in pestle mortar and grounded material was placed in conical 250 milliliter conical glass flask. The weight of sample along with grounded material was noted down by electronic weighing balance. 50 milliliter of hexane was added to the conical flask and level of flask was marked. Placed the solution to for 10-15 minutes so that fat or oil gets dissolved in hexane. The conical flasks were placed in sonicator for sonication for 30-45 minutes at 30-45 degree celsius temperature. After sonication, place the solution to settled down for 10- 15 minutes and shifted the supernatant solution to another conical flask and process was repeated to 2-3 times for more oil yield from seed. The conical flask containing the supernatant solution of hexane was placed in sonivapor to complete vacuum removal of hexane. After completed removal of hexane, remained oil was weighed along flask and noted down. The oil content was determined by:

$$\text{Oil content (\%)} = \frac{W1 - W2}{\text{weight of seed used}} \times 100$$

Where,

- W1 = weight of grounded sample along with flask
 W2 = weight of extracted oil along with flask after removal of hexane

3.6.2 Estimation of Iodine and acid Value of Oil: Estimation of Iodine and Acid value was determined by standard AOAC (1990) methods.

1. Iodine value: 1 gram of sample was weighed into dry 500 ml mistoppered glass flask. 10 ml of chloroform was added so that oil or fats gets dissolved. Blank treatment was prepared by adding 10 ml of chloroform to glass flask. Pipette 25 ml iodine solution to the flask. Quantity of iodine must be 50-60 per cent in excess of that absorbed by the fat. After occasional shaking, flasks were placed in dark for 30 minutes. 20 ml of potassium iodide was added in the solution after incubation in the dark. Wash down any free iodine on the stopper with the help of freshly boiled and cold water. Sample were titrated with standard sodium thiosulfate solution, with constant shaking until yellow colour disappeared, then 1-2 ml of starch indicator was added and titrated until the blue colour disappear. The volume of titrant was recorded. Iodine value was determined as:

$$\text{Iodine Value} = \frac{(B - S) \times N \times 126.9 \times 100}{\text{Weight of sample}}$$

Where,

B = volume in ml of standard solution needed for blank

S = volume in ml of standard solution of Sodium thiosulfate

Solution necessary for sample.

N = normality of standard Sodium thiosulfate Solution required for sample.

W = weight of sample

126.9 = molecular weight of sample.

Plate 3.2 Process of oil extraction from selected linseed mutants

Grounded sample of linseed with hexane



Placement of sample in sonicator for sonication.



Supernatant solution separated after sonication



Sample placed in sonivapor to vacuum removal of hexane



Oil extracted from selected linseed mutants

2. **Acid Value:** Neutralization of alcohol was carried out by titrating agent with 0.1 N of Potassium hydroxide solutions. 25-50 ml of neutralized alcohol was added to 2 g of sample to dissolve the oil. The content was warmed on boiling water bath followed by the addition of phenolphthalein indicator. The acid value was estimated by titration of sample against 0.25 N of potassium hydroxide solution as:

$$\text{Acid value (mg KOH/g oil)} = \frac{\text{Vol. of alkali consumed} \times N \times 56.2}{\text{Sample weight}}$$

Where,

N = normality of potassium hydroxide solution.

56.2 = molecular weight of potassium hydroxide

3.6.3 Estimation of fatty acid profile:

The profiling of fatty acid i.e. both poly saturated and unsaturated fatty acid was done by using GS-MC method (Gas Chromatography coupled with Mass spectrometry analysis) according to AOAC standards. 10µl volume of each sample was injected in gas chromatograph coupled with mass selective detector.

Therefore, above all methods were followed in the research experiment for the efficient results.

4. RESULTS AND DISCUSSION

The overall scenario of the present research work was to study the effect of mutagens on physical and inherent capacity of linseed genotypes. Such experiments could be useful to increase the potential of existing variability by changing the genetic makeup of crops by altering one or many gene. The after effect can be unpredictable but using suitable selection method, the mutant lines with higher potential can be selected and used in future breeding programmes for generation of elite varieties/cultivars. Therefore, present experiments was carried out to achieve the aforesaid objectives and discussed below in the light of available literature to attain the desirable results:

- 4.1. Determination of lethal dose (LD_{50})**
- 4.2. Biological effect of mutagens in M_1 generation**
- 4.3. Spectrum and frequency of macro mutations in M_2 generation**
- 4.5. Estimates of variability in M_2 generation**
- 4.6. Estimates of mean performance of M_3 mutant lines**
- 4.7. Estimates of morphological variation in M_3 mutant lines**
- 4.8. Estimates of ANOVA (Analysis of Variance)**
- 4.9. Estimates of variability parameters in M_3 mutant lines**
- 4.10. Disease reaction of M_3 mutant lines to Powdery Mildew**
- 4.11. Selection of M_3 mutant lines for biochemical analysis**
- 4.12. Estimates of biochemical analysis of selected M_3 mutant lines**

4.1 Determination of lethal dose (LD_{50}):

The first stage in mutation breeding is to identify the dose, since the radio sensitivity of every species, varieties and genotypes are completely different that depends upon the physical and inherent quality of material. LD_{50} is the optimum dose that causes high frequency of favorable mutation with minimum damage to the plant

since doses lower than LD₅₀ favours the plants recovery after treatments, while the use of higher doses increases the possibility to induce mutations either in positive or negative ways. Therefore, the estimation of LD₅₀ of linseed is necessary to find out the optimum doses of both physical and chemical mutagens.

Germination and survival percentage of treated linseed varieties i.e. Baner, Him Alsi-2 and Surbhi along with the control (untreated) were observed in lab condition at 3rd and 7th DAT (Table 4.1). The LD₅₀ of different mutagen at different concentration of EMS (0.2 %, 0.4 %, 0.5 %, 0.6 %, 0.8 %) and gamma rays (20 kR, 30 kR, 40 kR, 50 kR and 60 kR) was calculated from seedling survival percentage with the help of probit analysis.

The results indicated that the average percent germination and survival were reduced with increasing concentration of mutagens (EMS and gamma rays) in lab condition (Table 4.1). The percent germination was reduced from 84, 86 and 80 to 65, 58 and 49 percent with increase of concentration from 20 kR to 60 kR (kilo Roentgen) for cultivars Baner, Him Alsi-2 and Surbhi respectively. As per probit analysis based on survival percentage, the LD₅₀ of gamma rays showed variation in different cultivars, which is 96.63 kR (Baner), 72.94 kR (Him Alsi-2) and 53.22 kR (Surbhi) as shown in Table 4.2. The lowest LD₅₀ of cultivar Surbhi may be due to different seed characters as compared to other two cultivars (Fig. 4.1). The maximum percent reduction over control in both seed germination and survival percentage was shown at 60 kR followed by 50 kR indicating a clear pattern in all the varieties studied, showing that with increase in concentration of gamma rays, percent reduction over control also increased.

Similarly, there was decrease in germination and survival percentage with increase of concentrations of EMS as shown in Table 4.1. The percent germination was reduced from 87, 94 and 79 to 49, 50 and 10 percent in varieties namely, Baner, Him Alsi-2 and Surbhi respectively. Also, lethality (LD₅₀) of EMS varied among cultivars i.e. Baner (0.86 %, EMS), Him Alsi- 2 (0.72 %, EMS) and Surbhi (0.35 %, very less as compare to LD₅₀ concentration of different cultivars (Table 4.2). With increase in concentration of EMS, there was increase in percent reduction over the

control. The maximum percent reduction over control for seed germination and survival were observed in Surbhi (89.58 %, germination and 92.39 %, survival) at 0.8 percent EMS concentration, which was far more than maximum percent reduction over control of Baner and Him Alsi-2. The variation in LD₅₀ was also observed in other crops such as soybean (Karthika and Lakshmi 2006), mungbean (Jain and Khandelwal 2008) and urdbean (Selvam et al. 2010).

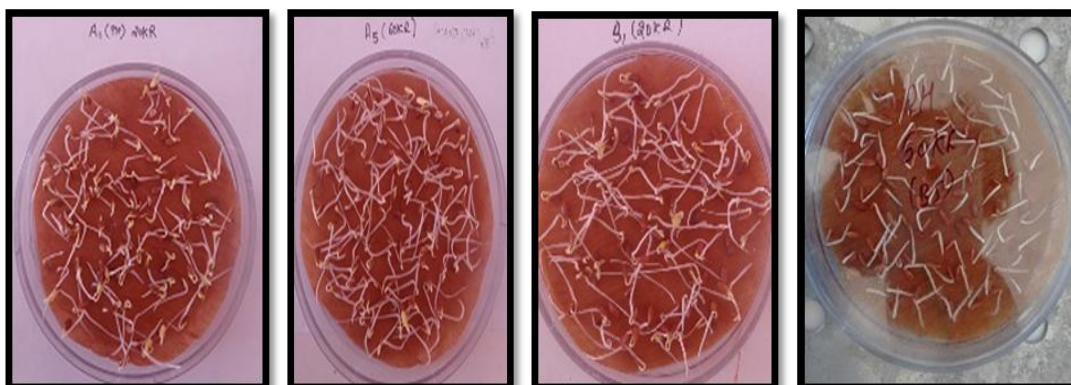
Overall, LD₅₀ values of different mutagens was lowest in Surbhi i.e. 53.22 kR (gamma rays) and 0.35 percent (EMS) (Figure 4.1). There may be possible reasons for germination and survival inhibition as reported by different authors. Gamma irradiation generates free radicals that may bring metabolic disorders in the seeds leading to growth retardation. It also affects enzyme activity since higher seed vigour is related to higher germination efficiency. It is also reported that high α -amylase activity increased metabolic activity leading to enhanced seed vigour. Hence it may be inferred that gamma irradiation may decrease α -amylase activity. Yellow and brown seeded linseed have different morphology and characteristics, so this may be the reason that the effect of EMS and gamma rays were observed more in Surbhi (Yellow seeded) as compared to Baner and Him Alsi-2 (both are of brown seed). The value of lethal dose was found different in all three cultivars, showing that lethal dose varies not only from crop to crop but also with variety to variety also (Fig. 4.1 & Table 4.2).

To check the similarity pattern of these mutagens at different concentration between controlled and natural condition (field condition), all the treatments were carried forward to field condition to observe the biological effect of EMS and gamma rays in M₁ generation.

Table 4.1. Effect of different doses of gamma rays and EMS on germination and survival percentage (Lab condition)

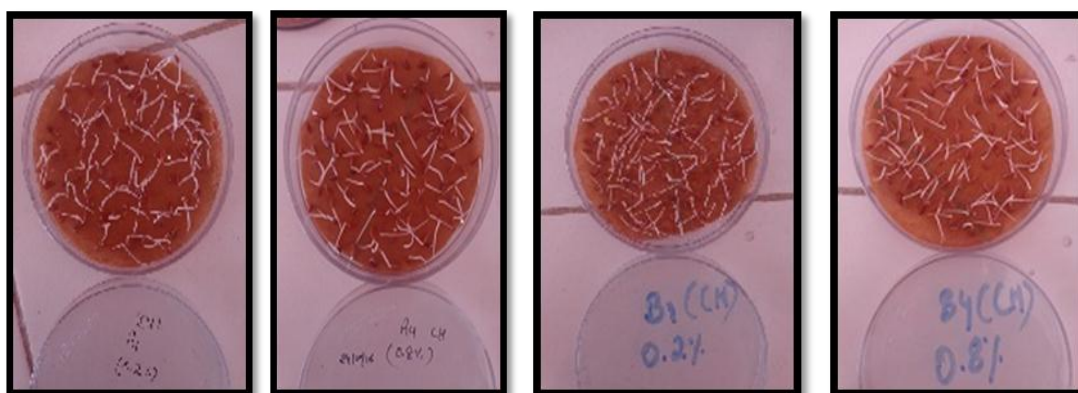
Treatment	Per cent germination	Per cent reduction over control	Plant survival	Per cent reduction over control
Baner				
Gamma rays (kR)				
20	84	14.29	82	16.33
30	80	18.37	78	20.41
40	76	22.45	75	23.47
50	70	28.57	66	32.65
60	65	33.67	64	34.69
Control	98		98	
EMS (%)				
0.2	87	11.22	86	11.34
0.4	85	13.27	84	13.4
0.5	71	27.55	70	27.84
0.6	67	31.63	60	38.14
0.8	49	50.00	48	50.52
Control	98		97	
Him Alsi-2				
Gamma rays (kR)				
20	86	12.24	83	14.43
30	81	17.35	78	19.59
40	69	29.59	65	32.99
50	66	32.65	62	36.08
60	58	40.82	56	42.27
Control	98		97	
EMS (%)				
0.2	94	5.05	91	6.19
0.4	85	14.14	76	21.65
0.5	71	28.28	65	32.99
0.6	53	46.46	52	46.39
0.8	50	49.49	42	56.7
Control	99		97	
Surbhi				
Gamma rays (kR)				
20	80	17.53	78	18.75
30	75	22.68	74	22.92
40	64	34.02	60	37.5
50	53	45.36	51	46.88
60	49	49.48	45	53.13
Control	97		96	
EMS (%)				
0.2	79	17.71	71	22.83
0.4	61	36.46	56	39.13
0.5	42	56.25	40	56.52
0.6	17	82.29	12	86.96
0.8	10	89.58	7	92.39
Control	96		92	

Plate 4.1 Germination of treated seed of linseed by gamma rays and EMS



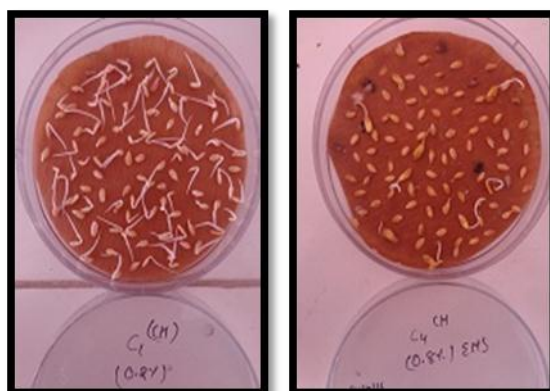
Pictures depicting germination in linseed cultivar Baner at 20 and 60 kR of gamma rays at 7th DAT

Pictures depicting germination in linseed cultivar Him Alsi-2 at 20 and 60 kR of gamma rays at 7th DAT



Pictures depicting germination in linseed cultivar Baner at 0.2% and 0.8% EMS at 7th DAT

Pictures depicting germination in linseed cultivar Him Alsi-2 at 0.2% and 0.8% EMS at 7th DAT



Pictures depicting germination in linseed cultivar Surbhi at 0.2% and 0.8% EMS at 7th DAT

Table 4.2. Lethal Dose (LD₅₀) of gamma rays and EMS in Baner, Him Alsi-2 and Surbhi

Mutagens/ Variety	Gamma rays			EMS		
	log LD ₅₀	LD ₅₀ conc. (kR)	V(m)	log LD ₅₀	LD ₅₀ conc. (%)	V(m)
Baner	1.96	96.63	0.012	0.068	0.86	0.002
Him Alsi-2	1.86	72.94	0.0040	-0.140	0.72	0.001
Surbhi	1.72	53.22	0.0020	-0.460	0.35	0.003

Figure 4.1. Differences in LD₅₀ dose of different linseed cultivars (Baner, Him Alsi-2 and Surbhi)

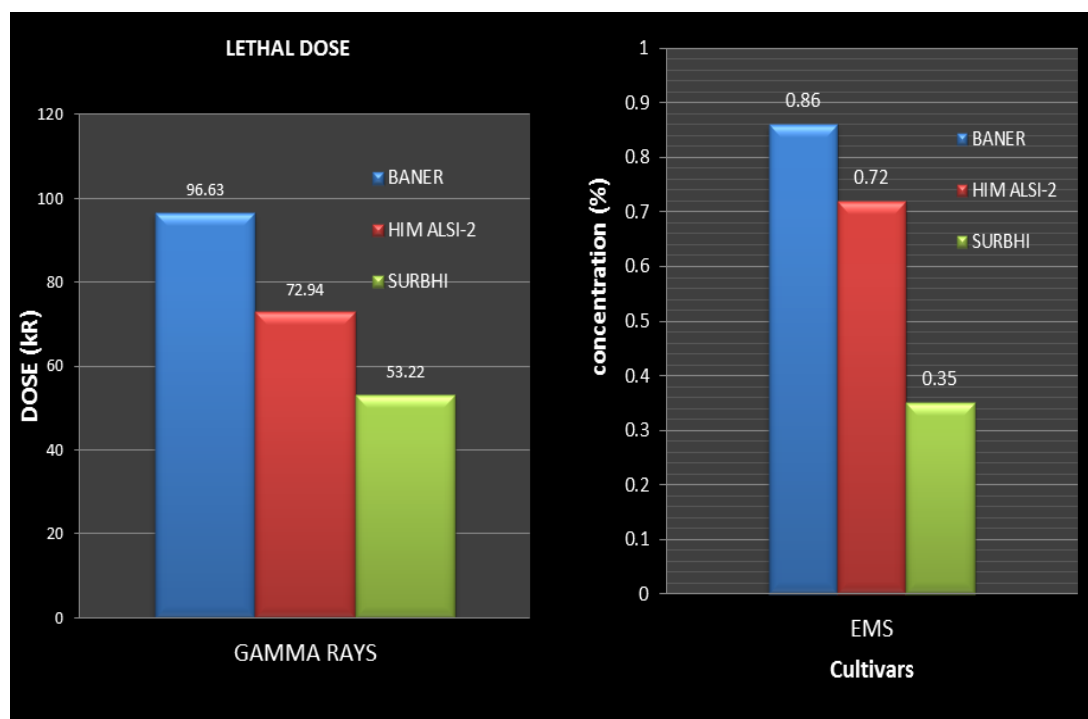
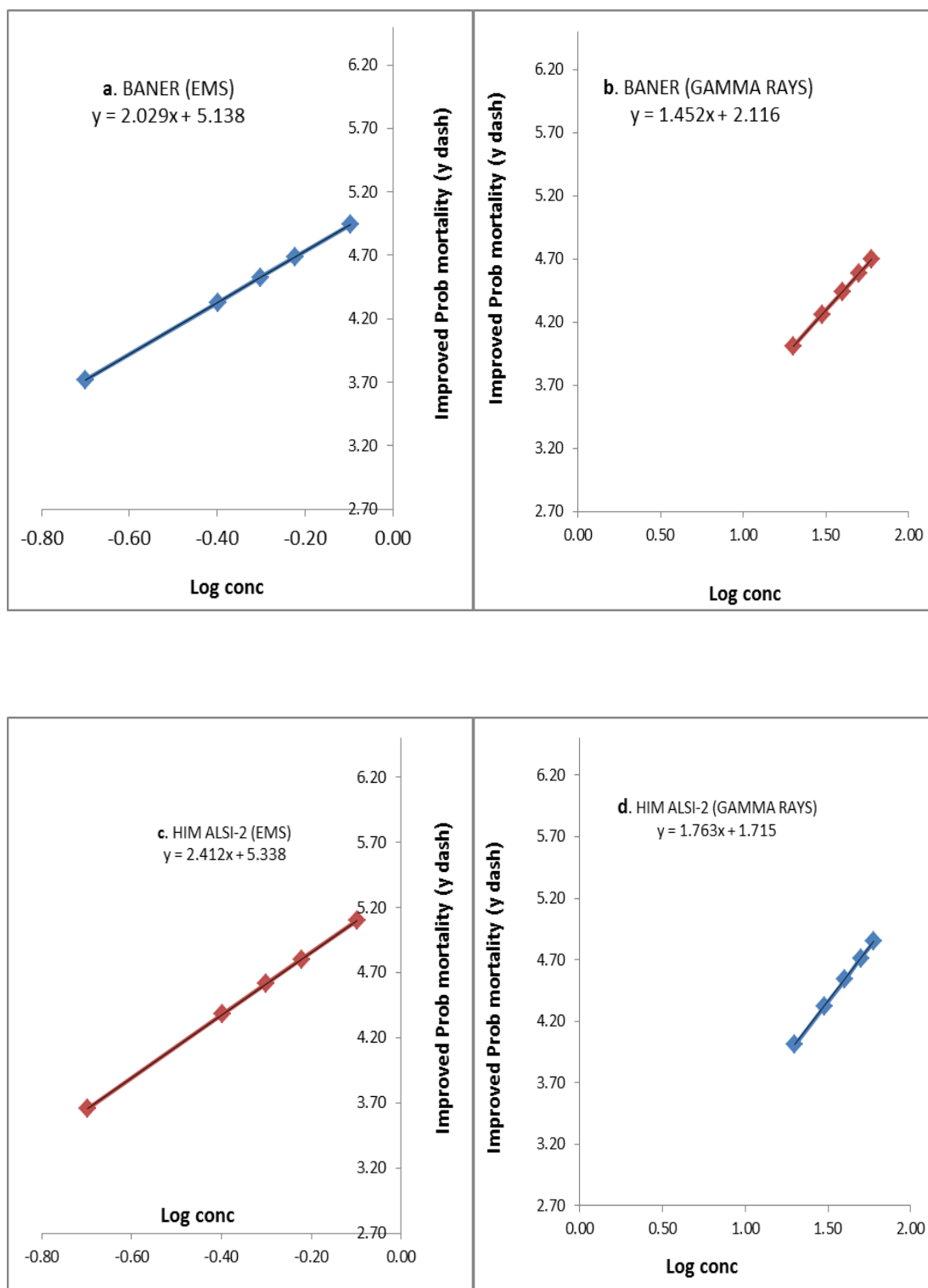
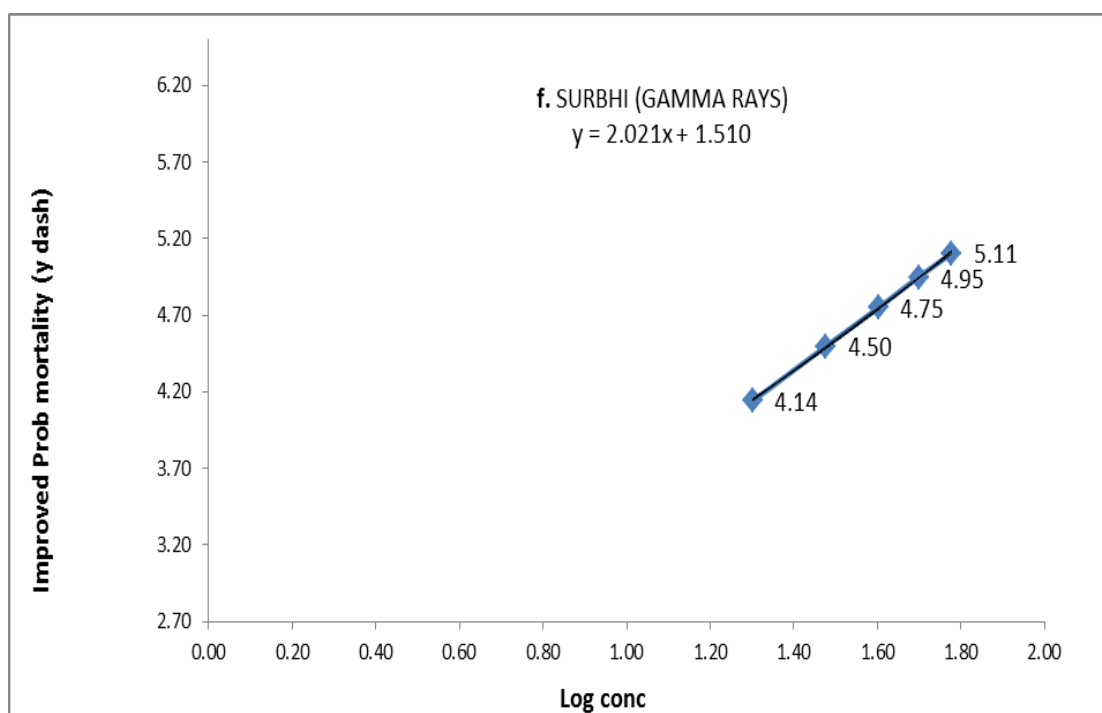
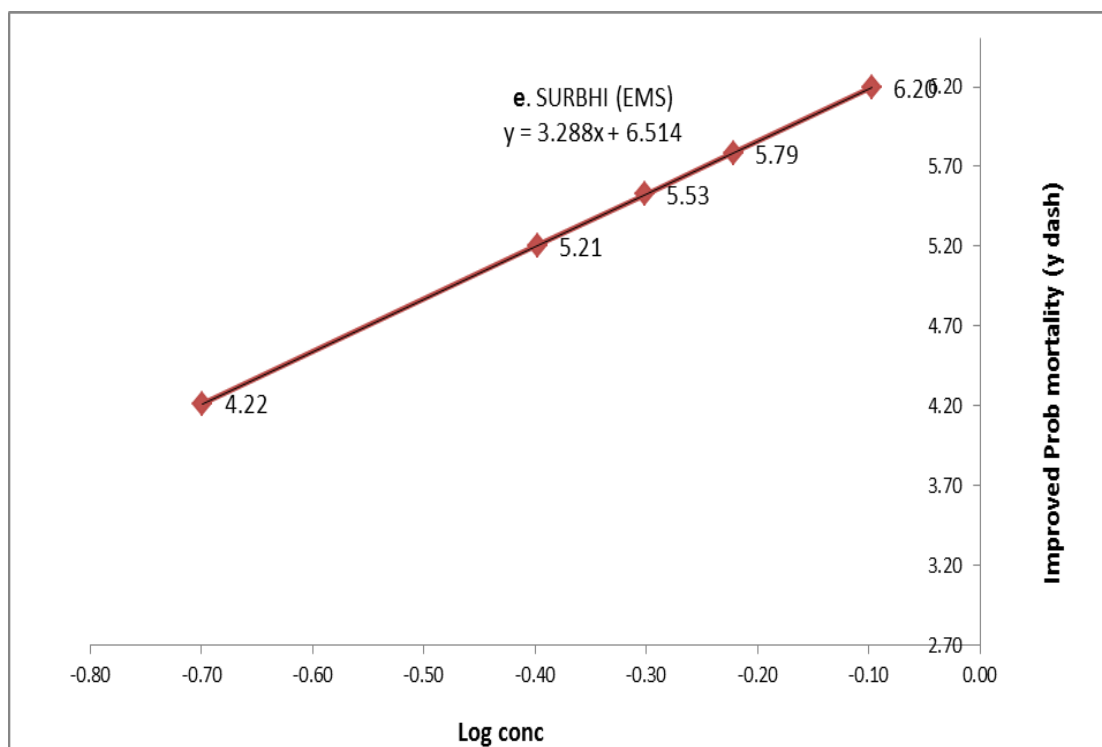


Fig. 4.2. (a,b,c,d,e & f) Probit log graphs dose effect of EMS and Gamma rays on different linseed cultivars.





4.2. Biological effect of mutagens in M₁ generation:

4.2.1 Germination and survival percentage under field condition:

Treated seeds of different concentration of EMS and gamma rays were evaluated in field condition (Table 4.3). Results showed that each concentration of gamma rays have significant effect on seed germination and survival of M₁ mutants. Percent germination in field condition decreased in same pattern in all the treated varieties i.e. Baner (83.00 % to 62.40 %), Him Alsi-2 (90.60 % to 64.80 %) and Surbhi (84.40 % to 60.40 %) from 20 kR to 60 kR respectively. Percent reduction over the control was found maximum due to 60 kR across all the treated varieties viz. Baner (36.33 %), HimAlsi-2 (34.28 %) and Surbhi (36.82 %).

Similar results were found for the parameter survival percentage of treated gamma rays plant and showed that survival percentage decreased with increase in concentration of gamma rays from 20 kR to 60 kR (Table 4.3). Also it was found that the percent reduction over control in survival percentage was maximum in 60 kR in all the varieties.

In case of EMS treated M₁ plants, the germination and survival percentage followed same pattern as in gamma rays. But germination and survival percentages reduced drastically in Surbhi as compared to Baner and Him Alsi-2. Percent reduction over control was found maximum in 0.8 percent EMS in Surbhi (100 %). The Surbhi has lowest germination and survival percentage as compared to Baner and Him Alsi-2 in different concentration of gamma rays and EMS. The plant germination decreased with increase in the dose of mutagen doses. Wani (2009), Jagadeesan (2004), Jonnada (2006) and Deka (2016) also recorded decreased germination with increase of doses of mutagens in chickpea, sunflower, groundnut and linseed, respectively.

4.2.2 Pollen fertility in M₁ generation:

Pollen fertility as represented in percentage of control was greatly affected by different concentration of EMS and gamma rays. Higher concentration of EMS (0.8 %) and gamma rays (60 kR) reduced the pollen fertility to considerable level in all the treatments (Table 4.4). Observed plants showed that maximum reduction of pollen fertility was observed at 0.5 percent EMS (62.50 %) and 60 kR (63.75%) in Surbhi as compared to Baner and Him Alsi-2. This showed that effect of mutagen doses varies not only with species but also with genotypes.

Table 4.3. Effect of different doses of gamma rays and EMS on germination and survival percentage (field condition)

Treatment	Per cent germination	Per cent reduction over control	Plant survival Percent	Per cent reduction over control	Percent Lethality
Baner					
Gamma rays (kR)					
20	83.00	15.31	79.60	17.94	20.40
30	82.60	15.71	77.80	19.79	22.20
40	69.00	29.59	71.00	26.80	29.00
50	66.80	31.84	63.20	34.85	36.80
60	62.40	36.33	60.40	37.73	39.60
Control	95.00		94.00		
EMS (%)					
0.2	92.25	2.89	90.00	5.01	17.80
0.4	90.00	5.26	89.50	5.54	19.80
0.5	83.50	12.11	82.00	13.46	22.20
0.6	80.00	15.79	75.25	20.58	26.80
0.8	65.00	31.58	64.25	32.19	35.20
Control	94.00		93.75		
Him Alsi-2					
Gamma rays (kR)					
20	90.60	8.11	82.20	15.95	17.80
30	85.00	13.79	80.20	18.00	20.40
40	75.60	23.33	77.80	20.45	35.40
50	73.40	25.56	73.20	25.15	39.80
60	64.80	34.28	64.80	33.74	40.20
Control	94.60		93.80		
EMS (%)					
0.2	92.75	4.13	91.75	4.92	10.00
0.4	89.00	8.01	87.50	9.33	10.50
0.5	78.00	19.38	75.50	21.76	18.00
0.6	76.50	20.93	74.00	23.32	24.75
0.8	67.25	30.49	65.00	32.64	35.75
Control	95.75		94.50		
Surbhi					
Gamma rays (kR)					
20	84.40	11.72	82.20	12.55	8.25
30	80.40	15.90	79.60	15.32	12.50
40	65.20	31.80	64.60	31.28	24.50
50	62.20	34.94	60.20	35.96	26.00
60	60.40	36.82	59.80	36.38	35.00
Control	95.60		94.00		
EMS (%)					
0.2	70.0	7.3	57.5	14.2	42.5
0.4	65.0	13.9	50.3	25.0	49.8
0.5	33.5	55.6	28.0	58.2	72.0
0.6	3.0	96.0	0.5	99.3	99.5
0.8	1.0	98.7	0.0	100.0	100.0
Control	75.5		67.0		

Table 4.4 Effect of different doses of gamma rays and EMS on pollen and ovule fertility in M₁ generation

Treatment	Pollen fertility	Percent Pollen fertility	Ovule fertility	Percent seed sterility	Percent reduction over control (Pollen fertility)	Percent reduction over control (Ovule fertility)
Baner						
Gamma rays (kR)						
20	95.50	4.40	64.40	25.81	4.40	25.81
30	89.75	10.16	67.30	22.47	10.16	22.47
40	89.25	10.66	69.90	19.47	10.66	19.47
50	82.25	17.67	48.90	43.66	17.67	43.66
60	75.13	24.80	49.50	42.97	24.80	42.97
Control	99.90		86.80			
EMS (%)						
0.2	95.88	6.91	64.00	25.06	3.93	25.06
0.4	91.13	9.53	70.30	17.68	8.69	17.68
0.5	85.88	14.29	62.90	26.35	13.95	26.35
0.6	79.88	18.17	49.50	42.04	19.96	42.04
0.8	74.75	24.05	41.50	51.41	25.10	51.41
Control	99.80		85.40			
Him Alsi-2						
Gamma rays (kR)						
20	93.00	14.79	65.70	25.26	6.72	25.26
30	90.38	16.67	63.30	27.99	9.35	27.99
40	85.63	24.55	57.00	35.15	14.12	35.15
50	81.75	32.06	52.60	40.16	18.00	40.16
60	75.88	36.19	55.60	36.75	23.90	36.75
Control	99.70		87.90			
EMS (%)						
0.2	95.50	4.03	76.40	6.37	4.50	6.37
0.4	90.00	8.78	59.60	26.96	10.00	26.96
0.5	84.00	14.04	52.00	36.27	16.00	36.27
0.6	79.50	20.05	58.00	28.92	20.50	28.92
0.8	74.50	25.18	50.00	38.73	25.50	38.73
Control	100.00		81.60	0.00		
Surbhi						
Gamma rays (kR)						
20	85.13	4.40	62.40	27.27	14.88	27.27
30	83.25	9.91	51.90	39.51	16.75	39.51
40	75.38	15.92	62.20	27.51	24.63	27.51
50	67.88	20.42	52.70	38.58	32.13	38.58
60	63.75	25.43	47.70	44.41	36.25	44.41
Control	100.00		85.80			
EMS (%)						
0.2	89.00	10.91	77.60	2.76	8.25	2.76
0.4	74.50	25.43	62.60	21.55	23.20	21.55
0.5	62.50	37.44	51.60	35.34	35.57	35.34
Control	97.00		79.80			

Also, it was observed that percent reduction over control (pollen fertility) was found maximum in highest concentration of treatment of gamma rays and EMS. With increase in concentration of gamma rays from 20 kR to 60 kR, the increase in percent reduction over control (pollen fertility) was observed i.e. Baner (4.40 % to 24.80 %), Him Alsi-2 (6.72 % to 23.90 %) and Surbhi (14.88 % to 36.25 %). The same pattern was observed in EMS in all the treated varieties. In comparison to EMS with gamma rays, the effect of EMS in different concentration is slightly higher than the effect of gamma rays. The increase in lethality as pollen sterility was reported by many investigators in different crops i.e. green gram (Das et al. 2006; Das and Prusti 2020; Das et al. 2021), blackgram (Sagade and Apparao 2011). They concluded that probable reason for increase in pollen sterility might be meiotic irregularities.

4.2.3 Ovule fertility in M₁ generation:

As expressed in percentage of control, ovule fertility was measured in terms of seed setting per capsule (Table 4.4). Marked variations in number of seeds per capsules were observed in treatments with gamma rays and EMS. With increase in dose of gamma rays and EMS, decrease in ovule fertility was observed in Baner, Him Alsi-2 and Surbhi. The minimum ovule fertility was observed at 60 kR gamma rays viz. Baner (61.25 %), Him Alsi-2 (55.60 %) and Surbhi (65.00 %); 0.8 percent EMS in Baner (60.00 %), Him Alsi-2 (60.00 %); 0.5 per cent in Surbhi (51.00 %). Data pertaining to percent reduction over control in pollen and ovule fertility showed that there was inverse relation with the doses of EMS and gamma rays with the pollen and ovule fertility which proves that with increase in concentration of mutagens, the pollen and ovule fertility got reduced drastically. Also it was observed that different concentrations have their own biological effects, and effect varies with genotypes based upon the physiological and genetic properties of seeds of cultivars.

4.3. Spectrum and frequency of macro mutations in M₂ generation

4.3.1. Spectrum and frequency of chlorophyll mutations

Chlorophyll mutations are the most conspicuous and easily detectable, as they have been extensively used to find out the sensitivity of crop plants to mutagens and to elucidate effectiveness and efficiency of mutagens (Gaul 1960). The frequency of chlorophyll mutations in M₂ has been suggested to provide the most reliable index of mutation rate because of greater accuracy on scoring and have also been noted to depict the damage caused to the treated material (Raja Ramodoss 2014). The frequency of chlorophyll mutations was calculated as the percentage of chlorophyll mutants isolated in the whole population of a particular treatment. The frequency of chlorophyll mutants in general was low in this crop and it may be due to the fact that oilseed crops are resistant to induced chlorophyll mutations as reported by Rajan (1969) and Rangaswamy (1973).

Based on the intensity of pigmentation at seedling stage, four types of chlorophyll mutations viz. chlorina, albina, xantha and radina were recorded amongst the segregating M₂ plants in all three varieties of linseed as shown in Table 4.5.

Albina: A lethal mutation with entirely white leaves with no carotenoids, survived up to 2 - 4 leaf stages. Some albina mutants had sectorial white color leaf blade and were viable up to maturity stage.

Xantha: Leaf colour was yellow to whitish yellow with prevailing carotenoids or chlorophyll was not evenly produced and survived up to 10 - 60 days.

Radina: Purplish radish colour was observed in whole seedling, survived upto initiation of flowering.

Chlorina: Light green colour appeared on leave which gradually spread to whole plant.

The spectrums of all type of chlorophyll mutation were observed at different concentration of gamma rays and EMS in treated Baner, Him Alsi-2 and Surbhi. In Baner, spectrum of chlorophyll mutations was almost same in all concentrations i.e. one, while frequency of chlorophyll mutations differed in each treatment. At 20 kR gamma rays and 0.5 per cent EMS, one radina type chlorophyll mutant was observed

having frequency of 0.162 per cent and 0.212 per cent respectively. Also one xantha type mutant with frequency of 0.152 per cent was found in 30 kR gamma rays treatment. The maximum frequency of chlorophyll mutations was induced by 60 kR (0.490 %) gamma rays and 0.6 per cent EMS (0.476 %).

In Him Alsi-2, at 30 kR gamma rays, xantha (1), albino (1) and chlorina (1) type of chlorophyll mutants having frequency of 0.212 per cent was observed (Table 4.5). In 40 kR treatment only xantha (1) and radina (1) type of mutant was observed having frequency of 0.205 per cent. At 20, 50 and 60 kR, no chlorophyll mutant was observed in Him Alsi-2. On the other hand, except 0.2 per cent EMS, all EMS treatments i.e. 0.4, 0.5, 0.6 and 0.8 per cent showed chlorophyll mutations. At 0.4 per cent EMS, only one chlorina type mutant was observed having frequency of 0.238 per cent. Among all concentration of EMS and gamma rays, maximum frequency of chlorophylls mutations was observed to be induced by 30 kR (0.667 %) followed by 0.6 per cent EMS (0.667 %) having spectrum three and two respectively.

In Surbhi, except 20 kR gamma rays, all the doses of gamma rays and EMS produced chlorophyll mutants. Highest spectrum of chlorophyll mutant was observed in 0.5 per cent EMS i.e. radina (2) having frequency of 0.800 per cent. Among all doses of gamma rays and EMS, maximum frequency of chlorophyll mutation was induced by 0.5 per cent EMS (1.200 %) followed by 30 kR gamma rays (0.714 %).

Among all the treated population of Baner, Him Alsi-2 and Baner at different concentrations of gamma rays and EMS, the maximum frequency of chlorophyll mutation was observed in 0.5 per cent EMS (1.200 %) in Surbhi, followed by 30 kR gamma rays (0.714 %) in Surbhi, 30 kR gamma rays (0.667 %) in Him Alsi-2. It was found that the spectrum and frequency of chlorophyll mutation not only depends upon concentration of EMS and gamma rays but also the nature and magnitude of physiological and genetics properties of varieties. Also the spectrum and frequency varies between varieties and no dose dependent relationship was observed by different researchers. Levan (1941), Marki and Bianu (1969), Beard (1971), Deka (2016) and many others also reported chlorophyll mutants in M_2 generation of mutagenized population. Bianu and Marki (1970) reported more albino and fewer xantha mutants in linseed which were in line with the present findings. The appearance of radina

mutants in our experiment was also in line with the studies of Deka (2016) in linseed. The occurrence of chlorophyll deficient chimeras might be due to changes in genes responsible for the chlorophyll mutation (Das et al. 2021; Monika and Seetharaman 2017).

Table 4.5 Spectrum and frequency of chlorophyll mutation induced by EMS and gamma rays in M₂ generation

Treatments	Xantha	Albino	Radina	Chlorina	Total
Baner					
Gamma Rays (kR)					
20	0	0	1(0.167)	0	1(0.167)
30	1(0.156)	0	0	0	1(0.156)
40	1(0.161)	1(0.161)	0	0	2(0.322)
50	1(0.227)	0	0	1(0.227)	2(0.454)
60	1(0.243)	0	1(0.243)	0	2(0.490)
EMS (%)					
0.2	0	1(0.175)	0	1(0.175)	2(0.350)
0.4	1(0.172)	0	1(0.172)	0	2(0.344)
0.5	0	0	1(0.212)	0	1(0.212)
0.6	1(0.238)	1(0.238)	0	0	2(0.476)
0.8	0	1(0.212)	0	1(0.212)	2(0.425)
Him Alsi-2					
Gamma Rays (kR)					
20	0	0	0	0	0
30	1(0.212)	1(0.212)	0	1(0.212)	3(0.667)
40	1(0.205)	0	1(0.205)	0	2(0.408)
50	0	0	0	0	0
60	0	0	0	0	0
EMS (%)					
0.2	0	0	0	0	0
0.4	0	0	0	1(0.238)	1(0.238)
0.5	1(0.232)	0	1(0.232)	0	2(0.465)
0.6	1(0.212)	0	1(0.212)	0	2(0.425)
0.8	0	1(0.333)	0	1(0.333)	2(0.667)
Surbhi					
Gamma Rays (kR)					
20	0	0	0	0	0
30	0	1(0.357)	0	1(0.357)	2(0.714)
40	0	0	1(0.312)	0	1(0.312)
50	0	1(0.312)	0	0	1(0.312)
60	1(0.312)	0	0	1(0.312)	2(0.625)
EMS (%)					
0.2	1(0.400)	0	0	0	1(0.400)
0.4	0	0	1(0.400)	0	1(0.400)
0.5	1(0.400)	0	2(0.800)	0	3(1.200)

Plate 4.2 Different type of chlorophyll mutants observed in M₂ generation



Xantha type mutant



Chlorina type mutant



Radina type mutant



Albino type mutant

4.3.2 Spectrum and frequency of morphological mutations

Different types of morphological changes have been noticed in M₂ generation in field condition in all three varieties studied as shown in Table 4.6. The changes in morphological features like plant type, flower color, flower aestivation and seed colour were caused by different concentrations of gamma rays and EMS in linseed varieties.

In variety Baner, spreading and erect type plants were observed in both EMS and gamma rays induced mutants. Mutants treated with gamma rays showed more spreading and erect type plants than mutants treated with EMS. Maximum frequency of spreading type (3) was observed in 60 kR (0.732 %), while maximum number of erect type plants (3) were observed in 50 kR having frequency of 0.682 per cent. Only one spreading type mutants was observed in 0.6 per cent EMS having frequency of 0.238 per cent. Plants having different corolla arrangement were found in Baner i.e. free, overlapping and abnormal types. Frequency of free type flower type mutants was observed in 40 kR (0.968 %) followed by 30 kR (0.781 %). Frequency of free type corolla arrangement mutants was found more than the frequency of overlapping and abnormal type mutants. The treatments 30, 50 and 60 kR gamma rays showed flowers with abnormal corolla arrangement having either defective petals or increased number of petals (more than 5). The 0.4 per cent and 0.5 per cent EMS treated mutants has shown all type of plants having flower with corolla arrangement of free, overlapping and abnormal type. Frequency of abnormal type mutants was found more in EMS treated population than the gamma irradiated population.

In variety Him Alsi-2, maximum spectrum of morphological mutations was induced by 60 kR gamma rays (18) and 0.8 per cent EMS (17) having frequency of 4.000 per cent and 4.333 per cent respectively. Spreading and erect type of plant were found in different concentration of gamma rays and EMS and it was observed that spectrum of morphological mutation was more by gamma rays than EMS. Velvate, twisted and abnormal type of flower aestivation was also noticed in M₂ mutants. At 50 and 60 kR, highest numbers of funnel shape flowers were observed with frequency of 0.626 per cent. No star shape plant was observed in gamma rays treatment while except 0.2 per cent EMS, all other concentration of EMS (0.4, 0.5, 0.6, 0.8 %)

induced one star shaped flowering mutants, with highest frequency at 0.8 per cent EMS (0.333). Maximum number of fawn seed colour mutants was caused by 60 kR gamma rays with frequency of 0.626 per cent.

In variety Surbhi, spectrum of morphological mutation was highest at 60 kR gamma rays (20) and 0.5 per cent EMS (10) having a frequency of 6.250 per cent and 4.000 per cent respectively showing that the higher doses of gamma rays and EMS caused more mutations than the lower doses. There was no change in seed colour of mutants which was similar to respective control parents i.e. golden yellow. Spreading and erect type of mutants were found in M₂ mutant of Surbhi.

Overall observations showed that various type mutants having altered morphological characteristics than respective control were recorded in all three cultivars (Baner, Him Alsi-2 and Surbhi) in M₂ generation.

Table 4.6. Spectrum and frequency of morphological macro mutations induced by EMS and gamma rays in M₂ generation

Variety/ Treatment	Plant Type		Flower Aestivation			Flower Shape						Seed Colour			Total
	Spreading	Erect	Velvate	Twisted	Abnormal	Funnel	Disc	Star	Tubular	Abnormal	Sterile	Yellow	Dark Brown	Fawn	
1	2	3	4	5	5	7	8	9	10	11	12	13	14	14	(2 to 14)
BANER															
GAMMA RAYS (kR)															
20	0	0	1 (0.166)	1(0.166)	0	1(0.166)	0	1(0.166)	1(0.166)	1(0.166)	0	0	1(0.166)	0	7 (1.167)
30	1 (0.156)	1 (0.156)	5 (0.781)	0	1(0.156)	2 (0.313)	0	2 (0.313)	1(0.156)	2 (0.313)	0	0	1(0.156)	0	16(2.500)
40	2(0.323)	1(0.161)	6(0.968)	2(0.323)	0	3(0.484)	0	1(0.161)	0	4(0.645)	0	0	0	0	19(3.065)
50	0	4(0.800)	0	2(0.400)	1(0.200)	0	0	1(0.200)	2(0.200)	1(0.200)	0	0	1(0.200)	1(0.200)	18 (4.091)
60	3(0.732)	2(0.488)	4(0.976)	1(0.244)	1(0.244)	3(0.732)	0	1(0.244)	2(0.488)	0	4(0.976)	0	1(0.244)	1(0.244)	23 (5.610)
EMS (%)															
0.2	0	0	0	0	0	0	0	0	0	0	0	0			0 (0.000)
0.4	0	1(0.172)	1(0.172)	2(0.345)	3(0.517)	1(0.172)	0	1(0.172)	2(0.345)	0	0	0	0		11 (1.897)
0.5	0	0	1(0.213)	2(0.426)	4(0.851)	1(0.213)	0	1(0.213)	1(0.213)	1(0.213)	2(0.426)	0	1(0.213)	0	14 (2.979)
0.6	1(0.238)	1(0.238)	0	0	2(0.476)	1(0.238)	0	1(0.238)	1(0.238)	0	0	0	1(0.238)	0	8 (1.905)
0.8	0	1(0.213)	0	3(0.638)	3(0.638)	0	0	2((0.426)	2(0.426)	3(0.638)	1(0.213)	0	1(0.213)	2(0.426)	18 (3.830)
Control		Semi erect	Semitwisted				Disc						Brown		
HIM ALSI-2															
GAMMA RAYS (kR)															
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.000)
30	0	1(0.357)	0	0	0	1(0.357)	0	0	0	0	0		0	0	2 (0.444)
40	1(0.313)	0	1(0.313)	1(0.313)	2(0.313)	1(0.313)	0	0	2(0.626)	0	2(0.626)			1(0.313)	10 (2.041)
50	1(0.313)	1(0.313)	1(0.313)	1(0.313)	2(0.626)	2(0.626)	0	0	2(0.626)	2(0.626)	1(0.313)			0	13 (2.600)
60	2(0.626)		2(0.626)	2(0.626)	2(0.626)	2(0.626)	0	0	3(0.938)	2(0.626)	1(0.313)			2(0.626)	18 (4.000)
EMS (%)															
0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.000)
0.4	0	0	0	0	0	0	0	1(0.238)	0	1(0.238)	1(0.238)	0	0	0	3 (0.714)
0.5	1(0.233)	0	1(0.233)	1(0.233)	1(0.233)	1(0.233)	0	1(0.233)	2(0.466)	0	2(0.466)	0	1(0.233)	0	11 (2.558)
0.6	1(0.222)	0	1(0.222)	2(0.444)	1(0.222)	1(0.222)	0	1(0.222)	3(0.666)	0	0	0	1(0.222)	0	11 (2.444)
0.8	0	1(0.333)	0	1(0.333)	1(0.333)	1(0.333)	0	1(0.333)	2(0.666)	1(0.333)	2(0.666)	0	3(1.00)	0	13 (4.333)
Control		Semi erect	Semitwsited				Disc						Brown		

SURBHI															
GAMMA RAYS (kR)															
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.000)
30	0	1(0.357)	0	0	0	1(0.357)	0	0	0	0	0	0	0	0	2 (0.714)
40	1(0.313)	0	1(0.313)	1(0.313)	2(0.313)	1(0.313)	0	0	2(0.625)	0	2(0.625)	0	0	1(0.313)	11 (3.438)
50	1(0.313)	1(0.313)	1(0.313)	1(0.313)	2(0.625)	2(0.625)	0	0	1(0.313)	2(0.625)	1(0.313)	0	0	0	12 (3.750)
60	2(0.625)	2(0.625)	2(0.625)	2(0.625)	2(0.625)	2(0.625)	0	0	3(0.938)	2(0.625)	1(0.313)	0	0	2(0.626)	20 (6.250)
EMS (%)															
0.2	1(0.400)	0	0	0	1(0.400)	1(0.400)	0	0	0	1(0.400)	1(0.400)	0	0	1(0.400)	6 (2.400)
0.4	1(0.400)	0	1(0.400)	0	0	1(0.400)	1(0.400)	0	1(0.400)	1(0.400)	1(0.400)	0	0	1(0.400)	8 (3.200)
0.5	1(0.400)	0	1(0.400)	0	2(0.800)	1(0.400)	1(0.400)	0	1(0.400)	1(0.400)	1(0.400)	0	0	1(0.400)	10 (4.000)
Control	Spreading		Velvate			Star								Golden yellow	

Plate 4.3 Different type of flower shapes observed in M₂ generation



Abnormal flower type



Abnormal flower type



Funnel shape flower type



Abnormal flower type



**Dark blue and abnormal
with disc flower type**



**Light Blue and Star shape
flower type**



Twisted type dehiscence



**White flower with star shape
flower type**



Blue flower with disc shape flower



Light Blue and Blue flower colour with disc and star shape

Plate 4.4 Different type of morphological mutants observed in M₂ generation



Abnormal mutant with bushy type



Curly plant



Single stem plant



Plant with higher No. of primary branches



Abnormal plant



Control

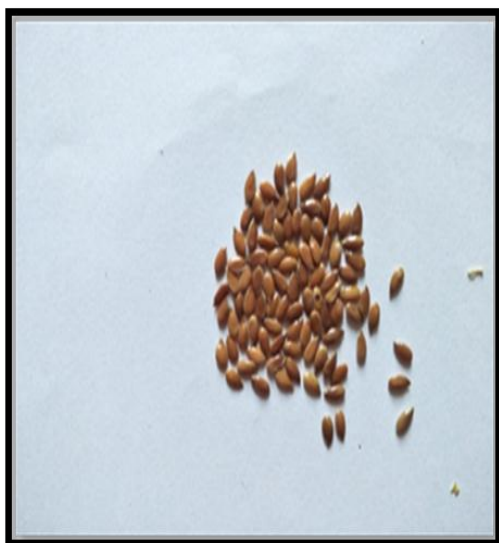


Control

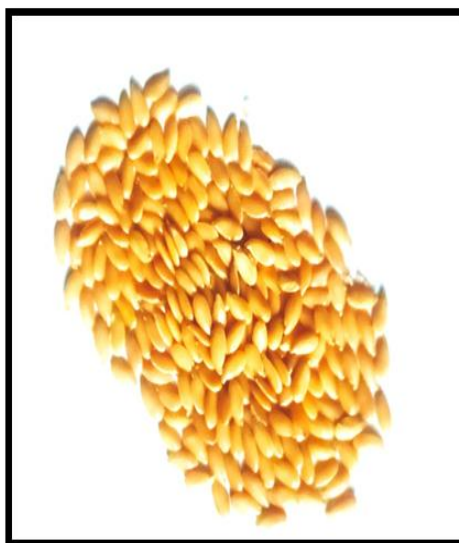


Control

Plate 4.5 Different type of seed colour observed in M₂ generation



Light brown colour seed



Golden yellow colour seed



Dark brown colour seed



Fawn colour seed

4.3.3 Spectrum and frequency of total macromutations (chlorophyll + morphological) mutations

In M₂ generation, spectrum of total macro-mutations was found maximum at higher concentration of EMS and gamma rays across all three cultivars i.e. Baner, Him Alsi-2 and Surbhi as shown in Table 4.7.

In Baner, spectrum of total macro mutations was recorded as maximum at 60 kR gamma rays (25) and 0.8 per cent of EMS (20) having frequency of 6.100 per cent and 4.255 per cent respectively. However, observation showed that except 20 kR and 0.2 per cent EMS, other doses of EMS and gamma rays also showed higher spectrum and frequency. However, dose dependent relationship was not found and occurrence of macro mutations in the present study was independent of mutagen concentrations which is in conformity with other reports of Deka (2016) in linseed and Sheeba et al. (2004) in sesame.

In Him Alsi-2, observations showed that with increase of doses of gamma rays and EMS, the spectrum and frequency of total macro mutations also increased which clearly revealed the presence of dose dependent relationship in population. The spectrum of total macro mutations was found maximum at 60 kR of gamma rays (18) and 0.8 per cent EMS (15) having frequency of 4.000 per cent and 5.000 per cent respectively.

In Surbhi, similar pattern as recorded in Him Alsi-2 was found for relative frequency and spectrum of total macro mutation with the increasing doses of gamma rays and EMS. Highest spectrum was found at 60 kR gamma rays (22) and 0.5 per cent EMS (13) with frequency of 6.875 per cent and 5.200 per cent respectively. Similar results were also reported by Das et al. (2006); and Das and Baisakh (2020).

Table 4.7 Spectrum and mutation frequency (M_f) of total macro mutations induced by gamma rays and EMS in M_2 generation

Treatments	Chlorophyll mutation frequency (M_f) (%)	Viable macromutation frequency (M_f) (%)	Total M_f (%)
Baner			
20 kR	1(0.167)	1(0.167)	2(0.334)
30 kR	16(2.500)	1(0.156)	17(2.656)
40 kR	19(3.065)	2(0.322)	21(3.387)
50 kR	18 (4.091)	2(0.454)	20(4.545)
60 kR	23 (5.610)	2(0.490)	25(6.100)
0.2 %	0 (0.000)	2(0.350)	2 (0.350)
0.4 %	11 (1.897)	2(0.344)	13(2.241)
0.5 %	14 (2.979)	1(0.212)	15(3.191)
0.6 %	8 (1.905)	2(0.476)	10(1.381)
0.8 %	18 (3.830)	2(0.425)	20(4.255)
Him Alsi-2			
20 kR	0 (0.000)	0(0.000)	0(0.000)
30 kR	2 (0.444)	3(0.667)	5(1.081)
40 kR	10 (2.041)	2(0.408)	12(2.449)
50 kR	13 (2.600)	0(0.000)	13(2.600)
60 kR	18 (4.000)	0(0.000)	18(4.000)
0.2 %	0 (0.000)	0(0.000)	0(0.000)
0.4 %	3 (0.714)	1(0.238)	4(0.952)
0.5 %	11 (2.558)	2(0.465)	13 (3.023)
0.6 %	11 (2.444)	2(0.425)	13(2.869)
0.8 %	13 (4.333)	2(0.667)	15 (5.000)
Surbhi			
20 kR	0 (0.000)	0(0.000)	0(0.000)
30 kR	2 (0.714)	2(0.714)	4(1.428)
40 kR	11 (3.438)	1(0.312)	12(3.750)
50 kR	12 (3.750)	1(0.312)	13(4.062)
60 kR	20 (6.250)	2(0.625)	22(6.875)
0.2 %	6 (2.400)	1(0.400)	7(2.800)
0.4 %	8 (3.200)	1(0.400)	9(3.600)
0.5 %	10 (4.000)	3(1.200)	13(5.200)

4.4 Mutagenic effectiveness and efficiency of EMS and gamma rays in M₂ population

The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. Mutagenic effectiveness is a measure of frequency of mutations induced by unit mutagen dose, whereas mutagenic efficiency is the measure of proportion of mutations in relation to undesirable changes like lethality, injury, sterility and chromosomal aberrations induced by the mutagens in M₁ generation. A highly effective mutagen may not necessarily show high efficiency and vice-versa. However, after continuous work done over the generations, it was observed that only a few mutagenic treatments have been effective for inducing high frequency of mutation. Therefore, selection of effective and efficient mutagen is very essential to recover high frequency of desirable mutations in any mutation breeding programme (Usharani and Kumar 2015; Vairam 2017). In M₂ generation of treated population, mutagenic effectiveness and efficiency were worked out for chlorophyll mutations, viable macromutations and macromutations (chlorophyll + viable macromutations) to find the most effective and efficient dose of mutagens as shown in Table 4.8.

Mutagenic effectiveness showed presence of no dose dependant relation for gamma rays and EMS which means it varied according to doses of mutagen and varieties under study (Table 4.8). In Baner, mutagenic effectiveness was maximum at 50 kR (0.909) followed by 20 kR (0.833) gamma rays. On the other hand, all the doses of EMS showed less effectiveness as compare to doses of gamma rays. In EMS treatment, minimum effectiveness was observed at 0.5 per cent (0.085), while maximum was observed at 0.2 per cent (0.351). In Him Alsi-2, only two doses of gamma rays were found effective i.e. 30 kR (2.222) and 40 kR (1.020), while other doses i.e. 20, 50 and 60 kR were found ineffective for inducing chlorophyll mutations. Except 0.2 per cent EMS, all other (0.3, 0.4, 0.5 and 0.6 per cent) EMS treatments were found effective with maximum effectiveness at 0.5 per cent (0.186). In Surbhi, except 20 kR, all other doses of gamma rays and EMS were found effective for causing chlorophyll mutations with maximum effectiveness at 30 kR (2.381).

followed by 60 kR (1.042) while minimum was observed at 0.4 per cent EMS (0.200). Similar results were obtained by Sharma et al. (2005), Das and Baisakh (2020), and Das and Prusti (2021).

Mutagenic efficiency provides the best available measure to evaluate different mutagenic treatments (Konzak et al. 1965). It varies with the dose of mutagens, physiological and genetical characteristic of cultivars. The efficiency at lower concentration of mutagens is due to the fact that biological injury (lethality and sterility) generally increases with increase in doses of mutagens (Konzak et al. 1965; Nilan and Konzak 1961). Mutagenic efficiency based on biological injury i.e. lethality varies from 0.015 to 0.072 (Table 4.8). In treatment of Baner, it varied from 0.015 to 0.051 in which maximum efficiency was found in 0.2 per cent EMS (0.051) followed by 20 kR gamma rays (0.038). In Him Alsi-2, two doses of gamma rays i.e. 30 kR (0.040) and 40 kR (0.017) was found efficient, and other doses i.e. 20, 50 and 60 kR were found inefficient for chlorophyll mutation. Similarly, except 0.2 per cent EMS, all other treatments (0.4, 0.5, 0.6 and 0.8 %) were found efficient. In Surbhi, similar pattern was observed as observed in Him Alsi-2. Based on pollen fertility in M_1 generation, the mutagenic efficiency of gamma rays and EMS in M_2 generation ranged from 0.07 to 0.033 and concluded that doses of gamma rays and EMS showed similar behavior of mutagenic efficiency as shown due to lethality (M_1) (Table 4.8). Similar results were obtained by Das et al. (2021) showing uneven pattern and no dependent relationship of doses of gamma rays and EMS.

Effectiveness of mutagens for morphological mutations showed that it varied according to varieties of linseed. Among all the doses of EMS and gamma rays, most effective doses were 60 kR (9.330) and 0.5 per cent (11.915) in Baner, 60 kR (6.667) and 0.8 per cent (10.833) in Him Alsi-2, and 60 kR (10.417) and 0.2 per cent (24.000) in Surbhi. The effectiveness was found maximum at 60 kR gamma rays in all cultivars, while it varied with particular cultivar in case of EMS treatments.

Table 4.8 Mutagenic effectiveness and efficiency of gamma rays and EMS in M₂ generation

Treat ment	Effectiveness		Efficiency of Chlorophyll Mutation		Efficiency of Morphological Mutation	
	Chlorophyll Mutation	Morphological Mutation	Based on Lethality	Based on Pollen	Based on Lethality	Based on Pollen
Baner						
Gamma rays (kR)						
20	0.833	5.833	0.037	0.008	0.265	0.057
30	0.521	8.333	0.015	0.007	0.246	0.112
40	0.806	7.661	0.030	0.011	0.287	0.105
50	0.909	8.182	0.025	0.012	0.231	0.111
60	0.813	9.350	0.019	0.012	0.226	0.141
EMS (%)						
0.2	0.351	0.000	0.050	0.019	0.000	0.000
0.4	0.172	9.483	0.036	0.017	0.199	0.095
0.5	0.085	11.915	0.014	0.009	0.208	0.134
0.6	0.159	6.349	0.026	0.017	0.104	0.071
0.8	0.106	9.574	0.017	0.012	0.159	0.108
Him Alsi-2						
Gamma rays (kR)						
20	0.000	0.000	0.000	0.000	0.000	0.000
30	2.222	1.481	0.040	0.032	0.026	0.021
40	1.020	5.102	0.016	0.011	0.083	0.057
50	0.000	5.200	0.000	0.000	0.081	0.065
60	0.000	6.667	0.000	0.000	0.110	0.099
EMS (%)						
0.2	0.000	0.000	0.000	0.000	0.000	0.000
0.4	0.119	3.571	0.027	0.022	0.081	0.068
0.5	0.186	10.233	0.033	0.025	0.182	0.142
0.6	0.148	8.148	0.021	0.017	0.121	0.098
0.8	0.167	10.833	0.026	0.018	0.172	0.121
Surbhi						
Gamma rays (kR)						
20	0.000	0.000	0.000	0.000	0.000	0.000
30	2.381	2.381	0.072	0.057	0.072	0.057
40	0.781	8.594	0.019	0.012	0.216	0.140
50	0.625	7.500	0.015	0.012	0.183	0.144
60	1.042	10.417	0.024	0.017	0.245	0.178
EMS (%)						
0.2	0.400	24.000	0.036	0.009	0.220	0.056
0.4	0.200	16.000	0.015	0.008	0.125	0.064
0.5	0.480	16.000	0.032	0.016	0.106	0.055

For morphological mutations, the efficiency of gamma rays ranged from 0 to 0.287 in Baner, 0 to 0.110 in Him Alsi-2 and 0 to 0.245 in Surbhi based on M_1 lethality. Out of these, highest gamma rays efficiency was found with 40 kR (0.287) in Baner, 60 kR (0.110) in Him Alsi- and 60 kR (0.245) in Surbhi. It was observed that higher doses of gamma rays were more efficient in all the varieties for linseed for morphological macro mutations. On the other hand, maximum efficiency of EMS was observed at 0.2 per cent EMS (0.220) in Surbhi, followed by 0.5 per cent EMS (0.208) in Baner. Based on lethality, efficiency of EMS varied according to variety i.e. in Surbhi with increase in concentration of EMS efficiency decreased, whereas in Baner and Him Alsi-2, no dose dependent relationship was found. Based on M_1 pollen sterility, most efficient dose of gamma rays was 60 kR with efficiency values of (0.141, 0.099 and 0.178) in Baner, Him Alsi-2 and Surbhi Respectively. On the other hand, in EMS treatments, 0.5 percent (0.134) in Baner, 0.5 per cent (0.142) in Him Alsi-2 and 0.4 per cent (0.064) in Surbhi were found as most efficient for inducing morphological macro mutations.

4.5 Estimates of variability in M_2 generation

Variability in M_2 generation was observed by estimating the mean value, standard deviation and coefficient of variance for each treatment of EMS and gamma rays during *Rabi* 2017-18. The relative coefficient of variation (CV) was estimated based on the CV of mutagen treated population (CV_t) and the non-treated control sample (CV_{nt}), according to the method of Montalvan and Andro (2005). Estimates revealed drastic variation in the mean values of various traits in the treated groups when compared with the control. The trait wise comparisons of the means are as follows:

4.5.1. Days to 50 per cent flowering

Days to flowering were measured and early flowering mutant plants are desirable for this trait. Hence negative shift was followed. Observed data showed that there was no marked variation in days to flowering in both EMS and gamma rays (Table 4.9). However slight variation in flowering time was observed. Range of observed data showed that gamma rays and EMS affected each plant differently since the range in each treatment was different compared to untreated plants.

Table 4.9 Estimates of variability for days to 50 per cent flowering in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent Superiority	CV _t /CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	128-142	138.76 $\pm$ 0.95	2.81	-1.38	5.85	1.95
30	122-143	135.46 $\pm$ 2.60	9.92	-3.72	20.67	1.26
40	125-148	140.29 $\pm$ 2.26	6.02	-0.29	12.54	1.09
50	122-142	135.23 $\pm$ 2.49	6.64	-3.89	13.83	1.20
60	121-142	138.69 $\pm$ 1.48	3.84	-1.43	8.00	0.71
EMS (%)						
0.2	139-141	140.60 $\pm$ 0.22	0.50	-0.07	1.04	0.46
0.4	134-141	139.29 $\pm$ 0.61	1.63	-1.00	3.40	1.25
0.5	139-141	140.23 $\pm$ 0.20	0.52	-0.33	1.08	0.42
0.6	139-141	139.92 $\pm$ 0.24	0.62	-0.55	1.29	0.50
0.8	139-141	139.92 $\pm$ 0.14	0.35	-0.55	0.73	0.28
Control	139-141	140.70 $\pm$ 0.21	0.48			0.44
Him Alsi-2						
Gamma rays (kR)						
20	126-148	139.55 $\pm$ 1.97	4.68	0.90	7.80	4.11
30	125-140	133.71 $\pm$ 1.90	4.02	-3.32	6.70	4.01
40	121-148	133.69 $\pm$ 2.59	6.99	-3.33	11.65	5.37
50	129-140	131.44 $\pm$ 1.13	2.58	-4.96	4.30	2.38
60	129-140	136.67 $\pm$ 1.26	3.58	-1.18	5.97	2.60
EMS (%)						
0.2	120-141	137.91 $\pm$ 1.44	4.91	-0.28	8.18	4.02
0.4	128-140	138.12 $\pm$ 0.74	2.69	-0.13	4.48	2.05
0.5	124-141	139.41 $\pm$ 0.57	2.20	0.80	3.67	1.56
0.6	138-142	139.95 $\pm$ 0.21	0.67	1.19	1.12	0.59
0.8	134-140	139.71 $\pm$ 0.25	0.93	1.02	1.55	0.69
Control	138-140	138.30 $\pm$ 0.21	0.60			0.49
Surbhi						
Gamma rays (kR)						
20	123-140	136.87 $\pm$ 1.66	4.69	1.24	6.01	3.43
30	138-145	140.90 $\pm$ 0.77	1.72	4.22	2.21	1.61
40	139-141	139.60 $\pm$ 0.27	0.60	3.25	0.77	0.56
50	139-142	140.70 $\pm$ 0.26	0.59	4.07	0.76	0.55
60	129-140	137.20 $\pm$ 1.21	2.79	1.48	3.58	2.54
EMS (%)						
0.2	139-140	140.00 $\pm$ 0.14	0.38	3.55	0.49	0.28
0.4	139-141	139.89 $\pm$ 0.20	0.43	3.47	0.55	0.42
0.5	138-140	138.91 $\pm$ 0.16	0.39	2.74	0.50	0.34
Control	134-138	135.20 $\pm$ 0.33	0.78			0.70

In Baner, minimum mean value for days to 50 per cent flowering was found in 50 kR (135.23 days) followed by 30 kR (135.46 days) of gamma rays. In case of EMS, there was very less difference in days to 50 per cent flowering as compare to control. Percent superiority was found maximum in 50 kR (-3.89 %) followed by 30 kR (-3.72 %) gamma rays, while found minimum in 0.2 percent EMS (-0.07 %). Relative coefficient of variation was found maximum in 50 kR (20.67 %) followed by 30kR (13.83 %) gamma rays, while minimum was found in 0.8 per cent EMS (0.73 %).

In Him Alsi-2, result showed that gamma rays were more effective than EMS for days to 50 percent flowering as negative shift was found in more in gamma rays treated M₂ population then EMS treated population (Table 4.9). The minimum mean value was found in 50 kR (131.44 days) followed by 40 kR (133.69 days), while maximum mean value was found in 0.6 percent EMS (139.95 days) as compare to control (138.30 days). Percent superiority over control follows the same pattern as mean value. Relative coefficient of variation was found maximum in 40 kR gamma rays (11.65 %) followed by 0.2 percent EMS (8.18 %), while minimum relative coefficient of variation was found in 0.6 percent EMS (1.12 %).

In Surbhi, there was no single treatment of gamma rays and EMS which showed significant negative shift for days to 50 percent flowering as compare to control. The relative coefficient was found maximum in 20 kR gamma rays (6.01 %), while minimum in 0.2 percent EMS (0.49 %).

4.4.2 Number of primary branches per plant

Numbers of primary per plant is indirectly related to seed yield and were presented in Table 4.10.

In Baner, perusal of data showed that only three gamma rays treatments i.e. 20 kR (8.82), 40 kR (13.14) and 50 kR (9.31) were significant in showing positive shift in numbers of primary branches per plant from control. The maximum number of primary branches per plant was found in 40 kR (13.14) followed by 50 kR (9.31) of gamma rays, while minimum was observed in 0.2 percent EMS. Percent superiority over control was also found significant positive in 20 kR (16.05 %), 40 kR (72.89 %)

Table 4.10 Estimates of variability for number of primary branches per plant in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV _t /CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	4–18	8.82 $\pm$ 1.004	48.79	16.05	5.30	2.15
30	2–15	5.69 $\pm$ 0.20	66.66	-25.13	7.25	0.51
40	3–23	13.14 $\pm$ 1.33	41.87	72.89	4.55	0.64
50	3–22	9.31 $\pm$ 1.80	69.54	22.50	7.56	0.86
60	2–8	4.77 $\pm$ 0.50	37.46	-37.24	4.07	0.24
EMS (%)						
0.2	2–5	3.40 $\pm$ 0.40	37.20	-55.26	4.04	0.84
0.4	2–11	4.36 $\pm$ 0.63	53.72	-42.63	5.84	1.29
0.5	3–7	5.08 $\pm$ 0.35	24.73	-33.16	2.69	0.72
0.6	1–7	4.08 $\pm$ 0.54	47.46	-46.32	5.16	1.11
0.8	2–6	4.62 $\pm$ 0.33	25.85	-39.21	2.81	0.68
Control	7–9	7.60 $\pm$ 0.22	9.20			0.46
Him Alsi-2						
Gamma rays (kR)						
20	3–13	7.27 $\pm$ 1.05	47.67	-11.34	3.78	2.18
30	2–14	8.14 $\pm$ 1.44	49.85	-0.73	3.96	3.03
40	2–16	11.08 $\pm$ 1.30	42.42	35.12	3.37	2.70
50	9–19	12.78 $\pm$ 1.12	26.18	55.85	2.08	2.34
60	3–17	8.93 $\pm$ 1.29	55.88	8.90	4.43	2.66
EMS (%)						
0.2	3–11	6.27 $\pm$ 0.46	34.15	-23.54	2.71	1.27
0.4	4–9	6.40 $\pm$ 0.25	19.66	-21.95	1.56	0.70
0.5	2–11	6.45 $\pm$ 0.30	25.41	-21.34	2.02	0.83
0.6	2–7	4.95 $\pm$ 0.30	27.40	-39.63	2.17	0.85
0.8	2–11	7.19 $\pm$ 0.43	31.29	-12.32	2.48	1.11
Control	6–9	8.20 $\pm$ 0.33	12.60			0.67
Surbhi						
Gamma rays (kR)						
20	3–10`	4.93 $\pm$ 0.51	40.15	-32.47	2.53	1.06
30	2–6	3.60 $\pm$ 0.54	47.57	-50.68	3.00	1.14
40	2–6	4.70 $\pm$ 0.45	30.17	-35.62	1.90	0.94
50	3–7	4.60 $\pm$ 0.40	27.50	-36.99	1.73	0.84
60	4–18	7.50 $\pm$ 1.38	58.37	2.74	3.68	2.91
EMS (%)						
0.2	3–5	3.93 $\pm$ 0.18	19.28	-46.16	1.21	0.49
0.4	3–5	3.67 $\pm$ 0.24	0.32	-49.73	0.02	0.49
0.5	3–5	3.64 $\pm$ 0.20	18.54	-50.14	1.17	0.42
Control	6–9	7.30 $\pm$ 0.35	15.88			0.73

and 50 kR (22.50 %) gamma rays, while remaining treatments showed negative shift in number of primary branches per plant. The relative coefficient of variation was found maximum in 50 kR gamma rays (7.56 %), while it was found minimum in 0.5 percent (2.69 %).

In Him Alsi-2, maximum number of primary branches per plant was found in 50 kR (12.78) followed by 40 kR (11.08) showing positive shift of means from control (9.93) and only three treatments i.e. 40 kR, 50 kR and 60 kR showed positive shift of mean from control, while other treatment of gamma rays and EMS showed negative shift of mean from control. Percent superiority over control was found maximum in 50 kR gamma rays (55.85 %), while it was minimum in 0.6 percent EMS (-39.63 %). The relative coefficient of variation was found maximum in 60 kR gamma rays (4.43), while minimum was found in 0.4 percent EMS (1.56).

In Surbhi, all the treatments of gamma rays and EMS showed negative shift from control mean except 60 kR gamma rays (7.50) which showed positive shift from control in primary branches per plants.

It was observed that gamma rays treated plants of all three cultivars showed more positive response as compared to EMS treated plants. Also, cultivar Surbhi was affected more negatively than Baner and Him Alsi-2.

4.4.3 Number of secondary branches per plant:

The data pertaining to number of secondary branches per plant are presented in Table 4.11 and based on data following observations were recorded:

In Baner, perusal of data from different doses of gamma rays and EMS showed that only three treatments i.e. 20 kR, 40 kR and 50 kR gamma rays were having positive shift in mean values of 12.29, 15.86 and 12.31 respectively, while rest of the treatments showed negative shift of mean value from control (10.90) for this trait. Percent superiority over control was found maximum in 40 kR (45.50 %) followed by 50 kR (12.94 %) of gamma rays, while it was found minimum in 0.6 percent EMS (-64.04 %). Relative coefficient of variation was found maximum in 50 kR gamma rays (6.56 %), while minimum in 0.5 percent EMS (2.22 %).

Table 4.11 Estimates of variability for number of secondary branches per plant in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV _t /CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	4–18	8.82 $\pm$ 1.004	48.79	16.05	5.30	2.15
30	2–15	5.69 $\pm$ 0.20	66.66	-25.13	7.25	0.51
40	3–23	13.14 $\pm$ 1.33	41.87	72.89	4.55	0.64
50	3–22	9.31 $\pm$ 1.80	69.54	22.50	7.56	0.86
60	2–8	4.77 $\pm$ 0.50	37.46	-37.24	4.07	0.24
EMS (%)						
0.2	2–5	3.40 $\pm$ 0.40	37.20	-55.26	4.04	0.84
0.4	2–11	4.36 $\pm$ 0.63	53.72	-42.63	5.84	1.29
0.5	3–7	5.08 $\pm$ 0.35	24.73	-33.16	2.69	0.72
0.6	1–7	4.08 $\pm$ 0.54	47.46	-46.32	5.16	1.11
0.8	2–6	4.62 $\pm$ 0.33	25.85	-39.21	2.81	0.68
Control	7–9	7.60 $\pm$ 0.22	9.20			0.46
Him Alsi-2						
Gamma rays (kR)						
20	3–13	7.27 $\pm$ 1.05	47.67	-11.34	3.78	2.18
30	2–14	8.14 $\pm$ 1.44	49.85	-0.73	3.96	3.03
40	2–16	11.08 $\pm$ 1.30	42.42	35.12	3.37	2.70
50	9–19	12.78 $\pm$ 1.12	26.18	55.85	2.08	2.34
60	3–17	8.93 $\pm$ 1.29	55.88	8.90	4.43	2.66
EMS (%)						
0.2	3–11	6.27 $\pm$ 0.46	34.15	-23.54	2.71	1.27
0.4	4–9	6.40 $\pm$ 0.25	19.66	-21.95	1.56	0.70
0.5	2–11	6.45 $\pm$ 0.30	25.41	-21.34	2.02	0.83
0.6	2–7	4.95 $\pm$ 0.30	27.40	-39.63	2.17	0.85
0.8	2–11	7.19 $\pm$ 0.43	31.29	-12.32	2.48	1.11
Control	6–9	8.20 $\pm$ 0.33	12.60			0.67
Surbhi						
Gamma rays (kR)						
20	3–10`	4.93 $\pm$ 0.51	40.15	-32.47	2.53	1.06
30	2–6	3.60 $\pm$ 0.54	47.57	-50.68	3.00	1.14
40	2–6	4.70 $\pm$ 0.45	30.17	-35.62	1.90	0.94
50	3–7	4.60 $\pm$ 0.40	27.50	-36.99	1.73	0.84
60	4–18	7.50 $\pm$ 1.38	58.37	2.74	3.68	2.91
EMS (%)						
0.2	3–5	3.93 $\pm$ 0.18	19.28	-46.16	1.21	0.49
0.4	3–5	3.67 $\pm$ 0.24	0.32	-49.73	0.02	0.49
0.5	3–5	3.64 $\pm$ 0.20	18.54	-50.14	1.17	0.42
Control	6–9	7.30 $\pm$ 0.35	15.88			0.73

In Him Alsi-2, except 20 kR (10.18), all the treatments of gamma rays showed positive shift of mean value from control (13.70), while there was negative shift in all the treatments of EMS from control indicating that gamma rays have more positive effect than those treated with EMS. The maximum number of secondary branches was found in 50 kR gamma rays (21.56), followed by 40 kR (19.08), while minimum number of secondary branches per plant was found in 0.6 percent EMS treated population i.e. 3.20 per cent. All the EMS treated population showed inferiority over control, while in gamma rays treated population only 20 kR showed inferiority over control. Relative coefficient of variation varied in different treatments and was found maximum in 60 kR (7.81 %) and minimum in 0.4 percent EMS (2.20 %).

In Surbhi, both gamma rays and EMS treated populations at different concentrations showed negative shift of mean of number of secondary branches per plant from the control (14.50). There was presence of very low of percentage of relative coefficient of variation in mutant population of Surbhi as compared to mutant population of Baner and Him Alsi-2. This showed that the effect of gamma rays and EMS not only depends of its concentration but also physiological and genetic makeup of plants.

4.5.4 Plant height:

Data pertaining to plant height expressed in centimeters and is presented in Table 4.12.

In Baner, all the treatment of gamma rays showed positive shift of plant height from the control, whereas except 0.4 percent EMS (57.85 cm), all other EMS treatment showed negative shift from control (51.60 cm). Maximum superiority over control in plant height per plant was observed in 40 kR gamma rays (18.82 %) followed by 0.4 percent EMS (12.11 %), while minimum percent superiority over control was observed in 0.5 percent EMS (-13.88 %). Relative coefficient of variation was found more in gamma rays treatments than EMS treatments. The highest relative coefficient of variation was found in 50 kR gamma rays (4.52 %), while minimum was found in 0.6 percent EMS (1.17 %).

Table 4.12 Estimates of variability for plant height per plant in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV_l/ CV_{nt}	CD
Baner						
Gamma rays (kR)						
20	36.40-79.00	53.04 $\pm$ 2.70	20.98	2.79	4.18	5.56
30	41.40-70.60	55.74 $\pm$ 2.83	18.31	8.02	3.65	1.37
40	40.00-89.50	61.31 $\pm$ 3.39	22.77	18.82	4.54	1.64
50	40.00-77.40	57.36 $\pm$ 3.61	22.70	11.16	4.52	1.74
60	41.40-70.60	53.05 $\pm$ 2.86	19.40	2.81	3.86	1.37
EMS (%)						
0.2	39.40-51.00	47.41 $\pm$ 1.34	8.95	-8.12	1.78	2.82
0.4	51.50-73.00	57.85 $\pm$ 1.59	10.28	12.11	2.05	3.29
0.5	32.00-55.00	44.44 $\pm$ 1.80	14.60	-13.88	2.91	3.73
0.6	47.00-57.00	50.82 $\pm$ 0.83	5.89	-1.51	1.17	1.72
0.8	39.60-49.80	46.29 $\pm$ 0.92	7.15	-10.29	1.42	1.90
Control	49.00-56.00	51.60 $\pm$ 0.82	5.02			1.70
Him Alsi-2						
Gamma rays (kR)						
20	56.00-89.30	69.49 $\pm$ 3.37	16.08	9.78	7.62	7.04
30	48.80-79.00	69.56 $\pm$ 3.74	15.22	9.89	7.21	7.90
40	56.40-83.00	72.65 $\pm$ 2.67	13.23	14.77	6.27	5.52
50	60.00-94.40	76.42 $\pm$ 4.19	16.44	20.73	7.79	8.79
60	60.00-85.40	66.97 $\pm$ 1.94	11.21	5.80	5.31	3.99
EMS (%)						
0.2	46.00-75.70	61.00 $\pm$ 1.68	12.95	-3.63	6.14	4.69
0.4	67.50-84.20	72.75 $\pm$ 0.85	5.87	14.93	2.78	2.36
0.5	38.50-74.50	57.78 $\pm$ 1.63	15.20	-8.72	7.20	4.47
0.6	46.50-71.60	59.87 $\pm$ 1.60	11.95	-5.42	5.66	4.49
0.8	50.20-79.50	70.00 $\pm$ 1.39	10.48	10.58	4.97	3.89
Control	61.00-65.00	63.30 $\pm$ 1.42	2.11			1.18
Surbhi						
Gamma rays (kR)						
20	29.00-61.00	46.17 $\pm$ 1.99	16.71	5.19	4.82	4.12
30	24.00-45.00	34.70 $\pm$ 1.75	15.96	-16.29	4.60	3.68
40	29.70-40.10	35.48 $\pm$ 0.86	7.65	-14.83	2.20	1.80
50	26.70-34.30	31.56 $\pm$ 0.79	7.90	-22.17	2.28	1.66
60	34.40-53.00	42.54 $\pm$ 1.90	14.11	-1.61	4.07	3.99
EMS (%)						
0.2	23.00-45.00	34.40 $\pm$ 1.22	13.76	-16.85	3.97	2.52
0.4	22.00-37.00	32.67 $\pm$ 1.46	13.43	-20.09	3.87	3.07
0.5	24.00-45.00	33.91 $\pm$ 1.63	15.91	-17.77	4.59	3.40
Control	40.00-45.00	43.40 $\pm$ 0.45	3.47			0.95

In Him Alsi-2, highest positive shift of mean in plant height per plant mean was found in 50 kR (76.42 cm) followed by 0.4 per cent EMS (72.75 cm), while negative shift of mean was found in 0.5 per cent EMS (57.78 cm) as compared to control (63.30 cm). The percent superiority over control followed the same pattern as found in mean values. The relative coefficient of variation was observed maximum in 50 kR (7.79 %) followed by 20 kR (7.62 %), while it was found minimum in 0.4 per cent EMS (2.78 %).

In Surbhi, except 20 kR gamma rays, all the treatments of gamma rays and EMS showed negative shift of mean from control. The relative coefficient of variation was found maximum in 20 kR gamma rays (4.82 %) followed by 30 kR (4.60 %) and 0.5 percent EMS (4.59 %), while it was found minimum in 40 kR gamma rays (2.20 %).

4.5.5 Number of capsules per plant

Capsules per plant measured in numbers are directly related to seed yield and data are presented in Table 4.13.

In Baner, highest mean performance of treated mutants was observed in 40 kR gamma rays (106.79) showing positive shift of mean than that of untreated plant (55.60). Percent superiority of mutants over control at 40 kR (92.07 %) was found at maximum. It was observed that all the EMS treatment caused negative shift of mean from control and was inferior to control. The relative coefficient of variation was found maximum in 50 kR (10.31 %) followed by 60 kR (10.21 %), while it was found minimum in 0.6 per cent EMS (1.60 %).

In Him Alsi-2, all the EMS treated populations derived from different concentration and 20 kR gamma rays treated population showed negative shift from mean performance of control and was found inferior than that of control mean (52.10). The highest mean performance was found in 40 kR (103.77) showing 99.17 per cent superiority over control. Relative coefficient of variation was found maximum in 30 kR (25.66 %) followed by 20 kR (22.80 %), while minimum was found in 0.4 per cent EMS (5.11 %). Also it was found that relative coefficient of variation in gamma rays treated population was higher than that of EMS treated populations.

Table 4.13 Estimates of variability for number of capsules per plant in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV _t / CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	17-217	69.12 $\pm$ 13.19	78.68	24.32	8.72	27.16
30	12-113	31.00 $\pm$ 7.86	91.40	-44.24	10.13	3.80
40	16-189	106.79 $\pm$ 10.93	42.18	92.07	4.68	5.28
50	10-164	58.46 $\pm$ 15.07	92.97	5.14	10.31	7.27
60	10-123	44.15 $\pm$ 11.28	92.13	-20.59	10.21	5.40
EMS (%)						
0.2	14-25	19.80 $\pm$ 1.00	15.94	-64.39	1.77	0.75
0.4	12-98	31.57 $\pm$ 5.00	66.33	-43.22	7.35	11.59
0.5	16-39	25.00 $\pm$ 1.83	26.33	-55.04	2.92	3.78
0.6	14-25	19.54 $\pm$ 0.78	14.42	-64.86	1.60	1.62
0.8	19-45	27.15 $\pm$ 2.08	27.55	-51.17	3.05	4.30
Control	48-62	55.60 $\pm$ 1.59	9.02			3.28
Him Alsi-2						
Gamma rays (kR)						
20	11-122	44.45 $\pm$ 11.89	88.68	-14.68	22.80	24.84
30	12-197	65.86 $\pm$ 23.34	99.80	26.41	25.66	49.03
40	16-206	103.77 $\pm$ 15.03	52.33	99.17	13.45	31.12
50	41-189	82.56 $\pm$ 14.49	52.67	58.46	13.54	30.44
60	26-289	78.87 $\pm$ 16.85	83.15	51.38	21.38	34.70
EMS (%)						
0.2	11-29	19.18 $\pm$ 1.09	25.56	-63.19	6.57	3.03
0.4	10-32	22.08 $\pm$ 0.88	19.87	-57.62	5.11	2.42
0.5	9-28	19.93 $\pm$ 0.91	24.62	-61.75	6.33	2.49
0.6	8-21	14.30 $\pm$ 0.91	28.35	-72.55	7.29	2.54
0.8	7-29	18.38 $\pm$ 1.13	32.53	-64.72	8.36	3.16
Control	50-56	52.10 $\pm$ 0.64	3.89			1.79
Surbhi						
Gamma rays (kR)						
20	26-78	48.27 $\pm$ 4.32	34.70	-10.11	8.05	8.95
30	12-35	21.80 $\pm$ 1.96	28.43	-59.40	6.60	4.12
40	11-55	26.80 $\pm$ 3.74	44.07	-50.09	10.23	7.87
50	11-14	14.60 $\pm$ 1.02	22.18	-72.81	5.15	2.15
60	23-120	58.70 $\pm$ 9.74	52.45	9.31	12.17	20.45
EMS (%)						
0.2	14-25	19.07 $\pm$ 0.87	17.67	-64.49	4.10	1.79
0.4	14-26	19.56 $\pm$ 1.29	19.82	-63.58	4.60	2.71
0.5	15-25	19.27 $\pm$ 1.02	17.53	-64.12	4.07	2.13
Control	50-56	53.70 $\pm$ 0.70	4.31			1.46

In Surbhi, there was only one treatment i.e. 60 kR gamma rays which showed positive shift of mean (58.70) from control (53.70) and was found 9.31 per cent superior to control. Relative coefficient of variation was found maximum in 60 kR gamma rays treated mutants (12.17 %), while it was found minimum in 0.5 percent (4.07 %) EMS treated mutants.

4.5.6 Day to 75 per cent maturity

For this character earliness is required therefore, superiority of the treatments in negative direction are preferred. Observation of data of M₂ mutants was showed in Table 4.14

In Baner, maximum negative shift of mean for days to 75 per cent maturity was observed in 50 kR gamma rays (187.22 days) having range of 181 to 216 days which was 13.64 percent superior compared to untreated population followed by 30 kR gamma rays (192.57 days). However, the relative coefficient of variation was found maximum in 40 kR (41.47 %) followed by 20 kR (32.42 %), while minimum was observed in 0.5 per cent EMS (1.05 %). The difference may be due to range observed in various mutant populations under different treatments of EMS and gamma rays.

In Him Alsi-2, maximum negative shift of mean was observed in 50 kR gamma rays (187.22 days) followed by 30 kR (192.57 days) with maximum per cent superiority over control of 12.80 per cent and 10.31 per cent respectively. The gamma rays treatment showed higher per cent superiority then the EMS treatments over control. Relative coefficient of variation was found maximum in 40 kR (13.13 %) followed by 20 kR (10.40 %) and 0.2 per cent EMS (10.15 %).

In Surbhi, most of the treatments showed positive shift of mean from mean of untreated population which is undesirable for earliness except 20 kR and 60 kR of gamma rays which respectively showed 6.08 per cent and 0.65 per cent superior over control. Similarity relative coefficient of variation was found maximum in 20 kR gamma rays (13.60 %) followed by 60 kR gamma rays (6.82 %).

Table 4.14 Estimates of variability for number of days to 75 per cent maturity in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV _t / CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	178-218	210.24 $\pm$ 3.14	6.16	-3.03	32.42	6.47
30	179-209	192.57 $\pm$ 4.01	5.90	-11.18	31.05	8.47
40	177-122	201.15 $\pm$ 4.39	7.88	-7.22	41.47	9.10
50	181-216	187.22 $\pm$ 3.67	5.89	-13.64	31.00	7.71
60	185-210	200.20 $\pm$ 2.60	5.02	-7.66	26.42	5.35
EMS (%)						
0.2	220-221	220.70 $\pm$ 0.15	0.22	1.80	1.16	0.32
0.4	201-220	216.21 $\pm$ 1.65	2.86	-0.27	15.05	3.43
0.5	219-221	219.54 $\pm$ 0.22	0.35	1.26	1.84	0.45
0.6	219-222	220.00 $\pm$ 0.39	0.64	1.48	3.37	0.82
0.8	219-220	219.23 $\pm$ 0.12	0.20	1.12	1.05	0.25
Control	216-217	216.80 $\pm$ 0.13	0.19			0.28
Him Alsi-2						
Gamma rays (kR)						
20	180-221	206.00 $\pm$ 2.87	6.24	-4.05	10.40	8.09
30	179-209	192.57 $\pm$ 4.01	4.90	-10.31	8.17	8.47
40	177-222	201.15 $\pm$ 4.39	7.88	-6.31	13.13	9.10
50	181-216	187.22 $\pm$ 3.67	5.89	-12.80	9.82	7.71
60	185-210	200.20 $\pm$ 2.60	5.02	-6.75	8.37	5.35
EMS (%)						
0.2	180-219	213.14 $\pm$ 2.77	6.09	-0.73	10.15	7.72
0.4	198-219	216.32 $\pm$ 1.12	2.59	0.75	4.32	3.10
0.5	187-220	217.17 $\pm$ 1.10	2.73	1.15	4.55	3.01
0.6	201-221	218.50 $\pm$ 0.96	1.97	1.77	3.28	2.70
0.8	199-219	217.71 $\pm$ 0.82	1.98	1.40	3.30	2.28
Control	214-218	214.70 $\pm$ 0.42	0.60		1.00	0.87
Surbhi						
Gamma rays (kR)						
20	173-209	202.40 $\pm$ 3.55	6.80	-6.08	13.60	7.36
30	209-219	216.40 $\pm$ 1.18	1.72	0.42	3.44	2.47
40	219-221	219.80 $\pm$ 0.25	0.36	2.00	0.72	0.52
50	219-222	220.70 $\pm$ 0.26	0.37	2.41	0.74	0.55
60	198-222	214.10 $\pm$ 2.31	3.41	-0.65	6.82	4.85
EMS (%)						
0.2	219.00	219.00 $\pm$ 0.00	0.00	1.62	0.00	0.00
0.4	220-222	220.33 $\pm$ 0.20	0.32	2.24	0.64	0.49
0.5	219-220	219.09 $\pm$ 0.09	0.14	1.67	0.28	0.19
Control	215-218	215.50 $\pm$ 0.33	0.50			0.68

Overall, all the treatments of gamma rays and EMS showed varied responses depending upon the dose/concentration of mutagens and the morphological and genetic characteristics of these cultivars.

4.5.7 Number of Seeds per capsules:

Data pertaining to number of seeds per capsules was recorded as shown in Table 4.15.

In Baner, all the mutant population at different concentrations of EMS showed negative shift from mean of untreated population, while in case of gamma rays treatments, 20 kR (8.55), 40 kR (8.53) and 50 kR (8.53) mutant populations showed positive shift from mean of untreated population. The percent superiority over control followed same pattern as of mean. The highest inferiority over control was found in 0.2 per cent EMS (-23.25 %) followed by 0.6 per cent EMS (-22.65 %). Maximum relative coefficient of variation was found in 30 kR (2.36 %) gamma rays followed by 0.4 per cent EMS (1.64 %), while minimum was found in 40 kR gamma rays (0.43 %).

In Him Alsi-2, perusal of data pertaining to seeds per capsule found that only 30 kR (8.86) and 50 kR (8.83) gamma rays treatments showed positive shift of mean of number of seeds per capsule from mean performance of control (8.23), while other treatments showed negative shift of mean from control. All the mutant population treated with EMS were found more inferior than gamma rays treated mutants showing that presence of negative effect of EMS in Him Alsi-2 then gamma rays. Maximum inferiority over control was found in 0.5 per cent EMS (-38.86 %). On the other hand, relative coefficient of variation was more in EMS treated mutants than those irradiated with gamma rays at different doses. The highest percentage of relative coefficient of variation was found in 0.5 per cent EMS (5.29 %), while lowest was found in 40 kR gamma rays (0.80 %).

In Surbhi, all the mutant population treated with gamma rays and EMS showed negative shift in mean performance over control and were found inferior compared to untreated population (control), which clearly indicates that effect of EMS and gamma rays was more in Surbhi then Baner and Him Alsi-2. The relative

Table 4.15 Estimates of variability for number of seeds per capsule in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV _t / CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	7.80-9.30	8.55 $\pm$ 0.23	5.59	3.01	0.96	0.24
30	4.90-8.90	7.62 $\pm$ 0.29	13.74	-8.19	2.36	0.14
40	8.20-8.90	8.53 $\pm$ 0.05	2.49	2.77	0.43	0.02
50	7.80-9.60	8.53 $\pm$ 0.13	5.57	2.77	0.96	0.06
60	6.90-8.90	8.22 $\pm$ 0.19	8.24	-0.96	1.42	0.09
EMS (%)						
0.2	5.90-6.80	6.37 $\pm$ 0.10	5.13	-23.25	0.88	0.22
0.4	6.00-8.90	7.96 $\pm$ 0.20	9.54	-4.10	1.64	0.42
0.5	7.30-8.80	7.93 $\pm$ 0.14	6.29	-4.46	1.08	0.29
0.6	5.90-6.80	6.42 $\pm$ 0.08	4.67	-22.65	0.80	0.17
0.8	6.30-7.40	6.83 $\pm$ 0.09	5.01	-17.71	0.86	0.20
Control	8.00-9.00	8.30 $\pm$ 0.15	5.82		0.88	0.32
Him Alsi-2						
Gamma rays (kR)						
20	8.20-9.60	8.63 $\pm$ 0.13	4.89	-1.93	1.02	0.27
30	7.70- 10.00	8.86 $\pm$ 0.28	9.03	0.68	1.89	0.60
40	7.80-8.80	8.41 $\pm$ 0.09	3.84	-4.43	0.80	0.19
50	8.30-9.80	8.83 $\pm$ 0.15	5.06	0.34	1.06	0.31
60	6.20-8.90	8.23 $\pm$ 0.21	9.66	-6.48	2.02	0.42
EMS (%)						
0.2	4.80-9.20	6.90 $\pm$ 0.27	18.23	-21.59	3.81	0.74
0.4	3.00-9.00	7.70 $\pm$ 0.29	21.68	-12.50	4.53	0.80
0.5	2.80-8.20	5.38 $\pm$ 0.25	25.36	-38.86	5.29	0.69
0.6	3.80-9.40	6.55 $\pm$ 0.35	23.85	-25.57	4.98	0.98
0.8	3.00-8.20	5.75 $\pm$ 0.27	24.53	-34.66	5.12	0.75
Control	8.00-9.00	8.80 $\pm$ 0.13	4.79			0.37
Surbhi						
Gamma rays (kR)						
20	7.60-8.60	8.10 $\pm$ 0.06	3.10	-2.41	0.53	0.13
30	6.90-8.60	7.68 $\pm$ 0.23	9.62	-7.47	1.65	0.49
40	5.00-9.00	7.50 $\pm$ 0.45	19.12	-9.64	3.29	0.95
50	5.00-8.00	6.70 $\pm$ 0.30	14.16	-19.28	2.43	0.63
60	7.30-8.70	8.22 $\pm$ 0.15	5.81	-0.96	1.00	0.32
EMS (%)						
0.2	5.00-7.00	6.51 $\pm$ 0.12	7.27	-21.57	1.25	0.25
0.4	5.22-7.89	6.44 $\pm$ 0.20	9.41	-22.41	1.62	0.42
0.5	5.90-7.20	6.90 $\pm$ 0.21	11.20	-16.87	1.92	0.45
Control	8.00-9.00	8.30 $\pm$ 0.15	5.82			0.30

coefficient of variation was found maximum in 40 kR gamma rays (3.29 %) while minimum was found in 20 kR (0.53 %).

4.5.7 Seed yield per plant

Data pertaining to seed yield per plant was shown in Table 4.16.

In Baner, among all the treatments of gamma rays and EMS, the maximum mean value was observed in 40 kR (5.26 g) followed by 20 kR (3.87 g) with percent superiority over control by 78.31 percent and 31.19 per cent respectively, and only these two treatments showed positive shift of mean values from the control (2.95 g). All other treatments showed negative shift from mean of control. EMS treated mutants were found more inferior than gamma rays treated mutants. Percent superiority over control was found maximum in 40 kR (78.31 %). The highest inferiority over control was found in 0.2 per cent EMS (-84.75 %) followed by 0.6 per cent EMS (-84.41 %). The highest percentage of relative coefficient of variation was found in 30 kR (46.41 %) followed by 60 kR (37.70 %), while minimum percentage of relative coefficient of variation was found in 0.6 per cent EMS (1.46 %).

In Him Alsi-2, among all the doses of gamma rays, 20 kR showed negative shift in mean value from mean of control, while all other showed positive shift. Among all EMS treatment, only 0.8 percent (2.65 g) showed positive shift of mean from control (2.53 g). The highest mean value was observed in 40 kR (4.96 g) followed by 50 kR (4.24 g). The percent superiority was found highest in 40 kR (96.05 %). Among all the treatment of gamma rays and EMS, highest relative coefficient of variation was observed in 20 kR (29.23 %) followed by 30 kR (24.88 %), while minimum was found in 60 kR (2.37 %).

In Surbhi, among all gamma rays and EMS treatment, treatment with 20 kR (2.47 g) only showed positive shift of mean than mean of control (2.37 g), whereas the other treatments showed negative shift of mean. The percent superiority was found maximum in 50 kR (94.94 %), followed by 0.2 percent and 0.3 percent EMS (-81.01 %). The relative coefficient of variation was found maximum in 30 kR (23.47 %), while minimum was found in 0.5 per cent EMS (1.18 %).

Table 4.16 Estimates of variability for seed yield per plant in M₂ generation

Treatments	Range	Mean $\pm$ error	CV (%)	Percent superiority	CV _t /CV _{nt}	CD
Baner						
Gamma rays (kR)						
20	1.02-11.79	3.87 $\pm$ 0.68	72.26	31.19	24.92	1.40
30	0.21-5.85	1.24 $\pm$ 0.46	134.58	-57.97	46.41	0.22
40	0.21-9.74	5.26 $\pm$ 0.67	52.45	78.31	18.09	0.32
50	0.14-8.13	2.76 $\pm$ 0.74	96.70	-6.44	33.34	0.36
60	0.21-5.99	1.98 $\pm$ 0.60	109.33	-32.88	37.70	0.29
EMS (%)						
0.2	0.45-0.49	0.45 $\pm$ 0.01	5.81	-84.75	2.00	0.02
0.4	0.12-3.31	1.26 $\pm$ 0.21	62.58	-57.29	21.58	0.44
0.5	0.21-1.56	0.87 $\pm$ 0.10	39.95	-70.51	13.78	0.20
0.6	0.43-0.50	0.46 $\pm$ 0.01	4.22	-84.41	1.46	0.01
0.8	0.43-0.50	0.47 $\pm$ 0.01	5.03	-84.07	1.73	0.01
Control	2.76-3.02	2.95 $\pm$ 0.03	2.90			0.06
Him Alsi-2						
Gamma rays (kR)						
20	0.09-7.34	2.21 $\pm$ 0.79	118.98	-12.65	29.23	1.66
30	0.12-8.18	2.93 $\pm$ 1.05	101.26	15.81	24.88	2.22
40	0.38-9.94	4.96 $\pm$ 0.69	50.42	96.05	12.39	1.44
50	2.01-8.14	4.24 $\pm$ 0.55	38.68	67.59	9.50	1.15
60	0.45-9.41	3.19 $\pm$ 0.61	9.66	26.09	2.37	1.26
EMS (%)						
0.2	1.27-3.14	2.22 $\pm$ 0.10	21.95	-12.25	5.39	0.29
0.4	1.23-3.20	2.30 $\pm$ 0.12	25.41	-9.09	6.24	0.32
0.5	1.32-3.44	2.10 $\pm$ 0.09	23.46	-17.00	5.76	0.25
0.6	1.20-2.94	2.20 $\pm$ 0.09	17.97	-13.04	4.42	0.25
0.8	1.78-3.42	2.65 $\pm$ 0.09	17.58	4.74	4.32	0.25
Control	2.40-2.78	2.53 $\pm$ 0.10	4.07			0.07
Surbhi						
Gamma rays (kR)						
20	1.47-4.21	2.47 $\pm$ 0.20	30.66	4.22	8.35	0.40
30	0.12-1.60	0.51 $\pm$ 0.14	86.14	-78.48	23.47	0.29
40	0.22-2.45	0.88 $\pm$ 0.21	75.16	-62.87	20.48	0.44
50	0.09-0.21	0.12 $\pm$ 0.01	28.75	-94.94	7.83	0.02
60	0.67-6.11	2.59 $\pm$ 0.58	70.42	9.28	19.19	1.21
EMS (%)						
0.2	0.43-0.50	0.45 $\pm$ 0.01	4.47	-81.01	1.22	0.01
0.4	0.43-0.50	0.45 $\pm$ 0.01	4.79	-81.01	1.31	0.02
0.5	0.45-0.50	0.47 $\pm$ 0.02	4.32	-80.17	1.18	0.03
Control	2.22-2.54	2.37 $\pm$ 0.03	3.67			0.05

Among three cultivars, Surbhi was more negatively affected than other two cultivars. The gamma rays were found more effective in increasing the yield than EMS.

4.5.8 Selection of M₂ Mutants

On the basis of performance of mutants in M₂ generation, 113 mutant lines were selected based on their yield and earliness in maturity and carried forward to M₃ generation for further evaluation for various quantitative and qualitative characters. Out of 113 lines, 28 lines exhibited earliness and 85 lines were high yielding as compared to their respective controls (Table 4.17).

Table 4.17 Selection of M₂ mutants based on their performance

Treatments	No. of mutant selected	Selection criteria	
		Earliness	High yield
T-1	10	5	5
T-2	8	3	5
T-3	11	3	8
T-4	4	1	3
T-5	6	1	5
T-6	4	0	4
T-7	6	2	4
T-8	10	2	8
T-9	6	0	6
T-10	9	0	9
T-11	10	5	5
T-12	2	0	2
T-13	5	2	3
T-14	3	0	3
T-15	2	1	1
T-16	2	1	1
T-17	2	0	2
T-18	5	0	5
T-19	8	2	6
Total	113	28	85

4.6 Mean performance of mutant lines in M₃ generation

Mean performance of M₃ mutants has been recorded based on their performance of characters controlling the genetic variation. The performance of these mutant lines was compared within the treatment and between treatments mean as well as their respective untreated one i.e. Baner, HimAlsi-2 and Surbhi. Significant from deviation of mean were observed in all the mutants in both negative and positives ways as shown in Appendix-IV.

In days to 50 per cent flowering, maximum negative shift from mean observed in treatment T-11 (60 kR of Him Alsi-2) i.e. 160.77 days, followed by T-13 (40 kR of Surbhi) i.e. 163.75 days, while maximum positive shift was observed in T-2 (30 kR of Baner) i.e. 174.33 days, followed by T-6 (20 kR of Him Alsi-2) 172.90 days. Since for early maturity negative shift from mean is favourable, gamma rays having dose of 40 to 60 kR showed favourable effect for days to 50 per cent flowering. Among all the mutant lines, 32 mutant lines showed significant negative shift from the mean, while other showed either negative shift or significant positive shift which is not favourable in generating early maturity. In T-11 and T-13 treatments, all the mutant lines showed significant negative shift of mean. Mutant lines M₃₋₁₁₋₂ showed maximum negative of shift mean of days to 50 per cent flowering i.e. 153.00 days followed by T₃₋₇₋₄ (154.00 days), M₃₋₁₁₋₉ (156.00 days) showing that theses mutant lines can be selected further for development of early maturing cultivar.

Plant height and technical height per plant can be used for differentiating the flax type and oil type mutant in linseed. Less technical height can lead to higher number of secondary branches so as the seed yield and vice versa, while high technical height of plants showed high fibre yield. Highest mean performance in plant height was found in T-8 of 40 kR gamma rays (77.27 cm), followed by -T2 (74.83 cm) of gamma rays while minimum mean performance in plant height was observed in T-13 (48.83 cm). Among all the mutant lines, the lines derived from treated variety Surbhi were found having negative shift from overall mean performance in plant height (Appendix-IV). Highest mean performance in plant height was observed in mutant line M₃₋₁₈₋₃ (86.95 cm) followed by M₃₋₁₉₋₅ (86.50 cm), M₃₋₃₋₉ (86.50 cm) and M₃₋₁₀₋₃ (84.25 cm), while lowest mean height was observed in M₃₋₁₃₋₄ (43.00 cm), followed by M₃₋₁₃₋₂ (45.50 cm). The mean performance of technical height per plant

was found maximum in treatment T-8 (38.38 cm), followed by T-7 (38.29), and while minimum was observed in T-13 (15.92 cm). Among all the mutant lines, the maximum positive shift of mean in technical height was found in M₃₋₁₋₇ (49.25 cm) followed by M₃₋₇₋₁ (46.50 cm), while minimum was found in M₃₋₁₃₋₂ (15.00 cm).

Number of primary branches per plant is indirectly related to the seed yield. More number of primary branches can bear more secondary branches so as the yield. Highest positive shift of mean performance was observed in treatment T-12 (10.67) followed by treatment T-10 (10.39) and T-15 (10.35), while lowest mean performance in primary branches per plant was found in T-2 (8.28) followed by T-8 treatment (8.38). All the mutant lines showed significant difference in mean performance of primary branches. The highest mean performance in primary branches per plant was observed in M₃₋₁₁₀₋₄ (12.50) followed by M₃₋₅₋₂ (12.00) and M₃₋₁₈₋₄ (11.80) mutant lines while minimum mean performance was observed in M₃₋₁₄₋₃ (6.00). Mean performance of number of secondary branches per plant of mutant lines and treatments showed that highest mean of secondary branches per plant as found in treatment T-12 (15.50) followed by T-8 (15.38), while minimum was found in T-1 (11.61). Most of the mutant lines showed negative shift from mean when compared to their respective control of Baner, Him Alsi-2 and Baner. Among all the mutant lines, lines of Him Alsi-2 i.e. M₃₋₁₀₋₃ (16.50), M₃₋₇₋₃ (16.25), M₃₋₇₋₄ (16.25) and M₃₋₁₁₋₅ (16.00); Baner i.e. M₃₋₁₈₋₄ (15.55), M₃₋₁₆₋₁ (15.50) and M₃₋₁₆₋₂ (14.50); Surbhi i.e. M₃₋₁₂₋₂ (16.50) showed positive shift in mean performance than their respective mean of control. While minimum performance was observed in mutants of Him Alsi-2 i.e. M₃₋₁₁₋₂ (9.00) and M₃₋₁₁₋₇ (9.50) in Treatment T-11.

Observations of days to 75 per cent maturity showed that treatment namely, T-11, T-13, T-14, T-7, T-8, T-12, T-15 and T-16 showed significant negative shift of mean performance in which treatments T-11 (208.95 days) followed by T-13 (214.33 days) and T-14 (215.25 days) showed maximum negative shift from the mean while all other treatments showed either positive shift or significant positive shift of mean performance. The maximum positive shift of mean in number of days to 75 per cent maturity was found in T-19 (227.11 days). Among all the mutant lines, M₃₋₂₋₁₁ (203.50 days), M₃₋₁₁₋₉ (201.00 days) and M₃₋₁₄₋₁ (196.50 days) showed maximum negative shift of mean from grand mean and mean of their respective control, thus indicating

that these lines can be used for development of early maturing varieties of linseed by choosing suitable breeding method.

Number of capsules per plant directly affects the seed yield of plant. More number of capsules leads to higher yield and vice versa. Data evaluation revealed that treatments T-12 (72.33), T-7 (66.43), T-13 (65.58), T-15 (65.00), T-17 (65.17) and T-10 (62.28) showed significant positive shift from the grand mean for number of capsules per plant. Treatments T-16 (57.33) and T-18 (55.79) showed positive shift from mean, while rest of other treatments showed negative shift from grand mean. Among all the mutant lines derived from EMS and Gamma rays treated varieties of linseed i.e. Baner, Him Alsi-2 and Surbhi, mutant line M₃₋₁₃₋₂ (92.00) showed highest number of capsules per plant followed by M₃₋₁₀₋₉ (76.50), while lowest number of capsules per plant was found in M₃₋₁₁₋₃ (32.50) followed by M₃₋₉₋₅ (36.25). Mean performance showed that the highest and lowest number of capsules per plant were found in treatments of gamma rays at different concentrations but in different mutant lines derived from different cultivar i.e. Surbhi and Him Alsi-2 respectively which indicates that effect of gamma rays depend upon its dose as well as morpho physiological and genetical characters of varieties of linseed.

The evaluation of mean performance of number of seeds per capsule showed that treatments i.e. T-17 (8.73), T-8 (8.69) and T-16 (8.68) showed significant positive shift from the mean and T-9 (8.64), T-14 (8.63), T-14 (8.62), T-6 (8.61), T-12 (8.58), T-19 (8.57), T-13 (8.54), T-18 (8.51) and T-5 (8.44) showed positive shift from mean, while other treatment showed negative shift from mean. Among all the mutant lines of different treatments, mutant M₃₋₉₋₃ (9.30) showed highest seed per capsule while lowest was observed in M₃₋₃₋₈ (7.00), M₃₋₈₋₆ (7.00), M₃₋₁₀₋₄ (7.00), M₃₋₁₁₋₄ (7.00) and M₃₋₁₁₋₇ (7.00). Based on data of number of seeds per capsule, lines which showed higher number of seed per capsules can be used in breeding programme to increase the seed yield per plant in linseed.

The biological yield per plant is indirectly related to both fibre and seed yield in linseed. The mean performance of mutant lines and treatment showed significant difference among mutant lines as well treatments. The treatments viz. T-16 (9.37 g), T-19 (9.19 g), T-12 (8.93 g), T-9 (8.87 g), T-19 (9.19 g), T-18 (8.70 g) and T-17 (8.67 g) showed significant positive shift from mean. Among all the mutant lines, highest

biological yield per plant was observed in M_{3-2-5} (13.50 g) while lowest was found in M_{3-11-8} (2.50 g).

Mean performance of seed yield per plant showed that treatments T-13 (3.44 g), T-12 (3.30 g), T-10 (3.28 g), T-15 (3.14 g), T-18 (3.11 g) and T-16 (3.04 g) showed significant positive shift of mean indicating that these doses of mutagen can be used for achieving higher yield in future breeding programme. Among all the mutant lines, 40 number of mutant lines showed significant positives shift from grand mean in which the line M_{3-13-2} (4.58 g) showed highest seed yield per plant followed by M_{3-10-8} (4.53 g), M_{3-19-4} (4.01 g) and M_{3-18-4} (3.98 g), while lowest seed yield per plant was observed in M_{3-4-4} (1.17 g) followed by M_{3-4-3} (1.26 g). It was found that lowest seed yield per plant was observed in mutant lines of treatment T-4 (40 kR gamma rays).

Harvest index was also estimated and data revealed that five treatments i.e. T-13 (0.53), T-1 (0.48), T-14 (0.45), T-15 (0.44) and T-10 (0.44) showed significant positive shift from mean. The mutant line M_{3-13-4} (0.94) showed highest harvest index followed by M_{3-11-8} (0.78) and M_{3-10-5} (0.78), while lowest harvest index was observed in M_{3-19-5} (0.18).

The trait of 1000 seed weight can also be used for selection of superior mutant lines and data revealed that among seven significant treatments, highest significant shift of mean was observed in treatment T-19 (6.57 g) followed by T-1 (6.55 g). Among all the mutant lines, M_{3-1-3} (7.10 g) and M_{3-3-1} (7.10 g) showed highest value of 1000 seed weight, while lowest was observed in M_{3-15-1} (5.15 g) depicting that the mutant lines having higher 1000 seed weight can be used in further breeding programmes or else can be directly selected for development of new variety of linseed.

4.7 Morphological variation of mutant lines in M_3 generation

Characterisation of M_3 mutant lines was done along with their respective control. Mutant lines were characterised for the flower colour, flower shape, flower aestivation and seed colour of mutant lines as shown in Appendix-IV and Table 4.18.

Table 4.18 Morphological variation of mutant lines in M₃ generation

Sr. No.	Morphological character	Number of lines	Mutant which showed deviation from control
1	Flower Colour		
I	light blue	7	M ₃₋₂₋₁ , M ₃₋₂₋₂ , M ₃₋₂₋₃ , M ₃₋₆₋₄ , M ₃₋₃₋₂ , M ₃₋₃₋₅ , M ₃₋₅₋₂
Ii	Dark Blue	4	M ₃₋₃₋₈ , M ₃₋₃₋₉ , M ₃₋₁₆₋₂ , M ₃₋₁₇₋₂
2	Flower shape		
I	Star shape	38	M ₃₋₁₋₄ , M ₃₋₁₋₅ , M ₃₋₁₋₇ , M ₃₋₁₋₉ , M ₃₋₁₋₁₀ , M ₃₋₂₋₄ , M ₃₋₂₋₆ , M ₃₋₂₋₇ , M ₃₋₃₋₆ , M ₃₋₃₋₇ , M ₃₋₃₋₈ , M ₃₋₃₋₁₁ , M ₃₋₄₋₄ , M ₃₋₅₋₁ , M ₃₋₅₋₂ , M ₃₋₅₋₄ , M ₃₋₅₋₅ , M ₃₋₇₋₄ , M ₃₋₇₋₅ , M ₃₋₈₋₁ , M ₃₋₈₋₂ , M ₃₋₈₋₅ , M ₃₋₈₋₆ , M ₃₋₈₋₈ , M ₃₋₈₋₁₀ , M ₃₋₉₋₁ , M ₃₋₉₋₂ , M ₃₋₉₋₃ , M ₃₋₉₋₄ , M ₃₋₁₀₋₂ , M ₃₋₁₀₋₃ , M ₃₋₁₀₋₆ , M ₃₋₁₀₋₇ , M ₃₋₁₀₋₈ , M ₃₋₁₉₋₁ , M ₃₋₁₉₋₃ , M ₃₋₁₉₋₄ , M ₃₋₁₉₋₅
Ii	Funnel shape	6	M ₃₋₄₋₃ , M ₃₋₆₋₂ , M ₃₋₆₋₃ , M ₃₋₆₋₄ , M ₃₋₈₋₄ , M ₃₋₁₁₋₁₀
3	Flower aestivation		
I	Velvate	15	M ₃₋₃₋₁ , M ₃₋₃₋₂ , M ₃₋₃₋₅ , M ₃₋₃₋₁₀ , M ₃₋₅₋₃ , M ₃₋₅₋₆ , M ₃₋₈₋₅ , M ₃₋₈₋₆ , M ₃₋₈₋₇ , M ₃₋₈₋₉ , M ₃₋₁₀₋₂ , M ₃₋₁₀₋₆ , M ₃₋₁₉₋₁ , M ₃₋₁₉₋₃ , M ₃₋₁₉₋₄
Ii	Twisted	9	M ₃₋₃₋₈ , M ₃₋₄₋₂ , M ₃₋₅₋₄ , M ₃₋₆₋₂ , M ₃₋₆₋₃ , M ₃₋₆₋₄ , M ₃₋₁₆₋₂ , M ₃₋₁₇₋₁ , M ₃₋₁₇₋₂
4	Seed Colour		
I	Dark Brown	5	M ₃₋₂₋₅ , M ₃₋₂₋₆ , M ₃₋₂₋₇ , M ₃₋₆₋₄ , M ₃₋₁₁₋₆
Ii	Light brown	5	M ₃₋₄₋₂ , M ₃₋₄₋₃ , M ₃₋₆₋₂ , M ₃₋₈₋₁ , M ₃₋₉₋₄
Iii	Fawn	4	M ₃₋₁₋₁ , M ₃₋₂₋₁ , M ₃₋₁₁₋₈ , M ₃₋₁₁₋₉

11 mutant lines namely M₃₋₂₋₁, M₃₋₂₋₂, M₃₋₂₋₃, M₃₋₆₋₄, M₃₋₃₋₂, M₃₋₃₋₅, M₃₋₃₋₈, M₃₋₃₋₉, M₃₋₅₋₂, M₃₋₁₆₋₂, M₃₋₁₇₋₂ show deviation in flower colour than control Baner, out of which, 7 lines namely M₃₋₂₋₁, M₃₋₂₋₂, M₃₋₂₋₃, M₃₋₆₋₄, M₃₋₃₋₂, M₃₋₃₋₅, M₃₋₅₋₂ showed light blue flower and M₃₋₃₋₈, M₃₋₃₋₉, M₃₋₁₆₋₂, M₃₋₁₇₋₂ lines showed dark blue flower colour. All other mutant lines showed no deviation in flower colour than their respective control i.e. Baner, Him Alsi-2 and Surbhi (Appendix-IV).

44 mutant lines namely M₃₋₁₋₄, M₃₋₁₋₅, M₃₋₁₋₇, M₃₋₁₋₉, M₃₋₁₋₁₀, M₃₋₂₋₄, M₃₋₂₋₆, M₃₋₂₋₇, M₃₋₃₋₆, M₃₋₃₋₇, M₃₋₃₋₈, M₃₋₃₋₁₁, M₃₋₄₋₃, M₃₋₄₋₄, M₃₋₅₋₁, M₃₋₅₋₂, M₃₋₅₋₄, M₃₋₅₋₅, M₃₋₆₋₂, M₃₋₆₋₃, M₃₋₆₋₄, M₃₋₇₋₄, M₃₋₇₋₅, M₃₋₈₋₁, M₃₋₈₋₂, M₃₋₈₋₄, M₃₋₈₋₅, M₃₋₈₋₆, M₃₋₈₋₈, M₃₋₈₋₁₀, M₃₋₉₋₁, M₃₋₉₋₂, M₃₋₉₋₃, M₃₋₉₋₄, M₃₋₁₀₋₂, M₃₋₁₀₋₃, M₃₋₁₀₋₆, M₃₋₁₀₋₇, M₃₋₁₀₋₈, M₃₋₁₁₋₁₀, M₃₋₁₉₋₁, M₃₋₁₉₋₃, M₃₋₁₉₋₄, M₃₋₁₉₋₅ showed deviation in flower shape than disc shape flower of control (Baner and Him Alsi-2), out of which six lines namely M₃₋₄₋₃, M₃₋₆₋₂, M₃₋₆₋₃, M₃₋₆₋₄, M₃₋₈₋₄, M₃₋₁₁₋₁₀ showed funnel type flower and 38 lines namely M₃₋₁₋₄, M₃₋₁₋₅, M₃₋₁₋₇, M₃₋₁₋₉, M₃₋₁₋₁₀, M₃₋₂₋₄, M₃₋₂₋₆, M₃₋₂₋₇, M₃₋₃₋₆, M₃₋₃₋₇, M₃₋₃₋₈, M₃₋₃₋₁₁, M₃₋₄₋₄, M₃₋₅₋₁, M₃₋₅₋₂, M₃₋₅₋₄, M₃₋₅₋₅, M₃₋₇₋₄, M₃₋₇₋₅, M₃₋₈₋₁, M₃₋₈₋₂, M₃₋₈₋₅, M₃₋₈₋₆, M₃₋₈₋₈, M₃₋₈₋₁₀, M₃₋₉₋₁, M₃₋₉₋₂, M₃₋₉₋₃, M₃₋₉₋₄, M₃₋₁₀₋₂, M₃₋₁₀₋₃, M₃₋₁₀₋₆, M₃₋₁₀₋₇, M₃₋₁₀₋₈, M₃₋₁₉₋₁, M₃₋₁₉₋₃, M₃₋₁₉₋₄, M₃₋₁₉₋₅ showed star shaped flower. Remaining mutant lines showed similar flower shape as shown by their respective control i.e. disc shape (Baner and Him Alsi-2) and star shape (Surbhi).

Different type of flower aestivations was observed in mutant lines. 24 mutant lines namely, M₃₋₃₋₁, M₃₋₃₋₂, M₃₋₃₋₅, M₃₋₃₋₈, M₃₋₃₋₁₀, M₃₋₄₋₂, M₃₋₅₋₃, M₃₋₅₋₄, M₃₋₅₋₆, M₃₋₆₋₂, M₃₋₆₋₃, M₃₋₆₋₄, M₃₋₈₋₅, M₃₋₈₋₆, M₃₋₈₋₇, M₃₋₈₋₉, M₃₋₁₀₋₂, M₃₋₁₀₋₆, M₃₋₁₆₋₂, M₃₋₁₇₋₁, M₃₋₁₇₋₂, M₃₋₁₉₋₁, M₃₋₁₉₋₃, M₃₋₁₉₋₄ showed deviation in flower aestivation than semi twisted flower aestivation of controls (Baner and Him Alsi-2). Out of 22 mutant lines, 15 lines namely M₃₋₃₋₁, M₃₋₃₋₂, M₃₋₃₋₅, M₃₋₃₋₁₀, M₃₋₅₋₃, M₃₋₅₋₆, M₃₋₈₋₅, M₃₋₈₋₆, M₃₋₈₋₇, M₃₋₈₋₉, M₃₋₁₀₋₂, M₃₋₁₀₋₆, M₃₋₁₉₋₁, M₃₋₁₉₋₃, M₃₋₁₉₋₄ showed velvate type flower aestivation and 9 lines namely M₃₋₃₋₈, M₃₋₄₋₂, M₃₋₅₋₄, M₃₋₆₋₂, M₃₋₆₋₃, M₃₋₆₋₄, M₃₋₁₆₋₂, M₃₋₁₇₋₁, M₃₋₁₇₋₂ showed twisted type flower aestivation. Remaining lines showed similar type of flower aestivation as shown by their control i.e. semi twisted (Baner and Him Alsi-2) and velvate (Surbhi).

Seed colour of M₃ population was observed which revealed that 14 mutants showed deviation in seed colour. Out of 14 mutants, four lines i.e. M₃₋₁₋₁, M₃₋₂₋₁, M₃₋₁₁₋₈, M₃₋₁₁₋₉ showed fawn colour seed, five lines i.e. M₃₋₂₋₅, M₃₋₂₋₆, M₃₋₂₋₇, M₃₋₆₋₄, M₃₋₁₁₋₆ showed dark brown seed and five mutants i.e. M₃₋₄₋₂, M₃₋₄₋₃, M₃₋₆₋₂, M₃₋₈₋₁, M₃₋₉₋₄ light brown seed. While other remaining mutant lines showed similar seed colour as shown by their respective control i.e. Brown (Baner and Him Alsi-2) and golden yellow (Surbhi).

Observed data showed that there were no changes in flower colour, aestivation and seed colour of mutant lines of Surbhi indicating less morphological variation than Baner and Him Alsi-2 when subjected to EMS and gamma rays mutagen. This might be due to morphological and genetical behaviour of varieties.

4.8 Analysis of variance

Analysis of variance in compact family block design was used for comparison between progenies belonging to the same family and between progenies belonging to different families (Table 4.19). ANOVA for quantitative characters of mutants lines over the parents and mutants showed significant difference for the mean values among accessions for all characters studied except secondary branches per plant. The characters that showed significant difference among blocks (replication) was plant height and harvest index. The ANOVA between families revealed significant difference in mean values for all the characters except number of secondary branches per plant. The analysis of variance of the mutants within each families indicated the significant difference in mean value for all the characters i.e. Days to 50 per cent flowering, plant height, technical height, number of primary branches, number of secondary branches, number of capsules per plants, days to 75 per cent maturity, number of seeds per capsule, biological yield per plant, seed yield per plant, harvest index and 1000- seed weight. These results were in accordance with Bindra et al.(2016), Chaudhary et al (2017), Meena et al (2020) with the indication that substantial variability was present in linseed mutants and were amenable for further genetic and selection parameters.

Table 4.19 Analysis of variance (ANOVA) of M₃ mutants for yield and its related components in Compact Family Block Randomised Design.

Character / components	Degree of freedom	Day to 50 percent Flowering (No's)	Plant height (cm)	Technical height (cm)	Primary Branches (No's)	Secondary branches (No's)	Capsules per plant (No's)	Days to 75 percent maturity (No's)	Number of seeds per capsule (No's)	Biological yield per plant (g)	Seed yield per plant (g)	Harvest index	1000 Seed weight (g)
Replication	1	40.19	82.52***	42.08	0.11	7.10	0.02	39.41	0.16	1.50	0.00	0.03*	0.05
Treatment	18	200.72***	1025.53***	484.12***	7.53*	18.85	629.17***	282.37***	0.62***	16.96***	2.15***	0.06***	1.75***
Error A	18	15.98	2.93	11.09	1.38	11.34	49.70	43.30	0.35	1.01	0.13	0.00	0.02
Mutants	112	64.89***	150.25***	157.27***	2.70***	16.49***	179.64*	195.35***	1.82***	6.51***	0.86***	0.03***	0.09**
Treatemnts:Mutants	131	71.84***	218.69***	123.45***	2.66***	7.00***	230.99***	117.38***	0.67***	7.36***	0.89***	0.03***	0.30***
Error B	131	8.76	3.44	11.47	0.77	2.99	36.36	2.07	0.11	0.70	0.01	0.07	0.03

***0.001 % , ** 0.01 % , *0.05% significance Level

4.9 Estimates of variability in M₃ generation

The estimates of genetic parameters viz. Phenotypic and genotypic coefficient of variation (PCV and GCV), heritability in Broad sense and genetic advance as percentage of mean for twelve traits including yield and related attributes were computed. The analysis revealed that for all the characters studied, phenotypic coefficient of variation was higher than the genotypic coefficient of variation, so it is evident that expression of characters controlling the yield and attributes was governed by mutant/genotype itself along with meagre effect of environment. The similar effect of mutants/genotypes was interpreted by Venkateshwarlu (2001), Dikshit et al. (2002) and Tejbir et al. (2009). Since the seed yield per plant and early maturity are the most desirable characters for development of superior varieties, certain mutants were found distinctly much superior to other with regards to high yield and early maturity as shown in Table 4.20. Increase in variability following the mutagenic treatments of EMS and gamma rays were also reported by Ahmad et al (2014), Akbar et al. (2003), Jagdeesan et al (2008). In order to know breeding utility of this variability and selection values of various quantitative traits, it is essential to determine the various genetic components and heritable proportion of variability (Gottschalk and Kaul 1980). Heritability estimates coupled with estimated genetic advance are more helpful than the heritability alone itself (Jahnson et al. 1955) because heritability estimates are subjected to certain estimation errors (Kaul and Grag 1979). Mutagen derived variability for quantitative characters in crop plants is heritable and that the response to selections good. Delayed selection is preferred as deleterious mutations are generally eliminated in early generations (Frey 1969). The characters wise variability of mutant lines is presented in Table 4.20 and described as below:

4.9.1 Days to 50 per cent flowering:

Estimates of days to 50 percent flowering showed that wide range was found in mutant lines which varied from 151 days to 189 days. The lowest and highest values of days to mutant lines were already deciphered in mean performance. The variation among the mutant lines were observed which revealed that PCV (3.76 %) of mutants for days to 50 per cent flowering was slightly higher than GCV estimates (3.32 %) showing that days to 50 per cent flowering was controlled genetically with

meagre effect of environment. Low GCV and PCV (<10) was observed for days to 50 per cent flowering depicting that very narrow variation were present in M₃ mutant population. The difference between GCV and PCV was very less showing that the environment effect was very less for days to 50 per cent flowering in mutant lines. Similar finding were reported by Ahmad (2017), Chaudhary et al. (2017), and Kaur et al. (2018).

Further estimates of variability parameters revealed that high heritability (78.29 %) coupled with low genetic advance as percentage of mean percentage (6.08 %) was found in days to 50 per cent flowering among M₃ mutants which indicates presence of non additive type of inheritance for days to 50 per cent flowering and therefore limited improvement could be expected for improvement of such characters. These finding were also revealed by Ahmad et al. (2014) and Chandrawati (2016).

4.9.2. Plant height per plant:

Variability analysis revealed that large range of plant height per plant was found in mutant lines which varied from 42.00 centimetres to 88.50 centimetre. On further evaluation, it was found that moderate values of GCV (15.26 %) and PCV (15.50 %) were present in mutant lines for plant height. Also PCV was slightly higher than GCV showing that very less effect of environment was present in mutant population. High heritability (98.89 %) coupled with high genetic advance as percent of mean (30.94 %) was found in mutant population which showed that plant height is primarily controlled by an additive type gene action and therefore polygenic/quantitative type of inheritance was responsible for plant height. In such cases simple selection model would be advantageous to obtain the desired genetic gain. Similar observation was also found by Ahmad et al. (2014), Chandrawati (2016), Reddy et al. (2013), Kanwar et al. (2014), Tyagi et al (2014) and Meena et al. (2020).

4.9.3. Technical height per plant:

Estimation of variability revealed that the technical height varied from 13.00 cm to 56.00 cm in M₃ mutant population along with control. Higher values of GCV

(22.51 %) and PCV (24.71 %) indicated that large amount of variation in studied population. Also, estimates of PCV were slightly higher than GCV predicting that effect of genotypic variation was more than environmental effect on plant height.

High magnitude of heritability (82.99 %) coupled with high GAM (42.99 %) was observed in M_3 population showing that technical height was governed by additive gene action. This finding was also reported by Kanwar et al. (2014), Tyagi et al. (2014), Singh et al. (2015), Chandrawati et al. (2016), and Ahmed (2017). High heritability with high GAM showed the possibility of higher selection response in technical height owing to its high transmissibility.

4.9.4. Number of primary and secondary branches per plant:

Observed estimates in M_3 population revealed that very high range was present for number of primary branches per plant (5.00-13.00) and secondary branches per plant (8.00-21.00).

Moderate GCV (10.74 %) and PCV (14.47 %) were observed in population for number of primary branches per plant. Similarly, for number of secondary branches per plant, moderate GCV (11.36 %) and PCV (17.93 %) were present. The estimates of GCV and PCV revealed the PCV was high than GCV thus indicating that effect of environment was also present in population. The difference between GCV and PCV was found very less (<10 %), which indicates that genetic effect in population was more than environment and also shows that characters were genetically controlled.

High heritability (55.04 %) coupled with moderate GAM (16.41 %) were observed for number of primary branches per plant. Similarly same pattern was observed for number of secondary branches per plant i.e. heritability (40.16 %) and GAM (14.83 %). These patterns showed that the characters were controlled by additive type gene action thus suggesting that simple selection models following the selection of these genotypes are useful for maximum gains. These results were in contention with the results obtained by Reddy et al. (2013) and Ahmed et al. (2014).

Table 4.20 Estimates of variability of mutants in M₃ generation

Genetic parameters	Mean	Range	Genotypic coefficient of variation (GCV) (%)	Phenotypic coefficient of variation (%)	Heritability (%)	Genetic advance	Genetic Advance as percentage of mean (%)
Daye to 50 percent Flowering (No's)	168.96	153.00-188.50	3.32	3.76	78.27	10.23	6.08
Plant height (cm)	67.99	43.00-86.95	15.26	15.50	98.89	21.03	30.94
Technical height (cm)	33.26	13.50-55.50	22.51	24.71	82.99	14.04	42.49
Primary Branches (No's)	9.05	6.00-13.00	10.74	14.47	55.04	1.49	16.41
Secondary branches (No's)	12.47	8.00-21.00	11.36	17.93	40.16	1.84	14.83
Capsules per plant (No's)	54.71	6.00-100.00	18.03	21.13	72.79	17.33	31.69
Days to 75 percent maturity (No's)	219.46	196.50-239.00	3.46	3.52	96.52	15.37	7.00
Number of seeds per capsule (No's)	8.41	7.00-9.60	6.00	7.26	68.40	0.85	10.22
Biological yield per plant (g)	7.71	2.00-14.00	23.64	26.03	82.44	3.41	44.21
Seed yield per plant (g)	2.71	0.60-5.00	21.92	27.05	65.68	0.99	36.60
Harvest index	0.36	0.17-1.25	27.31	35.37	59.62	0.16	43.44
1000 Seed weight (g)	6.29	5.00-7.25	5.84	6.52	80.82	0.67	10.77

4.9.5. Number of capsules per plant:

Wide range for number of capsules per plant was observed in M_3 population i.e. 32.50 to 92.00. Further deciphering of variability parameters revealed that moderate values of GCV (18.03 %) and high PCV (21.13 %) in the population. The effect of environment was also present but difference between GCV and PCV was low depicting that genetic effect was more for controlling the character than environment. High heritability (72.79 %) coupled with high GAM (31.69 %) was found in M_3 population showing the presence of additive gene inheritance. Similar results were found by Chandrawati et al. (2016) and Ahmed (2017) in their study showing that number of capsules per plant may be used for selection of high yield mutant lines in later generation for development of superior cultivars.

4.9.6. Days to 75 percent maturity:

Days to 75 per cent maturity is the most important character for selection of early maturing lines and data revealed wide range (196.50 days to 239.00 days) for days to 75 per cent maturity in M_3 population which varied from. Further variability parameters showed that low GCV (3.46 %) and PCV (3.52 %) was present among M_3 population revealing that very narrow variation was there. The PCV estimates were slightly higher than GCV showing very less effect of environment in population. But high heritability (96.52 %) was found for days to 75 per cent maturity. Moderate genetic advance (15.37 %) but low GAM (7.00 %) was found in M_3 population. High heritability coupled with low GAM was found for days to 75 per cent maturity which indicates the presence of non additive type of gene action and narrow potential among M_3 population for improvement of this character. Similar result was obtained by Meena et al. (2020).

4.9.7. Number of seeds per capsule:

Data revealed that low range was found for number of seeds per capsule i.e. 7.00 to 9.60. On further perusal it was found that low GCV (6.00 %) and low PCV (7.26 %) was present in the population showing very less potential in the present material and therefore it will be difficult to improve the number of seeds per capsule in further generations. Effect of environment was also present but was genetically controlled as the GCV estimates were more than ECV. High heritability (96.52 %)

coupled with moderate GAM (10.22 %) was found for number of seed per capsules revealing additive type of inheritance. Similar result were observed by Kanwar et al. (2014), Tyagi et al. (2014), Singh et al. (2015), Chandrawati et al. (2016), Ahmed (2017) and Meena et al. (2020).

4.9.8. Biological yield per plant:

Data estimation revealed that very wide range of biological yield (2.50 g to 14.00 g). Further variability estimates revealed that high GCV (23.64 %) and high PCV (26.03 %) were found in the M_3 population indicating substantial variability in M_3 population which could be used for development of superior cultivar. As the estimates of PCV were slightly higher than GCV, effect of environment was less on biological yield. High heritability (82.44 %) coupled with high GAM (44.21 %) was found in population revealing that biological yield was controlled by additive genes and additive type of inheritance was present. This character can be used for selection of superior cultivar by suitable simple selection model.

4.9.9. Seed yield per plant:

Ultimate aim for development of variety is to develop variety with high seed yield. High yield is polygenic character and controlled by many genes. The data observations of M_3 population for seed yield per plant concluded wide range (1.17 g to 4.58 g) for seed yield per plant. The evaluation of variability parameters revealed that high GCV (21.92 %) and high PCV (27.05 %) were present in M_3 population and substantial amount of variability was found in population. The variation among mutant lines can be further explored in later generations for development of high seed yielding cultivars. The GCV estimate was slightly lower than PCV estimate (<10) which showed that effect of environment was negligible and seed yield was genetically controlled.

On further evaluation, it was found that high heritability (65.68 %) coupled with high GAM (36.60 %) was present in population revealing that seed yield per plant was governed by additive type of gene action and simple selection model would be advantageous for obtaining desired genetic gain.

Therefore results obtained showed that seed yield per plant was polygenically controlled which might be due to favorable gene mutation at more than one loci. Similar result were obtained by Badere and Choudary (2004), Alka and Ansari (2013), Rai et al. (2013), Reddy et al. (2013), and Rafeeq et al. (2017) in their study in which they isolated high yielding mutants with increased seed yield per plant. Increased Seed yield per plant might be due to increase in number of capsules per plant, number of primary and secondary branches and other related traits. Similar results for high yield per plant were also obtained by Tyagi et al. (2014), Singh et al. (2015), Chandrawati et al. (2016), Ahmed (2017), and Meena at al. (2020) in linseed.

4.9.10. Harvest Index:

The data evaluation revealed that harvest index ranged from 0.18 to 0.94. The estimates of variability showed that high GCV (27.31 %) and high PCV (35.27 %) were found among the M₃ population. The high heritability (59.92 %) coupled with high GAM (43.44 %) was present for harvest index showing that the character was controlled by additive gene action and can be used for selection of superior cultivars.

4.9.11. 1000 seed weight:

Data estimates revealed that 1000 seed weight ranged from 5.00 to 7.25 grams. The low GCV (5.84 %) and low PCV (6.52 %) were present showing very less potential and variation in the studied population and thus will be difficult in improvement of such character in future generation. The high heritability (80.82 %) coupled with moderate GAM (10.77 %) were found for 1000 seed weight in M₃ population showing additive type gene action. These were in contention with Chandrawati et al. (2016), Ahmed (2017), and Meena at al. (2020) in linseed.

The estimated variability parameters in M₃ population of mutant lines along with the control revealed that sufficient amount of genetic variability was present and characters were genetically controlled. The estimated PCV for all the characters studied revealed that PCV were slightly higher than GCV with very low differences indicating very less influence of environment and predominance of the genetic factors (Vardhan and Rao 2012; Rai et al. 2013; Reddy et al. 2013; Rafeeq et al. 2016). The high heritability was found for all the character studied revealing that based on performance the elite mutant lines could be selected from M₃ generatio onwards.

Utility of estimate of heritability increased when coupled with GAM, which provides the information about the degree of genetic gain in a character obtained under a particular selection pressure (Johnson et al. 1955). The above estimates were in contention with Rafeeq et al. (2016), Reddy et al. (2013), Rai et al. (2013), and Meena et al. (2020). Based on yield performance and early maturity these mutant lines can be further evaluated for development of superior cultivar.

4.10 Disease reaction of M₃ mutant lines to Powdery Mildew:

The entire M₃ population of mutants along with control i.e. Baner, Him Alsi-2 and Surbhi was observed for powdery mildew severity as shown in Table 4.21. On evaluation of the population, 10 mutant lines viz. M₃₋₁₋₆, M₃₋₂₋₇, M₃₋₅₋₄, M₃₋₅₋₆, M₃₋₁₀₋₆, M₃₋₁₁₋₆, M₃₋₁₄₋₂, M₃₋₁₅₋₁, M₃₋₁₈₋₇, M₃₋₁₉₋₃ showed resistant reaction to powdery mildew, while 14 number of mutant lines namely, M₃₋₄₋₁, M₃₋₄₋₃, M₃₋₅₋₅, M₃₋₁₀₋₃, M₃₋₁₀₋₄, M₃₋₁₀₋₅, M₃₋₁₁₋₉, M₃₋₁₂₋₃, M₃₋₁₄₋₃, M₃₋₁₆₋₁, M₃₋₁₆₋₂, M₃₋₁₇₋₂, M₃₋₁₉₋₆, M₃₋₁₉₋₇ exhibited moderate resistance reaction to powdery mildew.

On the other hand, 22 number of mutant lines i.e. M₃₋₃₋₁₁, M₃₋₄₋₂, M₃₋₄₋₄, M₃₋₅₋₁, M₃₋₅₋₂, M₃₋₅₋₃, M₃₋₆₋₁, M₃₋₆₋₃, M₃₋₆₋₄, M₃₋₇₋₄, M₃₋₉₋₄, M₃₋₉₋₆, M₃₋₁₀₋₇, M₃₋₁₀₋₈, M₃₋₁₀₋₉, M₃₋₁₁₋₁₀, M₃₋₁₂₋₄, M₃₋₁₂₋₅, M₃₋₁₄₋₁, M₃₋₁₅₋₂, M₃₋₁₇₋₁, M₃₋₁₉₋₈ were found moderately susceptible to powdery mildew, while 62 number of mutant lines namely M₃₋₁₋₁, M₃₋₁₋₂, M₃₋₁₋₃, M₃₋₁₋₄, M₃₋₁₋₅, M₃₋₁₋₇, M₃₋₁₋₈, M₃₋₁₋₉, M₃₋₁₋₁₀, M₃₋₂₋₁, M₃₋₂₋₂, M₃₋₂₋₃, M₃₋₂₋₄, M₃₋₂₋₅, M₃₋₂₋₆, M₃₋₂₋₈, M₃₋₃₋₁, M₃₋₃₋₂, M₃₋₃₋₃, M₃₋₃₋₄, M₃₋₃₋₅, M₃₋₃₋₆, M₃₋₃₋₇, M₃₋₃₋₈, M₃₋₃₋₉, M₃₋₃₋₁₀, M₃₋₆₋₂, M₃₋₇₋₁, M₃₋₇₋₂, M₃₋₇₋₃, M₃₋₇₋₅, M₃₋₇₋₆, M₃₋₈₋₁, M₃₋₈₋₂, M₃₋₈₋₃, M₃₋₈₋₄, M₃₋₈₋₅, M₃₋₈₋₆, M₃₋₈₋₇, M₃₋₈₋₈, M₃₋₈₋₉, M₃₋₈₋₁₀, M₃₋₉₋₁, M₃₋₉₋₂, M₃₋₉₋₃, M₃₋₉₋₅, M₃₋₁₀₋₁, M₃₋₁₀₋₂, M₃₋₁₁₋₁, M₃₋₁₁₋₂, M₃₋₁₁₋₃, M₃₋₁₁₋₄, M₃₋₁₁₋₅, M₃₋₁₁₋₈, M₃₋₁₈₋₃, M₃₋₁₈₋₄, M₃₋₁₉₋₁, M₃₋₁₉₋₂, M₃₋₁₉₋₄, M₃₋₁₉₋₅ along with control Baner and Him Alsi-2 were found susceptible to powdery mildew.

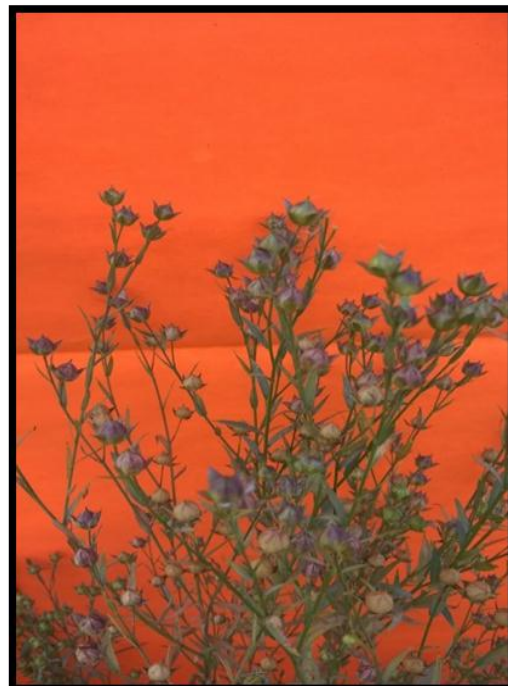
Five mutant lines namely M₃₋₁₁₋₇, M₃₋₁₂₋₁, M₃₋₁₂₋₂, M₃₋₁₈₋₁, M₃₋₁₈₋₂ along with control Surbhi were found highly susceptible to powdery mildew.

The mutant lines showing resistant and moderately resistant reaction to powdery mildew can be further subjected to assess the severity of disease in later generations and may be used as selection criteria for development of superior cultivar.

Table 4.21 Reaction of M₃ generation to Powdery Mildew Disease

Disease Score for Powdery Mildew	Disease reaction	Number of Mutants Lines along with parents	List of mutant lines
0	Highly resistance (HR)	0	0
1	Resistant (R)	10	M ₃₋₁₋₆ , M ₃₋₂₋₇ , M ₃₋₅₋₄ , M ₃₋₅₋₆ , M ₃₋₁₀₋₆ , M ₃₋₁₁₋₆ , M ₃₋₁₄₋₂ , M ₃₋₁₅₋₁ , M ₃₋₁₈₋₇ , M ₃₋₁₉₋₃
2	Moderately Resistant (MR)	14	M ₃₋₄₋₁ , M ₃₋₄₋₃ , M ₃₋₅₋₅ , M ₃₋₁₀₋₃ , M ₃₋₁₀₋₄ , M ₃₋₁₀₋₅ , M ₃₋₁₁₋₉ , M ₃₋₁₂₋₃ , M ₃₋₁₄₋₃ , M ₃₋₁₆₋₁ , M ₃₋₁₆₋₂ , M ₃₋₁₇₋₂ , M ₃₋₁₉₋₆ , M ₃₋₁₉₋₇
3	Moderately Susceptible (MS)	22	M ₃₋₃₋₁₁ , M ₃₋₄₋₂ , M ₃₋₄₋₄ , M ₃₋₅₋₁ , M ₃₋₅₋₂ , M ₃₋₅₋₃ , M ₃₋₆₋₁ , M ₃₋₆₋₃ , M ₃₋₆₋₄ , M ₃₋₇₋₄ , M ₃₋₉₋₄ , M ₃₋₉₋₆ , M ₃₋₁₀₋₇ , M ₃₋₁₀₋₈ , M ₃₋₁₀₋₉ , M ₃₋₁₁₋₁₀ , M ₃₋₁₂₋₄ , M ₃₋₁₂₋₅ , M ₃₋₁₄₋₁ , M ₃₋₁₅₋₂ , M ₃₋₁₇₋₁ , M ₃₋₁₉₋₈ ,
4	Susceptible (S)	64	M ₃₋₁₋₁ , M ₃₋₁₋₂ , M ₃₋₁₋₃ , M ₃₋₁₋₄ , M ₃₋₁₋₅ , M ₃₋₁₋₇ , M ₃₋₁₋₈ , M ₃₋₁₋₉ , M ₃₋₁₋₁₀ , M ₃₋₂₋₁ , M ₃₋₂₋₂ , M ₃₋₂₋₃ , M ₃₋₂₋₄ , M ₃₋₂₋₅ , M ₃₋₂₋₆ , M ₃₋₂₋₈ , M ₃₋₃₋₁ , M ₃₋₃₋₂ , M ₃₋₃₋₃ , M ₃₋₃₋₄ , M ₃₋₃₋₅ , M ₃₋₃₋₆ , M ₃₋₃₋₇ , M ₃₋₃₋₈ , M ₃₋₃₋₉ , M ₃₋₃₋₁₀ , M ₃₋₆₋₂ , M ₃₋₇₋₁ , M ₃₋₇₋₂ , M ₃₋₇₋₃ , M ₃₋₇₋₅ , M ₃₋₇₋₆ , M ₃₋₈₋₁ , M ₃₋₈₋₂ , M ₃₋₈₋₃ , M ₃₋₈₋₄ , M ₃₋₈₋₅ , M ₃₋₈₋₆ , M ₃₋₈₋₇ , M ₃₋₈₋₈ , M ₃₋₈₋₉ , M ₃₋₈₋₁₀ , M ₃₋₉₋₁ , M ₃₋₉₋₂ , M ₃₋₉₋₃ , M ₃₋₉₋₅ , M ₃₋₁₀₋₁ , M ₃₋₁₀₋₂ , M ₃₋₁₁₋₁ , M ₃₋₁₁₋₂ , M ₃₋₁₁₋₃ , M ₃₋₁₁₋₄ , M ₃₋₁₁₋₅ , M ₃₋₁₁₋₈ , M ₃₋₁₈₋₃ , M ₃₋₁₈₋₄ , M ₃₋₁₉₋₁ , M ₃₋₁₉₋₂ , M ₃₋₁₉₋₄ , M ₃₋₁₉₋₅ , Baner, Him Alsi-2
5	Highly Susceptible (HS)	6	M ₃₋₁₁₋₇ , M ₃₋₁₂₋₁ , M ₃₋₁₂₋₂ , M ₃₋₁₈₋₁ , M ₃₋₁₈₋₂ , Surbhi

Plate 4.6 Reaction of mutant lines to powdery mildew in M₃ generation



4.11 Selection and isolation of high yielding and early maturing mutant lines:

Based on the performance of mutants in M_3 generation, 17 mutants were isolated from the population and selected for further evaluation as shown in Table 4.22. Out of 17 mutants, nine high yielding M_3 mutants namely, M_{3-1-3} , M_{3-2-5} , M_{3-5-1} , M_{3-10-8} , M_{3-11-2} , M_{3-12-1} , M_{3-13-2} , M_{3-18-3} , M_{3-19-4} and eight early maturing mutants viz. M_{3-3-5} , M_{3-6-3} , M_{3-7-4} , M_{3-8-2} , M_{3-11-9} , M_{3-15-2} , M_{3-16-2} , M_{3-19-8} were isolated and further evaluated for biochemical analysis. The isolated mutants showed substantial potential than their respective control viz. Baner, Him Alsi-2 and Surbhi. Potential of these high yielding and early mutants can be exploited by selection and further evaluation in later generations.

Table 4.22: Isolation and selection of high yielding and early maturing mutant lines

Sr. No.	Character	No. of mutants	Name of selected mutant lines
1	High yield	9	M_{3-1-3} , M_{3-2-5} , M_{3-5-1} , M_{3-10-8} , M_{3-11-2} , M_{3-12-1} , M_{3-13-2} , M_{3-18-3} , M_{3-19-4}
2	Earliness	8	M_{3-3-5} , M_{3-6-3} , M_{3-7-4} , M_{3-8-2} , M_{3-11-9} , M_{3-15-2} , M_{3-16-2} , M_{3-19-8}

4.12 Estimation of biochemical analysis of selected M_3 mutant lines

4.12.1 Estimation of oil content:

Estimation of oil content was presented in Table 4.23. Extracted oil yield in 17 isolated mutant lines showed that EMS and gamma rays drastically changed the oil content in linseed which was also earlier reported by Badare and Chaudhary (2004), Green (1986), Nichterlin et al. (1988) and Rowland (1991) that mutagens effectively alter the oil content. Present results showed that the highest oil content was found in M_{3-3-5} (58.44 %) derived from 40 kR gamma rays of Baner followed by M_{3-13-2} (57.06 %) derived from 40 kR gamma rays of Surbhi. Among all the mutant lines, the lines M_{3-1-3} , M_{3-2-5} , M_{3-11-2} , M_{3-12-1} , M_{3-13-2} , M_{3-3-5} , M_{3-7-4} , M_{3-11-9} showed positive increase in oil content than the controls (Baner, Him Alsi-2 and Surbhi), while the mutant lines M_{3-5-1} , M_{3-19-4} , M_{3-15-2} and M_{3-16-2} showed negative shift of oil content from the all

three controls. Similar findings of increased oil content by induced mutation was reported by Badare and Chaudhary (2004), Green (1986), Nichterlin et al. (1988) showing that induced mutation are useful in generation of high oil yielding cultivars. Iodine value of mutants ranged from 164 to 195 and presence of high iodine value indicates that unsaturated acid percentage was more than the saturated fatty acids in mutant lines of linseed. Highest iodine value was found in M₃₋₁₃₋₂ (195), M₃₋₁₁₋₂ (195) while lowest was found in M₃₋₅₋₁ (164). On the other hand acid value showed that it ranged from 0.65 to 0.90. Mutant lines which showed high acid value were more prone to rancidity as high acid value indicates that oil has high reaction to oxidation.

4.23. Estimation of oil content, iodine and acid value of isolated M₃ mutant lines

Sr. No.	Mutant lines	Oil percentage	Iodine Value (g I ₂ /100g oil)	Acid value (mg KOH/g oil)
1	M ₃₋₁₋₃	48.99	177	0.80
2	M ₃₋₂₋₅	48.37	175	0.75
3	M ₃₋₅₋₁	36.75	164	0.70
4	M ₃₋₁₀₋₈	41.97	171	0.72
5	M ₃₋₁₁₋₂	55.41	195	0.90
6	M ₃₋₁₂₋₁	47.09	177	0.80
7	M ₃₋₁₃₋₂	57.06	195	0.90
8	M ₃₋₁₈₋₃	39.44	170	0.72
9	M ₃₋₁₉₋₄	33.86	165	0.70
10	M ₃₋₃₋₅	58.44	190	0.85
11	M ₃₋₆₋₃	39.40	170	0.70
12	M ₃₋₇₋₄	50.65	188	0.77
13	M ₃₋₈₋₂	39.18	170	0.65
14	M ₃₋₁₁₋₉	46.67	177	0.80
15	M ₃₋₁₅₋₂	38.05	178	0.70
16	M ₃₋₁₆₋₂	34.79	177	0.63
17	M ₃₋₁₉₋₈	42.85	172	0.65
18	Baner	39.00	170	0.75
19	Him Alsi-2	40.00	175	0.65
20	Surbhi	44.00	177	0.83

4.12.2. Fatty acid profile:

The results for fatty acid composition in present investigation were presented in Table 4.24. The performance of M₃ mutants which were selected based on their performance in yield and earliness showed presence of variation in fatty acid composition. Both saturated acids i.e. Palmitic (C16:0), Stearic (C18:0) and unsaturated acid i.e. Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3) were principal fatty acids present in varied amount in mutant lines. It was found that fatty acid composition of mutant lines were dominated by poly unsaturated fatty acid (PUFA), in which highest percentage was observed for linolenic acid ranging from 36.11 % to 53.01 %, followed by oleic acid (21.26 % to 28.50 %) and linoleic acid (14.55 to 25.30 %). The maximum reduction in linolenic acid percentage was found in M₃₋₁₉₋₈ (36.11 %) followed by M₃₋₁₉₋₄ (38.59 %) as compared to all the controls. On the other hand, maximum increase in linolenic acid was found in similar lines i.e. M₃₋₁₉₋₈ (25.30 %) and M₃₋₁₉₋₄ (23.30 %) which showed that with increase in percentage of linoleic acid, there was decrease in linolenic acid and vice versa. The same kind of observation was also observed by Green (1986), Green and Marshall (1984), Rowland (1990). The oleic acid was found maximum in M₃₋₁₉₋₄ (28.80 %) followed by M₃₋₆₋₄ (27.50 %) and M₃₋₁₁₋₉ (27.25 %). In saturated acids, palmitic acid (C16:0) ranged from 3.97 per cent to 5.23 per cent and Stearic acid ranged from 4.12 per cent to 6.56 per cent. The lines with reduced linolenic acid content can be further evaluated for stability and then can be released as cultivars.

Table 4.24 Fatty acid profiling of isolated M₃ mutant lines

Sr. No.	Mutant lines	Saturated fatty acid		Unsaturated fatty acid		
		Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)
1	2	3	4	5	6	7
1	M ₃₋₁₋₃	4.21	5.89	26.24	17.65	45.58
2	M ₃₋₂₋₅	4.81	5.45	24.33	16.34	48.68
3	M ₃₋₅₋₁	4.23	4.34	25.23	17.25	48.39
4	M ₃₋₁₀₋₈	4.12	4.55	22.12	16.70	52.16
5	M ₃₋₁₁₋₂	5.23	4.23	25.23	17.78	44.23
6	M ₃₋₁₂₋₁	4.44	6.41	24.34	17.50	46.89
7	M ₃₋₁₃₋₂	4.24	4.67	26.36	18.60	45.76
8	M ₃₋₁₈₋₃	4.56	5.12	21.26	17.36	51.32
9	M ₃₋₁₉₋₄	3.97	5.39	28.50	23.20	38.59
10	M ₃₋₃₋₅	5.12	4.12	24.00	15.56	50.72
11	M ₃₋₆₋₃	4.35	6.23	27.50	18.28	43.15
12	M ₃₋₇₋₄	4.58	4.56	26.34	16.28	47.65
13	M ₃₋₈₋₂	4.32	6.23	24.45	18.28	46.29
14	M ₃₋₁₁₋₉	5.11	4.57	27.25	23.00	39.31
15	M ₃₋₁₅₋₂	5.01	4.89	25.45	15.25	48.82
16	M ₃₋₁₆₋₂	4.43	5.78	21.67	14.55	53.01
17	M ₃₋₁₉₋₈	4.34	6.56	27.35	25.30	36.11
18	Baner	4.51	5.12	22.34	17.28	49.31
19	Him Alsi-2	4.46	5.68	24.20	17.20	48.00
20	Surbhi	4.23	5.34	24.00	16.29	48.68

5. SUMMARY AND CONCLUSIONS

Present investigation entitled “Genetic improvement of linseed for inducing earliness in maturity and reducing the undesirable compounds through mutation” was carried out in the experimental farm of Department of Genetics and Plant Breeding, COA, CSK HP KV, Palampur to assess the nature and magnitude of induced variability, determine the inheritance pattern of earliness in induced lines; determine the relative proportion of oil content of linseed mutants and isolate lines/mutants with reduced linolenic content in addition to earliness.

A chemical mutagen, Ethyl Methane Sulphonate (EMS) and a physical mutagen, gamma rays (Co^{60}) were utilized in the current investigation to induce mutations within the hand-picked material and to realize genetic variability with fascinating characters.

500 seeds of linseed cultivars (Baner, Him Alsi-2 and Surbhi) were irradiated with gamma rays of concentration of 20, 30, 40, 50 and 60 kR at government agency (NRL; Nuclear Research laboratory), ICAR-IARI, New Delhi. Additionally for EMS, 500 healthy and mature seeds of cultivars viz. Baner, Him Alsi-2 and Surbhi were treated with EMS concentration of 0.2, 0.4, 0.5, 0.6 and 0.8 percent. After treatment with Gamma radiation and EMS, 100 treated seeds of each treatment with controls of respected cultivars were placed on germination paper in petri dishes and placed in germination chamber for 7 days at $25 \pm 2^\circ \text{C}$. Then at 3rd and 7th days after treatments, germination and survival percentage were recorded. The LD_{50} was then determined based on survival percentage after treatment with different doses of gamma rays and EMS. Then, another 400 gamma rays treated and EMS treated seeds were exposed to field condition to spot the effect of different doses in linseed. In M_1 generation under lab as well as field condition there was decrease in germination and survival percentage with increase in concentration of EMS. Maximum germination and survival percentage was recorded in lower concentrations with minimum at higher concentrations.

M₂ generation was raised during the Rabi 2017-18 from the seeds harvested from individual M₁ plants in each dose/treatment in progeny rows. Data on single plant basis were recorded for different traits viz. plant survival, days to flower initiation, days to 75 per cent maturity, plant height, number of primary branches, number of secondary branches, capsules per plant, seeds per capsule, 1000 seed weight and seed yield per plant. Observations were also recorded for chlorophyll and viable macromutations.

Selected M₂ mutant plants after evaluation for particular characteristics were selected to carry forward to M₃ generation. These M₃ mutant lines were raised in compact family block design with two replications in *Rabi* 2018-19. Data of various qualitative and quantitative characters were recorded and analyzed.

Recorded observation revealed that the LD₅₀ of gamma rays showed variation in different cultivars, which was 96.63 kR (Baner), 72.94 kR (Him Alsi-2) and 53.22 kR (Surbhi). Lethality (LD₅₀) of EMS varied across three cultivars i.e. Baner (0.86 %, EMS), Him Alsi- 2 (0.72 %, EMS) and Surbhi (0.35 %)

Percent germination in field condition got reduced in same pattern in all the treated variety i.e. Baner (83.00 % (20 kR) to 62.40 % (60 kR)), Him Alsi-2 (90.60 % (20 kR) to 64.80 % (60 kR)) and Surbhi (84.40 % (20 kR) to 60.40 % (60 kR)). Percent reduction over the control was found maximum in 60 kR in all the treated varieties viz. Baner (36.33 %), HimAlsi-2 (34.28 %) and Surbhi (36.82 %).

Observed plants showed that maximum reduction of pollen fertility was observed 0.5 percent EMS (62.50 %) and 60 kR (63.75 %) in Surbhi as compared to Baner and Him Alsi-2. The minimum ovule fertility was observed at 60 kR gamma rays viz. Baner (61.25 %), Him Alsi-2 (55.60 %) and Surbhi (65.00 %); 0.8 percent EMS in Baner (60.00 %), Him Alsi-2 (60.00 %) and 0.5 per cent in Surbhi (51.00 %).

In Baner, the effectiveness of gamma arrays was found maximum in 50 kR (0.906 %) followed by 20 kR (0.833 %), while minimum was found in 0.8 per cent EMS (0.106 %). In Him Alsi-2, only 30 kR and 40 kR gamma rays were effective in creating chlorophyll mutation, while other treatment of gamma rays i.e. 20 kR, 50 kR and 60 kR were found having nil chlorophyll mutation. In Surbhi, except 20 kR all other treatments of gamma rays and EMS were found effective. The maximum

effectiveness was found in 30 kR (2.381 %) followed by 60 kR (1.042 %) gamma rays.

Variability in M_2 generation was observed by estimating the mean value, standard deviation and coefficient of variance for each treatment of EMS and gamma rays in the M_2 generation during the *rabi* 2017-18. In Baner, minimum mean value for days to 50 per cent flowering was found in 50 kR (135.23 days) followed by 30 kR (135.46 days) of gamma rays. In Him Alsi-2, results showed that the minimum mean value was found in 50 kR (131.44 days) followed by 40 kR (133.69 days), while maximum mean value was found in 0.6 percent EMS (139.95 days) as compare to control (138.30 days). In Surbhi, there was no single treatment of gamma rays and EMS which showed significant negative shift for days to 50 percent flowering as compare to control.

In Baner, maximum negative shift of mean in days to 75 per cent maturity was observed in 50 kR gamma rays (187.22 days) having range of 181 to 216 days which was 13.64 percent superior to the untreated population flowed by 30 kR gamma rays (192.57 days). In Him Alsi-2, maximum negative shift of mean was observed in 50 kR gamma rays (187.22 days) followed by 30 kR (192.57 days) with maximum per cent superiority over control of 12.80 per cent and 10.31 per cent respectively. In Surbhi, most of the treatments showed positive shift of mean from mean of untreated population which is undesirable for earliness except 20 kR and 60 kR of gamma rays which showed 6.08 per cent and 0.65 per cent superiority over control.

In Baner, among all the treatments of gamma rays and EMS, the maximum mean value of seed yield per plant was observed in 40 kR (5.26 g) followed by 20 kR (3.87 g) with percent superiority over control by 78.31 percent and 31.19 per cent respectively, and only these two treatments showed positive shift of mean values from the control (2.95). In Him Alsi-2, the percent superiority was found highest in 40 kR (96.05 %). Among all the treatments of gamma rays and EMS, highest relative coefficient of variation was observed in 20 kR (29.23 %) followed by 30 kR (24.88 %), while minimum was found in 60 kR (2.37 %). In Surbhi, percent inferiority was found maximum in 50 kR (94.94 %).

Mean performance of M_3 mutant lines has been observed based on their performance of characters controlling the genetic variation. In days to 50 per cent flowering, maximum negative shift from mean was observed in treatment T-11 (60 kR of Him Alsi-2) i.e. 160.77 days, followed by T-13 (40 kR of Surbhi) i.e. 163.75 days. Observation of days to 75 per cent maturity showed that treatments T-11, T-13, T-14, T-7, T-8, T-12, T-15 and T-16 showed significant negative shift of mean performance in which treatments T-11(208.95 days) followed by T-13 (214.33 days) and T 14 (215.25) showed maximum negative shift from the mean. Among all the mutant lines, M_{3-2-11} (203.50 days), M_{3-11-9} (201.00 days) and M_{3-14-1} (196.50 days) showed maximum negative shift of mean from grand mean and mean of their respective control. Mean performance of seed yield per plant showed that treatments T-13 (3.44 g), T-12 (3.30 g), T-10 (3.28 g), T-15 (3.14 g), T-18 (3.11 g) and T-16 (3.04 g) showed significant positive shift of mean indicating that these doses of mutagen can be used for developing higher yield in future breeding programme.

ANOVA on quantitative characters of mutant lines over the parents and between mutants showed significant difference for the mean values among accessions for all characters except secondary branches per plant.

The estimates of genetic parameters viz. Phenotypic and genotypic coefficient of variation (PCV and GCV), heritability in Broad sense and genetic advance as percentage of mean for twelve yield and yield related attributes were computed. The estimated PCV for all the characters studied revealed that PCV values were slightly higher than GCV with very low differences indicating the very less influence of environment indicating predominance of the genetic factors. High heritability (96.52 %) coupled with low GAM (7.00 %) was found in M_3 population for days to 75 per cent maturity. The evaluation of variability parameters revealed that high GCV (21.92 %) and high PCV (27.05 %) were observed for seed yield per plant. high heritability (65.68 %) coupled with high GAM (36.60 %) was present in population revealing that seed yield per plant was governed by additive type of gene action.

On evaluation of the population, 10 mutant lines viz., M_{3-1-6} , M_{3-2-7} , M_{3-5-4} , M_{3-5-6} , M_{3-10-6} , M_{3-11-6} , M_{3-14-2} , M_{3-15-1} , M_{3-18-7} , M_{3-19-3} showed resistant reaction to powdery mildew, while 14 number of mutant lines namely, M_{3-4-1} , M_{3-4-3} , M_{3-5-5} , M_{3-10-3} , M_{3-10-4} ,

M₃₋₁₀₋₅, M₃₋₁₁₋₉, M₃₋₁₂₋₃, M₃₋₁₄₋₃, M₃₋₁₆₋₁, M₃₋₁₆₋₂, M₃₋₁₇₋₂, M₃₋₁₉₋₆, M₃₋₁₉₋₇ were found having moderately resistant reaction to powdery mildew.

Based on the performance of mutant in M₃ generation 17 mutants were isolated, out of 17 mutants, nine high yielding mutants namely, M₃₋₁₋₃, M₃₋₂₋₅, M₃₋₅₋₁, M₃₋₁₀₋₈, M₃₋₁₁₋₂, M₃₋₁₂₋₁, M₃₋₁₃₋₂, M₃₋₁₈₋₃, M₃₋₁₉₋₄ and eight early maturing mutants namely, M₃₋₃₋₅, M₃₋₆₋₃, M₃₋₇₋₄, M₃₋₈₋₂, M₃₋₁₁₋₉, M₃₋₁₅₋₂, M₃₋₁₆₋₂, M₃₋₁₉₋₈ were isolated and further evaluated for biochemical analysis.

Present results showed that the highest oil content was found in M₃₋₃₋₅ (58.44 %) derived from 40 kR gamma rays of Baner followed by M₃₋₁₃₋₂ (57.06 %) derived from 40 kR gamma rays of Surbhi. The maximum reduction in linolenic acid percentage was found in M₃₋₁₉₋₈ (36.11 %) followed by M₃₋₁₉₋₄ (38.59 %) as compared to all the controls.

The following conclusions were drawn from the present investigation:

- EMS was found effective in reducing seed germination and plant survival in both generations. However, reduction was higher in M₁ in comparison to M₂. Plant survival showed a linear relationship with EMS and gamma rays.
- EMS was also found effective and efficient in producing chlorophyll and viable macromutations in both varieties. However, the spectrum and frequency of macromutations varied in three varieties.
- Total macromutation (Morphological and chlorophyll) data estimates showed that higher concentration of EMS (0.6 % and 0.8 % in Baner and Him Alsi-2, 0.5 % in Surbhi) and gamma rays (40 kR and 60 kR) showed high frequency in generating mutations in linseed.
- Early maturing lines i.e. M₃₋₁₄₋₂ (196.20 days) and M₃₋₁₁₋₂ (196.20 days) and high yielding line i.e. M₃₋₁₃₋₂ (4.58 g) can further evaluated to obtain maximum genetic gain.
- Ten number of mutant lines i.e. M₃₋₁₋₆, M₃₋₂₋₇, M₃₋₅₋₄, M₃₋₅₋₆, M₃₋₁₀₋₆, M₃₋₁₁₋₆, M₃₋₁₄₋₂, M₃₋₁₅₋₁, M₃₋₁₈₋₇, M₃₋₁₉₋₃ showed resistant reaction to powdery mildew.
- The analysis revealed that for all the characters phenotypic coefficient of variation was higher than the genotypic coefficient of variation, so it is evident

that expression of characters controlling the yield and attributes was governed by mutant/genotype itself along with meagre effect of environment. Characters having high heritability coupled with high/moderate genetic advance as percentage of mean can be used as selection criteria for new cultivar development in linseed.

- Mutant lines M_{3-3-5} (58.44 %) and M_{3-13-2} (57.06 %) showed highest oil percentage.
- Mutant line M_{3-19-8} (36.11%), showed maximum reduction of linolenic acid percentage than control.

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APPENDICES

APPENDIX-I

Meteorological data during the crop season from November 2016 to June 2017

Standard week	Temperature(°C)		Rainfall (mm)	Relative Humidity (%)
	Maximum	Minimum		
45	23.6	8.23	0	86.43
46	22.1	7.79	0	86.57
47	22.04	7.70	0	87.43
48	22.97	8.14	0	77.29
49	20.76	6.57	0	73.00
50	20.03	6.29	0	76.86
51	21.77	6.06	0	53.14
52	18.34	5.43	7.2	70.71
1	17.34	6.2	54.2	75.86
2	11.63	1.71	17.6	78.43
3	14.63	3.96	31.2	69.86
4	16.24	7.39	78.6	76
5	17.53	6.87	1	76.57
6	17.1	6.31	17	72.86
7	21.07	9.39	0	62.43
8	19.13	8.14	26.4	70.57
9	20.29	7.36	2.4	61.71
10	16.86	6.29	46.2	73.57
11	16.64	5.64	3.2	64.71
12	24.07	11.86	0	58.86
13	28.79	15.11	0	54
14	26.57	13.31	51.2	58.57
15	26.43	12.21	0	64.57
16	31.86	18.97	3.6	62.86
17	27.87	15.21	2.4	52.14
18	28.13	15.36	9.2	51.57
19	30.07	18.24	19.4	48.86
20	30.36	17.74	31.8	54.14

APPENDIX-II

Meteorological data during the crop season from October 2017 to April 2018

Standard week	Temperature (°C)		Rainfall (mm)	Relative Humidity (%)
	Maximum	Minimum		
45	23.6	8.23	0	86.43
46	22.1	7.79	0	86.57
47	22.04	7.70	0	87.43
48	22.97	8.14	0	77.29
49	20.76	6.57	0	73.00
50	20.03	6.29	0	76.86
51	21.77	6.06	0	53.14
52	18.34	5.43	7.2	70.71
1	17.34	6.2	54.2	75.86
2	11.63	1.71	17.6	78.43
3	14.63	3.96	31.2	69.86
4	16.24	7.39	78.6	76
5	17.53	6.87	1	76.57
6	17.1	6.31	17	72.86
7	21.07	9.39	0	62.43
8	19.13	8.14	26.4	70.57
9	20.29	7.36	2.4	61.71
10	16.86	6.29	46.2	73.57
11	16.64	5.64	3.2	64.71
12	24.07	11.86	0	58.86
13	28.79	15.11	0	54
14	26.57	13.31	51.2	58.57
15	26.43	12.21	0	64.57
16	31.86	18.97	3.6	62.86
17	27.87	15.21	2.4	52.14
18	28.13	15.36	9.2	51.57
19	30.07	18.24	19.4	48.86
20	30.36	17.74	31.8	54.14

APPENDIX-III

Meteorological data during the crop season from October 2018 to April 2019

Standard Weeks	Temperature (°C)		Mean relative Humidity (%)	Sunshine hours	Rainfall (mm)
	Maximum Temperature	Minimum Temperature			
40	26.6	14.0	68.9	8.9	2.0
41	25.6	12.4	72.0	7.6	2.0
42	25.2	11.9	81.1	9.5	0.0
43	23.6	10.1	52.6	9.5	0.0
44	22.6	10.5	70.9	6.3	23.8
45	20.4	7.3	74.6	7.3	1.0
46	19.7	7.6	74.6	5.0	22.0
47	21.0	7.0	70.0	8.7	0.0
48	20.1	7.1	82.7	6.4	0.0
49	17.1	4.9	74.7	7.4	0.0
50	14.9	4.5	68.6	4.9	6.2
51	17.9	3.6	65.0	9.2	0.0
52	15.1	1.4	71.3	8.4	0.0
1	13.9	4.0	64.4	3.4	27.8
2	14.4	3.0	80.1	5.4	7.4
3	16.9	5.5	58.9	5.3	0.0
4	13.9	3.7	64.6	5.1	49.0
5	15.7	4.3	67.6	4.7	104.0
6	16.1	6.4	74.6	3.0	59.0
7	15.7	6.3	79.9	3.7	82.0
8	13.7	4.6	72.3	4.9	24.2
9	17.7	5.6	73.1	7.4	4.0
10	17.8	6.8	68.3	4.9	18.8
11	21.6	9.0	58.0	6.1	3.4
12	24.1	11.5	58.6	7.6	1.0
13	27.3	13.7	57.4	9.7	4.2
14	26.4	14.3	55.4	6.3	0.0
15	26.4	14.3	55.4	6.3	0.0

APPENDIX-IV

Mean performance and morphological variation of M₃ mutant population

Treatment name	Treatments	Mutants	Daye to 50 percent Flowering (No's)	Plant height (cm)	Technical height (cm)	Primary Branches (No's)	Secondary branches (No's)	Capsules per plant (No's)	Days to 75 percent maturity (No's)	Number of seeds per capsule (No's)	Biological yield per plant (g)	Seed yield per plant (g)	Harvest index	1000 Seed weight (g)	Flower Colour	Flower Shape	Flower Aestivation	Seed Colour
20 kR	T-1	M ₃₋₁₋₁	182.50	65.25	30.50	8.75	11.00	50.00	231.50	8.50	7.20	2.73	0.38	6.25	Blue	Disc	Semitwisted	Fawn
	T-1	M ₃₋₁₋₂	181.00	64.75	41.50	9.50	12.00	48.00	229.50	9.00	6.50	3.04	0.46	6.33	Blue	Disc	Semitwisted	Brown
	T-1	M ₃₋₁₋₃	162.00	66.10	34.00	8.50	10.35	50.50	211.00	8.00	7.90	3.77	0.48	7.13	Blue	Disc	Semitwisted	Brown
	T-1	M ₃₋₁₋₄	167.50	56.70	23.00	8.50	12.00	49.00	215.50	8.00	5.50	2.09	0.38	6.88	Blue	Star	Semitwisted	Brown
	T-1	M ₃₋₁₋₅	170.00	60.85	27.50	9.50	13.00	48.00	226.50	8.50	3.60	2.08	0.71	6.40	Blue	Star	Semitwisted	Brown
	T-1	M ₃₋₁₋₆	168.00	82.25	54.50	8.20	10.20	46.50	217.50	8.50	4.50	1.73	0.39	6.45	Blue	Disc	Semitwisted	Brown
	T-1	M ₃₋₁₋₇	167.50	83.00	49.25	9.50	10.90	48.50	215.50	8.00	5.50	3.25	0.58	6.60	Blue	Star	Semitwisted	Brown
	T-1	M ₃₋₁₋₈	182.00	55.50	31.00	8.20	10.50	47.00	229.50	9.00	4.00	2.33	0.58	6.60	Blue	Disc	Semitwisted	Brown
	T-1	M ₃₋₁₋₉	162.00	54.50	27.50	8.25	11.50	47.00	216.50	7.00	5.00	2.49	0.50	6.50	Blue	Star	Semitwisted	Brown
	T-1	M ₃₋₁₋₁₀	164.00	62.25	26.00	8.80	10.75	48.50	221.50	9.00	5.00	2.43	0.49	6.60	Blue	Star	Semitwisted	Brown
	T-1	M _{3-1-BC}	168.50	62.50	37.50	9.40	15.50	57.00	215.00	9.05	6.50	1.84	0.28	6.35	Blue	Disc	Semitwisted	Brown
		Mean (T-1)	170.45	64.88	34.75	8.83	11.61	49.09	220.86	8.41	5.56	2.53	0.48	6.55				
30 kR	T-2	M ₃₋₂₋₁	177.50	75.75	47.00	8.00	10.50	44.00	223.50	8.50	8.20	2.00	0.24	6.90	Light Blue	Disc	Semitwisted	Fawn
	T-2	M ₃₋₂₋₂	175.00	77.75	33.50	8.00	10.25	59.00	226.50	8.00	5.50	3.56	0.65	6.25	Blue	Disc	Semitwisted	Brown
	T-2	M ₃₋₂₋₃	178.50	75.10	33.75	8.25	11.00	52.30	229.00	8.00	11.00	2.87	0.27	6.25	Light Blue	Disc	Semitwisted	Brown
	T-2	M ₃₋₂₋₄	178.50	78.15	34.75	9.00	11.70	56.00	230.00	7.50	8.20	3.40	0.42	6.25	Light Blue	Star	Semitwisted	Brown
	T-2	M ₃₋₂₋₅	172.00	78.25	34.60	7.80	10.50	52.25	224.00	8.50	13.50	3.92	0.29	6.15	Blue	Disc	Semitwisted	Dark Brown

	T-2	M₃₋₂₋₆	163.00	74.65	33.75	8.35	11.30	44.00	210.50	9.00	9.50	3.11	0.33	6.15	Blue	Star	Semitwisted	Dark Brown
	T-2	M₃₋₂₋₇	176.50	75.15	34.75	7.50	11.15	46.50	229.50	8.00	8.11	1.98	0.24	6.55	Blue	Star	Semitwisted	Dark Brown
	T-2	M₃₋₂₋₈	179.50	76.65	36.75	8.50	11.95	47.50	230.50	8.00	8.10	3.05	0.38	6.50	Blue	Disc	Semitwisted	Brown
	T-2	M_{3-2-BC}	168.50	62.00	36.00	9.10	14.50	55.50	215.00	8.95	6.50	2.61	0.39	6.25	Blue	Disc	Semitwisted	Brown
		Mean (T-2)	174.33	74.83	36.09	8.28	11.43	50.78	224.28	8.27	8.73	2.94	0.36	6.36				
40 kR	T-3	M₃₋₃₋₁	169.00	84.00	43.00	8.50	11.50	67.00	219.00	9.00	8.10	1.93	0.24	6.65	Blue	Disc	Velvate	Brown
	T-3	M₃₋₃₋₂	188.50	73.50	43.50	7.40	12.00	34.00	238.50	9.00	7.10	2.29	0.32	6.75	Light Blue	Disc	Velvate	Brown
	T-3	M₃₋₃₋₃	178.00	55.50	21.50	8.50	10.00	41.00	230.50	7.90	4.50	2.04	0.46	6.15	Blue	Disc	Semitwisted	Brown
	T-3	M₃₋₃₋₄	162.50	71.25	23.50	8.75	12.00	52.00	207.50	8.70	8.50	2.83	0.33	6.20	Blue	Disc	Semitwisted	Brown
	T-3	M₃₋₃₋₅	162.00	69.50	27.00	7.90	10.50	52.50	208.50	8.40	8.20	2.83	0.35	6.45	Light Blue	Disc	Velvate	Brown
	T-3	M₃₋₃₋₆	178.50	66.00	31.00	8.25	10.50	49.00	228.50	8.20	7.50	2.42	0.32	6.45	Blue	Star	Semitwisted	Brown
	T-3	M₃₋₃₋₇	173.00	62.50	34.50	8.75	11.00	53.50	227.00	7.50	7.90	2.93	0.37	6.45	Blue	Star	Semitwisted	Brown
	T-3	M₃₋₃₋₈	168.50	80.00	34.50	8.50	10.50	53.00	220.00	7.00	7.80	2.11	0.27	6.65	Dark Blue	Star	Twisted	Brown
	T-3	M₃₋₃₋₉	173.00	86.50	47.00	9.10	12.50	55.50	228.50	8.00	8.00	2.61	0.33	6.40	Dark Blue	Disc	Semitwisted	Brown
	T-3	M₃₋₃₋₁₀	170.00	66.50	35.00	9.15	11.00	63.50	223.00	8.00	8.10	2.42	0.30	6.00	Blue	Disc	Velvate	Brown
	T-3	M₃₋₃₋₁₁	162.00	70.50	37.50	8.00	10.50	72.00	203.50	9.00	7.10	1.92	0.27	6.15	Blue	Star	Semitwisted	Brown
	T-3	M_{3-3-BC}	168.50	63.50	34.00	8.50	15.50	56.50	213.50	9.05	8.50	2.51	0.30	6.15	Blue	Disc	Semitwisted	Brown
		Mean (T-3)	171.13	70.77	34.33	8.44	11.46	54.13	220.67	8.31	7.61	2.40	0.32	6.37				
50 kR	T-4	M₃₋₄₋₁	178.00	64.60	30.10	9.25	12.00	51.50	230.00	7.00	8.20	1.92	0.23	6.20	Blue	Disc	Semitwisted	Brown
	T-4	M₃₋₄₋₂	176.00	57.10	32.00	8.50	11.00	54.00	228.50	8.00	8.50	2.45	0.29	6.45	Blue	Disc	Twisted	Light Brown

	T-4	M₃₋₄₋₃	171.50	67.75	53.50	9.50	13.00	52.00	224.00	8.00	4.50	1.26	0.28	6.65	Blue	Funnel	Semitwisted	Light Brown
	T-4	M₃₋₄₋₄	173.50	66.50	30.00	8.50	11.50	53.50	223.50	8.00	4.10	1.17	0.27	6.20	Blue	Star	Semitwisted	Brown
	T-4	M_{3-4-BC}	168.50	62.00	30.50	9.25	14.50	55.50	215.00	9.05	7.50	2.22	0.30	6.35	Blue	Disc	Semitwisted	
		Mean (T-4)	173.50	63.59	35.12	9.00	12.40	53.30	224.20	8.01	6.56	1.80	0.27	6.37				
60 kR	T-5	M₃₋₅₋₁	180.00	70.50	32.50	9.00	11.00	49.00	230.50	8.00	8.10	3.38	0.41	6.35	Blue	Star	Semitwisted	Brown
	T-5	M₃₋₅₋₂	175.50	81.75	34.50	12.00	13.00	76.00	227.00	9.00	8.40	3.15	0.38	6.20	Light Blue	Star	Semitwisted	Brown
	T-5	M₃₋₅₋₃	173.00	83.60	32.20	8.50	11.00	55.50	223.50	8.00	8.50	2.93	0.34	6.45	Blue	Disc	Velvate	Brown
	T-5	M₃₋₅₋₄	175.50	66.50	36.50	8.00	11.00	43.50	226.50	8.50	7.45	2.45	0.33	6.45	Blue	Star	Twisted	Brown
	T-5	M₃₋₅₋₅	169.50	68.00	35.50	8.00	11.00	43.50	210.00	8.00	6.95	2.14	0.31	6.60	Blue	Star	Semitwisted	Brown
	T-5	M₃₋₅₋₆	164.00	66.00	44.00	7.50	12.50	44.00	212.00	8.50	6.40	1.83	0.29	6.25	Blue	Disc	Velvate	Brown
	T-5	M_{3-5-BC}	168.50	64.50	34.50	9.40	13.50	54.50	213.50	9.05	6.50	1.94	0.30	6.20	Blue	Disc	Semitwisted	Brown
		Mean (T-5)	172.29	71.55	35.67	8.91	11.86	52.29	220.43	8.44	7.47	2.55	0.34	6.36				
0.2 %	T-6	M₃₋₆₋₁	180.50	69.50	31.25	10.25	13.50	51.00	233.50	9.00	8.50	2.23	0.26	6.30	Blue	Disc	Semitwisted	Brown
	T-6	M₃₋₆₋₂	180.00	72.25	33.75	10.50	12.50	52.00	233.50	8.00	9.90	3.09	0.31	6.35	Blue	Funnel	Twisted	Light Brown
	T-6	M₃₋₆₋₃	166.50	69.00	40.75	9.40	11.50	42.00	210.50	8.00	8.20	2.98	0.36	6.50	Blue	Funnel	Twisted	Brown
	T-6	M₃₋₆₋₄	169.00	81.00	43.75	9.80	12.00	40.50	213.50	9.00	7.10	2.94	0.41	6.10	Blue	Funnel	Twisted	Dark Brown
	T-6	M_{3-6-BC}	168.50	64.00	43.00	9.25	14.00	57.00	215.00	9.05	7.00	1.97	0.28	6.25	Blue	Disc	Semitwisted	Brown
		Mean (T-6)	172.90	71.15	38.50	9.84	12.70	48.50	221.20	8.61	8.14	2.64	0.33	6.30				
20 kR	T-7	M₃₋₇₋₁	172.00	74.70	46.50	10.50	16.50	70.00	227.50	8.75	6.60	1.65	0.25	7.10	White	Disc	Semitwisted	Brown
	T-7	M₃₋₇₋₂	168.50	74.00	30.50	8.00	15.20	64.50	218.50	8.70	7.50	3.49	0.46	6.35	White	Disc	Semitwisted	Brown
	T-7	M₃₋₇₋₃	163.00	73.20	37.50	8.00	16.25	65.50	214.50	8.75	10.60	3.56	0.34	6.30	White	Disc	Semitwisted	Brown
	T-7	M₃₋₇₋₄	154.00	72.35	37.00	9.50	16.25	66.00	201.00	8.00	10.40	3.35	0.32	6.45	White	Star	Semitwisted	Brown

30 kR	T-7	M ₃₋₇₋₅	166.50	75.30	38.50	8.50	14.50	65.75	215.50	9.00	11.60	2.79	0.24	6.50	White	Star	Semitwisted	Brown
	T-7	M ₃₋₇₋₆	169.50	82.25	34.50	8.50	15.35	69.25	216.00	8.50	9.50	2.22	0.23	6.30	White	Disc	Semitwisted	Brown
	T-7	M _{3-7-HC}	165.50	65.00	43.50	9.75	13.50	64.00	215.50	9.10	6.50	2.08	0.32	6.60	White	Disc	Semitwisted	Brown
		Mean (T-7)	165.57	73.83	38.29	8.96	15.36	66.43	215.50	8.69	8.96	2.73	0.31	6.51				
	T-8	M ₃₋₈₋₁	163.50	78.75	37.80	9.00	12.00	46.50	215.50	9.00	8.10	2.51	0.31	6.45	White	Star	Semitwisted	Light Brown
	T-8	M ₃₋₈₋₂	160.50	76.75	36.20	7.75	11.00	50.00	208.50	8.00	8.20	2.40	0.29	6.45	White	Star	Semitwisted	Brown
	T-8	M ₃₋₈₋₃	165.50	77.10	37.35	8.60	11.00	49.00	215.50	9.00	6.00	2.16	0.41	6.50	White	Disc	Semitwisted	Brown
	T-8	M ₃₋₈₋₄	164.50	77.65	39.95	8.25	11.50	47.50	214.50	8.00	6.20	1.90	0.31	6.35	White	Funnel	Semitwisted	Brown
	T-8	M ₃₋₈₋₅	165.50	81.10	37.60	8.20	11.00	44.00	216.00	9.00	6.10	2.47	0.41	6.20	White	Star	Velvate	Brown
	T-8	M ₃₋₈₋₆	167.00	81.60	41.00	9.10	12.00	45.00	217.00	8.00	8.20	2.40	0.29	6.30	White	Star	Velvate	Brown
	T-8	M ₃₋₈₋₇	171.00	77.35	35.65	7.75	11.00	44.00	222.50	7.00	5.10	2.06	0.41	6.45	White	Disc	Velvate	Brown
	T-8	M ₃₋₈₋₈	171.50	76.45	38.50	7.95	10.00	45.00	220.50	8.00	6.20	1.86	0.30	6.50	White	Star	Semitwisted	Brown
	T-8	M ₃₋₈₋₉	170.00	79.60	39.60	7.25	12.00	45.00	222.50	8.00	5.90	2.22	0.41	6.30	White	Disc	Velvate	Brown
	T-8	M ₃₋₈₋₁₀	171.00	78.65	47.00	8.30	11.00	51.50	222.50	8.00	6.50	2.59	0.41	6.30	White	Star	Semitwisted	Brown
40 kR	T-8	M _{3-8-HC}	165.50	65.00	37.00	10.00	14.00	56.50	216.50	9.10	7.00	2.14	0.31	6.45	White	Disc	Semitwisted	Brown
		Mean (T-8)	166.86	77.27	38.38	8.38	11.50	47.64	217.41	8.28	6.68	2.25	0.35	6.39				
	T-9	M ₃₋₉₋₁	172.00	82.75	42.00	9.40	13.75	36.25	224.50	8.05	11.60	3.53	0.31	6.10	White	Star	Semitwisted	Brown
	T-9	M ₃₋₉₋₂	168.50	75.20	37.00	9.80	11.25	52.20	219.00	8.50	8.20	2.65	0.32	6.15	White	Star	Semitwisted	Brown
	T-9	M ₃₋₉₋₃	169.00	74.25	45.50	9.80	12.20	50.50	217.50	8.50	8.10	2.80	0.35	6.45	White	Star	Semitwisted	Brown
	T-9	M ₃₋₉₋₄	170.50	81.95	45.00	8.75	11.50	53.80	220.50	9.30	10.20	2.69	0.26	6.50	White	Star	Semitwisted	Light Brown
	T-9	M ₃₋₉₋₅	171.50	66.00	33.00	10.25	12.50	54.20	221.00	8.00	8.50	2.52	0.30	6.30	White	Disc	Semitwisted	Brown
	T-9	M ₃₋₉₋₆	174.50	77.15	34.00	8.50	12.00	51.00	222.50	9.00	9.50	2.66	0.28	6.30	White	Disc	Semitwisted	Brown
	T-9	M _{3-9-HC}	165.50	64.50	34.50	9.45	12.50	56.50	214.50	9.10	6.00	1.86	0.31	6.05	White	Disc	Semitwisted	Brown
		Mean (T-9)	170.21	74.54	38.71	9.42	12.24	50.64	219.93	8.64	8.87	2.67	0.30	6.26				

50 kR	T-10	M₃₋₁₀₋₁	168.00	66.25	33.35	9.50	13.50	50.50	214.50	9.00	7.90	2.96	0.38	6.20	White	Disc	Semitwisted	Brown
	T-10	M₃₋₁₀₋₂	174.00	73.00	35.50	8.25	10.50	43.50	224.50	8.00	8.10	2.70	0.33	6.35	White	Star	Velvate	Brown
	T-10	M₃₋₁₀₋₃	171.00	84.25	36.25	10.50	14.00	57.25	226.50	8.00	6.50	2.23	0.35	6.35	White	Star	Semitwisted	Brown
	T-10	M₃₋₁₀₋₄	173.50	81.75	40.25	12.50	16.50	68.50	222.50	7.00	8.20	3.75	0.46	6.40	White	Disc	Semitwisted	Brown
	T-10	M₃₋₁₀₋₅	169.00	72.75	31.00	11.25	12.50	55.00	214.50	9.00	4.50	3.39	0.78	6.45	White	Disc	Semitwisted	Brown
	T-10	M₃₋₁₀₋₆	173.50	74.50	35.00	10.50	12.00	72.50	224.00	8.00	8.50	3.21	0.38	6.35	White	Star	Velvate	Brown
	T-10	M₃₋₁₀₋₇	167.00	73.50	35.00	10.00	12.50	71.00	215.50	9.00	8.10	3.53	0.44	6.50	White	Star	Semitwisted	Brown
	T-10	M₃₋₁₀₋₈	167.00	72.50	24.50	11.00	14.00	74.00	217.00	8.00	9.90	4.53	0.46	6.40	White	Star	Semitwisted	Brown
	T-10	M₃₋₁₀₋₉	174.50	70.00	29.00	11.00	13.50	76.50	226.50	8.00	7.40	3.89	0.53	6.30	White	Disc	Semitwisted	Brown
	T-10	M_{3-10-HC}	165.50	64.50	39.50	9.35	14.50	54.00	214.50	9.10	8.50	2.62	0.31	6.15	White	Disc	Semitwisted	Brown
		Mean (T-10)	170.30	73.30	33.94	10.39	13.35	62.28	220.00	8.31	7.76	3.28	0.44	6.35				
60 kR	T-11	M₃₋₁₁₋₁	160.50	58.00	24.00	8.75	11.00	53.50	210.50	8.00	9.50	2.32	0.24	6.35	White	Disc	Velvate	Brown
	T-11	M₃₋₁₁₋₂	153.00	60.00	29.50	10.50	15.00	59.00	196.20	8.00	10.30	3.16	0.31	6.40	White	Disc	Velvate	Brown
	T-11	M₃₋₁₁₋₃	158.50	51.50	28.00	6.25	9.00	32.50	203.00	8.00	8.10	2.61	0.32	6.45	White	Disc	Velvate	Brown
	T-11	M₃₋₁₁₋₄	162.00	55.50	23.50	7.75	10.00	37.50	210.00	7.00	4.50	1.53	0.35	6.35	White	Disc	Velvate	Brown
	T-11	M₃₋₁₁₋₅	165.00	66.50	29.50	10.50	16.00	59.50	212.50	8.00	9.50	2.85	0.30	6.50	White	Disc	Velvate	Brown
	T-11	M₃₋₁₁₋₆	165.00	52.50	20.00	10.00	13.50	61.50	213.00	8.00	10.50	3.28	0.31	6.40	White	Disc	Velvate	Dark Brown
	T-11	M₃₋₁₁₋₇	161.50	56.00	23.50	7.00	9.50	38.50	212.50	7.00	4.50	1.78	0.41	6.45	White	Disc	Velvate	Light Brown
	T-11	M₃₋₁₁₋₈	159.50	53.50	25.50	7.25	10.50	36.50	203.50	8.00	2.50	1.83	0.78	6.35	White	Disc	Velvate	Fawn
	T-11	M₃₋₁₁₋₉	156.50	55.00	22.50	7.90	11.00	37.00	201.00	8.00	3.40	2.13	0.63	6.50	White	Disc	Velvate	Fawn
	T-11	M₃₋₁₁₋₁₀	161.50	58.75	27.00	8.60	12.00	51.00	214.50	9.00	8.50	2.94	0.35	6.40	White	Funnel	Velvate	Brown
	T-11	M_{3-11-HC}	165.50	62.00	33.50	9.55	14.50	64.00	214.50	9.10	6.50	2.00	0.31	6.75	White	Disc	Velvate	Brown
		Mean (T-11)	160.77	57.20	26.05	8.55	12.00	48.23	208.95	8.01	7.07	2.40	0.39	6.45				
20 kR	T-12	M₃₋₁₂₋₁	168.00	54.50	21.50	10.00	13.50	73.50	219.00	8.50	11.60	3.90	0.34	5.45	White	Star	Velvate	Golden

																		Yellow
	T-12	M₃₋₁₂₋₂	168.00	58.00	21.50	11.50	16.50	76.00	217.00	8.50	8.70	3.25	0.38	5.90	White	Star	Velvate	Golden Yellow
	T-12	M_{3-12-SC}	166.50	48.50	21.50	10.50	16.50	67.50	215.50	8.75	6.50	2.76	0.43	5.45	White	Star	Velvate	Golden Yellow
		Mean (T-12)	167.50	53.67	21.50	10.67	15.50	72.33	217.17	8.58	8.93	3.30	0.38	5.60				
30 Kr	T-13	M₃₋₁₃₋₁	163.50	53.75	20.50	8.50	11.00	55.00	214.50	8.50	8.10	3.04	0.38	5.20	White	Star	Velvate	Golden Yellow
	T-13	M₃₋₁₃₋₂	160.00	45.50	15.00	8.50	13.50	92.00	212.50	8.50	10.00	4.58	0.46	5.20	White	Star	Velvate	Golden Yellow
	T-13	M₃₋₁₃₋₃	164.00	53.25	15.50	8.50	11.50	58.00	214.50	8.00	4.50	2.64	0.59	5.35	White	Star	Velvate	Golden Yellow
	T-13	M₃₋₁₃₋₄	165.00	43.00	15.25	8.50	13.00	76.00	215.00	8.50	5.00	4.06	0.94	5.30	White	Star	Velvate	Golden Yellow
	T-13	M₃₋₁₃₋₅	163.50	53.00	15.75	8.50	11.50	44.00	214.00	9.00	8.50	2.74	0.32	5.35	White	Star	Velvate	Golden Yellow
	T-13	M_{3-13-SC}	166.50	44.50	13.50	11.50	16.50	68.50	215.50	8.75	7.50	3.59	0.48	5.25	White	Star	Velvate	Golden Yellow
		Mean (T-13)	163.75	48.83	15.92	9.00	12.83	65.58	214.33	8.54	7.27	3.44	0.53	5.28				
40 kR	T-14	M₃₋₁₄₋₁	175.00	47.50	19.50	8.40	13.50	50.50	196.50	8.50	4.50	2.64	0.59	5.30	White	Star	Velvate	Golden Yellow
	T-14	M₃₋₁₄₋₂	173.50	65.25	35.00	9.25	13.00	56.00	196.20	8.75	6.50	2.82	0.44	5.75	White	Star	Velvate	Golden Yellow
	T-14	M₃₋₁₄₋₃	176.00	51.50	15.50	6.00	10.00	42.50	226.00	8.50	6.20	2.07	0.33	5.55	White	Star	Velvate	Golden Yellow
	T-14	M_{3-14-SC}	166.50	44.50	20.50	10.50	16.00	67.00	215.50	8.75	9.00	3.85	0.43	5.15	White	Star	Velvate	Golden Yellow
		Mean (T-14)	172.75	52.19	22.63	8.54	13.13	54.00	197.75	8.63	6.55	2.85	0.45	5.44				
50 kR	T-15	M₃₋₁₅₋₁	177.00	55.50	33.00	9.50	11.50	64.50	226.50	8.75	10.20	3.52	0.35	5.15	White	Star	Velvate	Brown
	T-15	M₃₋₁₅₋₂	162.00	50.50	33.50	10.05	13.50	61.00	212.50	8.35	5.50	2.92	0.53	5.70	White	Star	Velvate	Brown

	T-15	M_{3-15-SC}	166.50	44.50	22.50	11.50	15.50	69.50	215.50	8.75	6.50	2.97	0.46	5.45	White	Star	Velvate	Brown
		Mean (T-15)	168.50	50.17	29.67	10.35	13.50	65.00	218.17	8.62	7.40	3.14	0.44	5.43				
0.5 %	T-16	M₃₋₁₆₋₁	172.00	65.75	34.25	10.50	15.50	57.50	227.50	8.00	9.70	3.23	0.33	6.75	Blue	Disc	Semitwisted	Brown
	T-16	M₃₋₁₆₋₂	161.50	63.60	33.10	9.75	14.50	57.50	212.00	9.00	9.90	3.45	0.35	6.70	Dark Blue	Disc	Twisted	Brown
	T-16	M_{3-16-BC}	168.50	61.50	31.50	9.40	13.00	57.00	215.00	9.05	8.50	2.40	0.28	6.40	Blue	Disc	Semitwisted	Brown
		Mean (T-16)	167.33	63.62	32.95	9.88	14.33	57.33	218.17	8.68	9.37	3.03	0.32	6.62				
0.6 %	T-17	M₃₋₁₇₋₁	173.00	60.75	31.50	8.50	12.75	72.00	227.00	8.75	8.70	3.12	0.36	6.25	Blue	Disc	Twisted	Brown
	T-17	M₃₋₁₇₋₂	174.50	77.25	37.00	8.50	11.60	64.00	226.00	8.40	8.30	2.70	0.33	6.15	Dark Blue	Disc	Twisted	Brown
	T-17	M_{3-17-BC}	168.50	64.50	34.50	9.40	14.50	56.50	215.00	9.05	9.00	2.69	0.30	6.25	Blue	Disc	Semitwisted	Brown
		Mean (T-17)	172.00	67.50	34.33	8.80	12.95	64.17	222.67	8.73	8.67	2.83	0.33	6.22				
0.8 %	T-18	M₃₋₁₈₋₁	171.00	65.75	33.10	10.00	11.00	43.75	230.00	8.50	8.75	2.87	0.33	6.50	Blue	Disc	Semitwisted	Brown
	T-18	M₃₋₁₈₋₂	167.50	73.75	35.50	9.75	14.00	41.50	219.50	7.75	9.75	3.03	0.31	6.45	Blue	Disc	Semitwisted	Brown
	T-18	M₃₋₁₈₋₃	167.50	86.95	43.25	11.50	13.75	71.50	214.50	8.50	8.50	3.80	0.45	6.75	Blue	Disc	Semitwisted	Brown
	T-18	M₃₋₁₈₋₄	169.00	81.00	44.00	11.80	15.55	73.50	221.00	8.50	8.75	3.98	0.46	6.45	Blue	Disc	Semitwisted	Brown
	T-18	M₃₋₁₈₋₅	166.50	72.95	31.00	9.90	11.25	48.00	215.50	8.75	8.95	2.80	0.31	6.65	Blue	Disc	Semitwisted	Brown
	T-18	M_{3-18-BC}	168.50	63.50	35.50	9.00	15.50	56.50	215.00	9.05	7.50	2.19	0.29	6.25	Blue	Disc	Semitwisted	Brown
		Mean (T-18)	168.33	73.98	37.06	10.33	13.51	55.79	219.25	8.51	8.70	3.11	0.36	6.51				
0.4 %	T-19	M₃₋₁₉₋₁	165.50	75.25	35.00	7.60	11.50	54.50	228.50	8.00	9.00	3.33	0.38	6.45	White	Star	Velvate	Brown
	T-19	M₃₋₁₉₋₂	166.00	71.35	32.00	8.00	10.00	49.50	228.00	9.00	8.60	2.96	0.35	6.75	White	Disc	Semitwisted	Brown
	T-19	M₃₋₁₉₋₃	170.00	71.00	31.50	8.00	10.00	48.75	230.50	9.00	8.70	3.25	0.38	6.45	White	Star	Velvate	Brown
	T-19	M₃₋₁₉₋₄	165.00	73.50	31.00	10.50	14.75	76.00	229.00	8.00	9.40	4.01	0.43	6.65	White	Star	Velvate	Brown
	T-19	M₃₋₁₉₋₅	167.00	86.50	31.50	8.50	11.50	57.50	228.50	9.00	10.25	1.84	0.18	6.75	White	Star	Semitwisted	Brown
	T-19	M₃₋₁₉₋₆	169.50	73.25	30.00	9.00	11.00	45.50	227.50	8.00	10.10	2.31	0.23	6.45	White	Disc	Semitwisted	Brown

	T-19	M₃₋₁₉₋₇	169.00	73.25	37.50	8.00	10.00	44.00	228.50	9.00	9.70	3.13	0.32	6.65	White	Disc	Semitwisted	Brown
	T-19	M₃₋₁₉₋₈	162.50	72.25	34.50	8.50	11.50	43.50	229.00	8.00	8.50	2.06	0.24	6.25	White	Disc	Semitwisted	Brown
	T-19	M_{3-19-HC}	165.50	66.00	36.50	9.55	14.50	64.00	214.50	9.10	8.50	2.73	0.32	6.75	White	Disc	Semitwisted	Brown
		Mean (T-19)	166.67	73.59	33.28	8.63	11.64	53.69	227.11	8.57	9.19	2.85	0.31	6.57				
		Grand mean	168.96	67.99	33.24	9.05	12.47	54.71	219.46	8.41	7.72	2.71	0.37	6.29				
		MIN	153.00	43.00	13.50	6.00	9.00	32.50	196.50	7.00	2.50	1.17	0.18	5.15				
		MAX	188.50	86.95	54.50	12.50	16.50	92.00	238.50	9.30	13.50	4.58	0.94	7.13				
		SE(m)	2.09	1.31	2.40	0.62	1.22	4.26	1.02	0.24	0.60	0.30	0.06	0.13				
		CV	1.75	2.73	10.19	9.71	13.87	11.02	0.66	4.08	10.91	15.84	22.48	2.90				
		CD	5.85	3.67	6.70	1.74	3.42	11.93	2.85	0.68	1.67	0.85	0.16	0.36				

Brief Biodata of student

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Academic Qualifications

Qualification	Year	School/Board/ University	Marks (%)	Division
10 th	2008	S.V.M Katrain	79.44	First
12 th	2010	GSSS (Boys), Dhalpur Kullu	84.80	First
B.Sc. (Hons.) Agriculture	2014	CSK HPKV, Palampur (H.P.)	74.80	First
M.Sc. (Genetics and Plant Breeding)	2016	SHIATS, Allahabad	95.00	First