DISCUSSION
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Mutagenic studies:

M₁ generation:

The important aspects of mutation research include enhancement of mutation frequency and the alteration of the mutation spectrum, which would increase the probability of isolating mutants of economic interest. For the enhancement of mutation frequency and spectrum, besides the basic information regarding the mutagenic sensitivity of various genotypes, effectiveness and efficiency of chemical/physical mutagen becomes necessary.

The effects of mutagens can be assessed through different biological parameters such as percentage of seed germination, seedling growth, pollen sterility, survival at maturity, chlorophyll deficient chimeras and morphological variations or abnormalities in M₁ generation. The above mentioned parameters are used as indices for testing mutagenic sensitivity of an organism (Ehrenberg 1955; Nilan et al.; 1968 and Tarar and Dnyansagar 1979). Hence, the results of the present study are analyzed and discussed as follows.

Seed Germination and Lethality:

The results of the present study have revealed a marked inhibition of seed germination in both the cultivars after the mutagenic treatments. The inhibitory effect on germination in both the cultivars of lentil could be seen in proportion with the increasing concentration of the mutagens. The slight increased germination percentage values of L-4611 as compared to L-4639 for the same mutagenic treatments substantiated the idea of varietal differences existing in mutagenic sensitivity (Sree Ramulu, 1970 and Reddy 1974).
The reduction in germination may be due to genetic and physiological processes inhibited by the mutagens resulting in cell mortality. Such increase in lethality values with respect to increasing dose/concentration values were also reported by Gregory (1955 and 1968) in peanut; Gottschalk and Schieb (1960) in peas; Sjodin (1962) in *Vicia faba*; Gaul (1967) in wheat; Bajaj (1970) in French bean; Singh and Chowdhary (1972) in cluster bean; Selim et al; (1974) in *Pisum sativum*; Hakande (1992) in winged bean; More (1992) in *Medicago sativa*; Satpute (1994) in safflower and Rayyan Asra (1995) in black gram.

Similar results have been obtained in lentil as well by Sarker and Sharma (1989); Vandana and Dubey (1990) and Solanki and Sharma (1992).

The decrease in germination percentage has been attributed mainly to the interference by the mutagen with metabolic activities of the seed (Micke 1958,1961, Gottschalk and Schieb 1960 and Sjodin 1962). The general inhibition of germination and increased lethality could be due to the lowering of the rate of mitotic proliferation and the consequent delay in cell division and repair of damaged DNA (Hutterman et al., 1978). According to Brock (1965) and Larik (1975), the mutagen induced chromosomal breakages may be the likely reason for reduced seed germination. Gaul (1964) stated that the pertinent effect is produced mainly through physiological damage.

Jana (1962), observed that germination was marginally affected even by very high dose of radiation. Blixt (1972), proposed that high doses of radiation are required for complete inhibition of germination of legumes. Prasad (1972) noted very little reduction in seed germination after gamma radiation and chemical mutagenic treatments in *Trifolium*. Subba Rao
(1988) studied the effects of gamma rays in *Cicer* and found that right from 10kR onwards the germination revealed a declining trend.

An increase in seed germination after gamma ray and EMS treatments was observed by Dutta (1969) and Rao (1983) in Okra and Kothekar (1978) in *Solanum nigrum*.


Aman (1968) opined that the endogenous growth regulators play an important role in seed germination and there exists a balance between the promoters and the inhibitors. Meherchandani (1975) correlated reduction in germination to the disturbance of balance between the promoters and the inhibitors.

Different researchers have reviewed the effect of alkylating agents and their mechanism of action in biological system, they are Ross (1962), Loveless (1966), Fishbein et al (1970), Lawley (1973 and 1974) and Sun and Singer (1975). These workers have ascribed the decrease in germination percentage to the lethality produced in seeds which possibly developed through physiological injuries, chromosomal aberrations and the hydrolytic products of the mutagens.

During the present investigation varietal differences in regard to mutagenic sensitivity, could be distinctly noticed.

According to Ashri and Herzog (1972), the differential varietal radiosensitivity originates from the sites of metabolic process affected at the embryonic level. They further indicated that the differential oil content of the seeds may be crucial as regards the radiosensitivity. Raghuvanshi and Singh (1979) recorded an inverse correlation between dose and germination in all the varieties of *Impatiens balsamina* except for the purple variety, which displayed better germination. Based on this, they inferred that a variation of physiological condition within one and same variety might affect the radiosensitivity.

**Seedling Height and Injury:**

Studying seedling growth as a parameter to assess the effects of mutagens was proposed by Ahnstrom (1974). It is clear from the results obtained in the present study on the seedling growth that the seedling height decreased with the increasing concentration of the mutagens. The cultivar L-4639 was more sensitive to EMS and SA as compared to L-4611 cultivar. According to Sax (1963) mutagen might inactivate the meristems and cause hormonal disturbances leading to reduction in plant height. It can also result in injury at cellular or chromosomal level (Sinha and Godward 1969).

Various environmental conditions under which seeds are irradiated i.e. physiological conditions of seeds themselves and the type of ionizing radiation, may all contribute to the stimulation or retardation of growth in seedlings (Caldecott and North 1961).

Pelc and Howard (1956) and Gorden (1957) have suggested that the possible interference of irradiation with synthesis of new DNA may lead to inhibition of growth. Gunckel and Sparrow (1962) indicated that although the genetic material of the cell is certainly sensitive to radiation damage, both primary and secondary physiological effects may be responsible for many changes. Evans (1965), while studying the effects of radiation on
meristematic cells considered growth reduction to be due to cumulative expression of mitotic
cycle delay, formation of chromosomal structural changes and loss of proliferation capacity
due to either premature differentiation or cell death.

Bhamburkar (1981) opined that the reduced seedling growth caused due to reduction in
number of cells contributing to seedling growth / cellular death is generated by mutagen
induced physiological processes.

Conger and Stevenson (1969) reported that, increased seedling injury at higher doses
could be correlated with chromosomal damage.

**Pollen Sterility:**

Sterility in M₁ generation is the first sign of genetical effectiveness of the mutagenic
treatment (Kivi 1962). The results in the present study showed that both the cultivars were
more sensitive to EMS than SA treatments. Chromosomal abnormalities resulting from
mutagenic treatments may actually induce pollen sterility (Konzak et al 1961; Sparrow and
Woodwell 1962; Gaul et al 1966; Gaul 1967; Sudhakaran 1971; Bhairava Murthy and
Venkataraman 1977). They advocated that ionizing radiations cause chromosomal
rearrangements leading to sterility.

The results obtained in the present study are in agreement with the results reported by
Vandana and Dubey (1990) in K-85 lentil cultivar. The induced pollen sterility by chemical
mutagens also has been noted by Froese Gertzén et al (1964), Gaul et al (1966), Sato and Gaul

According to Nilan et al (1964), gross injury due to gene controlled biochemical
processes or acute chromosomal aberrations or both may be the reason for pollen sterility. The
major cryptic changes in meiosis due to mutagenic treatments have been implicated for pollen sterility (Wanjari and Kutarekar 1977).

According to Sato and Gaul (1967), the radiation induced M₁ sterility might be due to detectable chromosomal aberrations and cryptic deficiencies, while the sterility induced by EMS might be due to the cryptic deficiencies and specific gene mutations. Sato and Gaul (1967) have divided the pollen sterility induced by EMS in three categories 1) Chromosomal 2) Genic and 3) Purely physiological.

Hakande (1992) observed that the variety JC/4198 showed higher pollen sterility after gamma rays, EMS and NEU treatments than variety iiHP sel-21 in winged bean.

**Plant Survival:**

An inverse relationship exists between concentration and survival percentage. In the present study EMS and SA reduced the survival percentage in both the cultivars. Similar results have been obtained by Dixit and Dubey (1986), Sarker and Sharma (1989); Vandana and Dubey (1990); Solanki and Sharma (1994) in lentil, Gustafsson, (1947) and Ehrenberg et. al, (1958) in barely, Gregory (1956) in peanuts, and Saric et al (1961) in wheat.

Gaul (1964) opined that chromosomal and extra chromosomal injury might lead to disturbances at physiological and cytological levels. Similar results have been reported by several workers, (Ehrenberg 1955; Siddiq and Swaminathan 1968; Chary 1983; Sudha rani 1990; Kiranmai 1992; Padmavati 1993; Asra Rayyan 1995 and Bale 1999).

In spite of good germinability, a decrease in the percentage of survival at maturity suggests the delayed effects of mutagen at a later stage of growth and development. Abnormal cell division with hampered metabolic activities causes disturbance to the normal cell leading
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to a crippled growth and later to death before maturity ( Ehrenberg 1955, Fuji and Matsumura 1964).

The general decline in the values of germination percentage, plant height, plant survival and an increase in pollen sterility with the increasing concentrations of the mutagen suggest mainly chromosomal damage. Fall in auxin levels due to breakage of highly sensitive enzyme system by the mutagen might also be the reason (Gorden 1957).

Chlorophyll Chimeras:

All the mutagenic treatments induced chlorophyll chimeras in lentil. They occurred at a higher frequency in the material treated with EMS while they were relatively fewer in number in material treated with SA mutagen.

Stadler (1930) was the first to observe that mutations induced by seed irradiation appeared in sectors in the M1 plants. Anderson et al (1949), Caldecott and Smith (1952) and Gaul (1961) also reported the occurrence of mutated sectors. The occurrence of chlorophyll deficient leaf spots in leguminous plants was reported by Kaplan (1954) and Zacharias (1956) in Glycine, Rayyan Asra (1995) in Black gram and Panchabhaye (1997) in Sunflower

Gaul (1958) proposed that chimeras arise due to differential responses of embryonic cells. This view was supported by Goud (1967). In the present study the chimeric plants did not breed true in the M2 generation. This suggests that the chimeric areas occur due to alteration in the DNA of the chloroplasts as proposed by Ehrenberg and Nybom (1954) and Swaminathan et al (1962). According to Freese et al (1963), there is a possibility that the M1 chloroplast streaks may be developing due to alkylation of the chloroplast DNA. The non-heritable nature of chimeras has been reported by Mackey (1954), Sjodin (1962), Swaminathan (1963) and
Ramanna and Natarajan (1965). Physiological changes could also bring about the formation of chimeras (Kothekar, 1978).

**Leaf Abnormalities:**

Leaf morphological changes could be seen in the M₁ population of both the cultivars. The variation comprised enhancement/reduction in the size of leaflets, adnation between two leaflets and leaflets displaying differential venation pattern. The mutagens successfully induced the varied leaf alterations. The EMS treatments showed maximum frequency of leaf changes than the SA treatment in both the cultivars.

The changes in leaf shape and size have been reported by a number of investigators in beans (Genter and Brown, 1941), pea (Gelin 1954), soybean, (Zacharias 1956; Bale 1999), black gram (Jana 1962; Appa Rao and Jana 1976a; Rayyan Asra 1995), winged bean (Hakande 1992) and in lucerne (More 1992).

Some of the workers also reported variability in leaf morphology e.g. Kothekar (1978) in *Solanum nigrum* and Satpute (1994) in safflower.

Chaturvedi and Singh (1978) found bifoliolate, tetrafoliolate, pentafoliolate and wrinkled leaves in *Phaseolus aureus* after EMS and DES treatments. Similar types of results were found by Rayyan Asra (1995) in black gram after gamma ray, Maleic hydrazide (MH) and Diethylsulphate (DES) treatments.

**M₂ Generation:**

**1 Chlorophyll mutations:**

Scoring of chlorophyll mutations has proved to be a dependable index for evaluating the genetic effects of mutagenic treatments. The chlorophyll frequency can be used to assess the efficiency of mutagen and also as an indicator to predict the rate of factor mutations.
Though the chlorophyll mutations do not yield viable seeds; they are useful in understanding different physical functions and pathological manifestations (Miller 1968). They also help in the study of the effects of specific gene products in differentiation (Robbelen 1968).

The spectrum of chlorophyll mutations became broader with increasing concentration of two mutagens in both the cultivars. The *viridis* type was observed in high frequencies while *albina* type could be observed to occur in very low frequencies in case of both the lentil cultivars. The *chlorina* type was followed by *xantha* type in both the cultivars as regards their frequency values.

Similar types of results were obtained by Sharma and Sharma (1981) and Rajput and Sarwar (1996) in lentil. But Singh et al. (1989) found that lower doses/ concentrations of gamma radiation and EMS resulted in higher frequency of chlorophyll mutations than higher doses/ concentrations in lentil.

The chlorophyll mutation frequency obtained in the present study increased with concentration of the mutagen. This is in conformity with the results of Ehrenberg (1960), Mackey (1961), Jana (1963), Blixt et al (1963), Blixt (1964), Konzak et al (1965), Blixt (1966), Sudha (1990), Gautam et al (1992), Vannirajan et al (1993) and Rayyan Asra (1995).

In the present study EMS treatments generated more chlorophyll mutants than SA in both the varieties. This was confirmed by the findings of Singh et al. (1989) in lentil that EMS was most efficient mutagen as compared with gamma rays and HA.

Sjodin (1962), Blixt et al (1963), Zannone (1965) Wellensiek (1965) and Monti (1968) have reported a higher frequency of chlorophyll mutations following treatments with chemical mutagens rather than radiations in several legumes. Monti (1968) specifically reported that DES was 3-4 times more efficient in black gram than X-rays in peas. Singh and Chaturvedi
(1988) observed that the chlorophyll mutations showed remarkable differences between the spectrum induced by alkylating agents (EMS and NMU) and radiations (Gamma rays).

According to Swaminathan (1964, 1965) chlorophyll development seems to be controlled by many genes located on different chromosomes. A variation in the number of loci (125-250) and (250-300) involved in chlorophyll synthesis has been proposed by Gustafsson (1963) and Nilan (1964), respectively.

**Mutagenic Effectiveness and Efficiency:**

A mutagen is useful only if it is effective as well as efficient. Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. In mutation breeding programmes, a high mutation rate accompanied by minimal deleterious effects is desirable. But, generally the mutagen dose that gives the highest mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Konzak et al 1965). Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of the mutagen and mutagenic efficiency gives an idea of mutation frequency in relation to biological damage such as lethality, injury, sterility and chromosomal aberrations etc. caused as a result of mutagenic treatment. Hence, a mutagen is useful only if it is effective as well as efficient since it results in the production of desirable changes (mutations) with minimum undesirable effects. In the present study, the SA mutagen has proved more effective than EMS.

Similar type of results were obtained by Reddy and Annadurai (1991) in three lentil cultivars where SA was most effective than EMS and gamma rays. Some researchers found that alkylating agents are more effective and efficient in inducing mutation than gamma rays, they are Blixt (1964), Wellensiek (1965), Konzak et al (1965), Monti (1968), Prasad (1972),

Higher mutagenic effectiveness and efficiency at lower doses have been reported by Siddiqi and Swaminathan (1968), Prasad (1972), Nerkar (1977b), Farook (1978), Bhamburkar (1981), Chary (1983), Sudha rani (1990), Reddy and Annadurai (1991) and Solanki and Sharma (1994). According to Konzak et al (1965), lower concentrations are more efficient as the injury, lethality or sterility increases with mutagen concentration at faster rate than mutations.

In the present study SA was more effective than EMS in both the cultivars, while EMS was observed to more efficient than SA. Maximum effectiveness in both the cultivars was observed at 0.05% EMS treatment where as 0.03% SA treatment was observed to be more efficient in both the cultivars.

When mutation rates based on efficiency were compared, EMS was most efficient as far as lethality; injury and pollen sterility are concerned in both the cultivars. Nilan (1972) has opined that physical and chemical agents can be helpful in the recovery of new mutants and possibly is an important step towards the goal of directed mutagenesis.

**Viable Mutants:**

A changed phenotype is due to several complex steps in the process of mutation, which should then be viable to be traced and recognized. Several mechanisms that could account for mutability differences have been proposed, but no evidence to explain the full range of the spectrum observed.

In the present investigation, several viable mutants exhibiting change in their morphology, such as plant structure, maturity, vegetative characters, seed characters, mutation
of polygenic interest and practical value were isolated. The spectra of viable mutations differed
in the different mutagenic treatments and also from one cultivar to another. The mutation
frequency was random for different characters.

The change in habit is the most common observation in any mutagenic study. Irradiation may also be the cause of alteration in the habit (Genter and Brown 1941; Gelin
1954; Down and Anderson 1956; Lamprecht 1957. Gunckel and Sparrow 1962 and Gunckel
1965) have opined that although the genetic material of cell is quite sensitive to radiation
damage, both primary and secondary physiological effects may be responsible for various
morphological changes.

In the present investigation, tall and dwarf mutants were obtained and these were
associated with various morphological changes in characters like days to flowering, days to
maturity, number of pods per plant and number of seeds per pod. These changes are the results
of pleiotropic effects of the mutated genes. The tallness is fundamentally due to an initial
increase in internode length, sometimes accompanied by an increase in internode number (Jana
1963). Stimulation in growth has been reported by Sax ((1963), Suss (1966), Torne (1967) and
Woodstock and Justice (1967). Moreover, increased length of the cells and also their number
per unit area contributes to tallness. Tall mutants have been observed by Tyagi and Gupta

Different workers have attributed reduction in plant height caused by mutations to
different factors. It may be due to irradiation (Konzak et al 1961; Moes 1961; Matsuo and
Onazawa 1961), decrease in growth of seedling due to destruction of auxins (Smith and
Kerstein 1942), interference with the synthesis of new DNA (Pelc and Howard 1955), genetic
loss due to chromosomal aberrations (Evans and Sparrow 1961), damage and deficiency of
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physiological pre-requisites to cell division (Stein and Sparrow 1963), cumulative expression of mitotic cycle delay, loss of proliferation capacity and cell death (Evans 1965), reduction in internodal length (Sushil Kumar et al, 1967) radiation damage caused to the nuclei (Thanki et al, 1970), inhibition of phytohormone responsible for normal growth at pre-synthesis level and RNA and DNA level (Tarar and Dnyansagar 1974). Dwarf mutants have been observed by several workers; Down and Anderson (1956) in Phaseolus vulgaris, Kundu (1980) and Rayyan Asta (1995) in black gram, Kothekar (1989) in Dolichos biflorus, Satpute (1994) in safflower and Ramesh and Dhananjay (1996) in lentil.

Early maturing mutants, obtained in the present study showed rapid growth and normal productivity. Early maturing mutant with increased yield was reported by Kawai (1969) in rice as a result of gamma irradiation. Jana (1962) explained that early maturity may be due to physiological changes caused by irradiation and increased production of flowering hormone. Early maturing mutants were also recovered by Down and Anderson (1956), Jana (1962), Gaul et al (1966) Prasad (1972), Swarup et al (1971), Dahiya (1973), Kaul (1977), Pawar et al (1979) and Thakur and Sethi (1993).

High yielding mutants were isolated during the course of present study. It is one of the most important characters for judging the agronomic value of the mutant as related to that of the mother variety. In this mutant, the yield attributes and yield components are increased. There is an increase in the number of seeds and number of pods. High yielding mutants have been reported in many plant genera following mutagen treatments. Dahiya (1973) in mung bean, Raghuvarshi and Singh (1974) in Trigonella, Bhatnagar et al (1979) in gram, Pawar et al (1979) and Chary (1983) in pigeon pea. Pawar and Wanjari (1994) induced high yielding variety of pigeon pea, mung bean and black gram. High yielding mutants in lentil have been

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Twining mutants, observed in the present study, were also recovered by Bhamburkar (1981), Sudha rani (1990) and Rayyan Asra (1995) in black gram. Such mutations can be considered as evolutionary conversion of the plant habit genes in lentil carrying substantial phylogenetic significance.

Variations in the seed coat colour and seed size were also observed in the present investigation in both the cultivars of lentil. The seed coat colour is affected by genetic factors like pigmentation factor, pigment complementary factor and modifying factors. The pigment factor by itself does not produce the seed colour but other complementary colour factors which depend upon the presence of the dominant pigment factor in order to express their colours. Once the dominant P is present, the complementary factors either produce a definite colour by themselves or interact to produce a wider range of colours.


Among all the mutants detailed above, the early maturing mutant, high yielding mutant and bold seed type mutant seem to be the most efficient ones for further improvement of the
lentil cultivars under study and there is scope for utilizing these characters for developing an improved variety of lentil.

II Quantitative Characters (M₂ and M₃ generations Micromutations)

Quantitative or metric traits are the characters which manifest continuous distribution of genotypes expressed as normal curve of frequency distribution. Variation of such traits increases from simultaneous segregation of several loci conditioning that trait, superimposed by the influence of non-genetic causes. All the agronomic characters in crop plants fall in this category of traits. These traits are complete in their inheritance.

Quantitative traits are generally continued by a balanced polygenic system involving a large number of minor genes. Micromutations affect the polygenic quantitative traits, which can only be detected in a group. They can be isolated and fixed only through the adoption of a suitable biometrical scale. Micro-mutants are either manifest or cryptic in their behavior. Because of their nature, micro-mutants are generally important from the plant breeding standpoint.

Micromutations are economically viable; they can be expressed or artificially induced with the help of appropriate mutagens. Baur (1924) stressed the importance of mutations in evolution of new quantitative characters. Mutagenic treatments increase the genetic variability, which can be utilized for selection, and improvement of plants. The utility of mutagens in providing efficient selection in treated population has been proved by Swaminathan (1963), Frey (1965), Borojevic (1966), Gaul and Aastveit (1966), Palenzona (1966) and Scossiroli et al., (1966).

According to Gregory (1961), coincident with induced visible mutations, numerous polygenic changes are also induced in the gene complex. Induced polygenic variability for
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They concluded that induced mutations bring about genetic variability in quantitative characters. Though the variance is enlarged (Khadar 1970, Khadar and Sukry 1972), the mean is usually either reduced, enhanced or equal to the control in all treated populations; and variability in different generations varies under different conditions.

In the present investigation, six quantitative parameters, viz. plant height, days to flowering, days to maturity, number of pods per plant, number of seeds per pod and 100 seed weight were analyzed to assess the extent of induced variability in M2 and M3 generations of the two cultivars of lentil. Normal looking M1 parents were selected for rising M2 generation and observations were made from M2 and M3 plants. For all the characters, there was significant difference between means of the control and treated populations.

The results obtained in the present investigation showed a shift of mean on positive and negative directions in almost all the characters studied compared to the control population. The variability in the treated population was increased with the increase in the concentration.

In the present study, the plant height decreased after mutagenic treatment in M2 and M3 generations significantly. The shift in mean was negative with increasing concentration. Reduction in plant height was also reported by Bajaj et al (1970) in Phaseolus vulgaris, Rajput

Evans and Sparrow (1961) correlated the reduction in height with the chromosomal injury, genic changes or both, Pelc and Howard (1955), on the other hand, related this to the inhibition in DNA synthesis or inhibition in auxin synthesis. Inhibition of growth of the apical meristem or partial failure of the internodes to elongate may also be the cause of reduction in plant height. The magnitude of variability was observed to increase along with the increasing concentrations. These results support the observations of various other workers (Micke 1961; Vig 1970; Siddiq 1972; Sree Ramulu 1974; Chary 1983; Reddy 1991; Padmavathi 1993). Increase in variability has been indicated due to enhanced crossover near the centromere (Wittinghill 1951).


Delay in flowering has been attributed to delay in germination (Bianchi et al 1963) or slowness in growth of the plant, Iqbal (1972).
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There was an increase in the number of pods per plant with few exceptions. This was supported by Hakande (1992), Rayyan Asra (1995) and Bale (1999) in various plant systems.

There was a decrease in the number of seeds per pod with few exceptions. The shift in mean was towards the negative as well as positive directions. However the variability increased in the treated plants. The results of negative shift in means and enhanced variability noticed in the present work support the observations of Brock (1985), but contradict the suggestion of Gaul and Aastveit (1966) which stresses that in a random mutation, the mean dose not shift away. Similar results of reduction in number of seeds were reported by several workers (Gregory 1955; Rajput 1973; Chary 1983; Vandana and Dubey 1990; Reddy 1991; Hakande 1992; Padmavathi 1993; Rayyan Asra 1995; Bale 1999).

The decrease in number of seeds in the treated plants may be due to chromosomal aberrations, which in turn induce sterility.

The data on 100-seed weight revealed that EMS and SA treatments resulted in increased seed weight in both the cultivars of lentil. The difference in mean either negative or positive, however has been found to be inappreciable. Variability was positive in case of both the mutagenic treatments and the varieties. This observation of increased variability without appreciable change in the mean is in conformity with the earlier results of Gaul (1965). This finding received support from several other researchers also; they were Sharma (1986), Vandana and Dubey (1990), Tyagi and Gupta (1991), Hakande (1992), Satpute (1994), Rayyan Asra (1955), Panchabhaye (1997) and Bale (1999).

The data on different quantitative characters studied, revealed that mutagen not only altered the mean values but also created genetic variability for polygenic traits. The increase in induced variation and decreased mean values were observed by Brock (1965), Goud et al
(1969, 1971), Perssons and Hagberg (1969), Khadr and Shukry (1972), Singh et al. (1977),
Tickoo and Jain (1979), Sahu et al. (1980), Reddy (1991), Hakande (1992), Padmavathi (1993),
Satpute (1994), Rayyan Asra (1995), Panchabhaye (1997) and Bale (1999). It is therefore,
inferred that mutagenic treatments are capable of inducing polygenic variability and this
feature can be exploited by the plant breeders for the genetic improvement of desirable traits
through proper selection.
Biochemical Studies:

Trypsin inhibitors in lentil cultivars:

Protease inhibitors are widely distributed among many species in several plant families, particularly among legumes (Liener and Kakade 1980). These inhibitors have been proposed to function as storage proteins, regulators of endogenous protease and as factors that protect plants from insects and pathogen attack (Liener and Kakade 1980; Ryan 1990).

Protease inhibitors in lentil seeds are considered to be antinutritional in human and animal diets. Trypsin inhibitor activity was first recorded by Jaffe (1950) in lentil where as chymotrypsin inhibitor activity was recorded by Mansfield et al (1959). Benken et al.(1980) studied varietal differences in 33 cultivars of lentil with respect to trypsin inhibitor activity which ranged from 0.8-1.9 mg/gm of flour. Weder et al (1985) studied 38 lentil accessions in regard to trypsin inhibitor content showing four iso inhibitors of trypsin out of which two iso inhibitors were inhibited to nearly the same extent as that of chymotrypsin.

Trypsin inhibitors in lentil mutants:

Very few reports are available in regard to mutation and trypsin, chymotrypsin inhibitor genes in plants. Lazaro et al (1985) reported adverse action of the high lysine mutation on TI genes in barley. The same mutation however revealed increased chymotrypsin inhibitor activity by 20 fold (Williamson et al 1987). An opaque 2 mutation in maize has been found with enhanced trypsin inhibitor level (Halim et al 1973). Lowered levels of trypsin inhibitor have been reported in barley mutants induced by pesticide treatments (Harsulkar, 1994). An extensive study of mutation and trypsin inhibitor genes has been carried out by Kothekar et al (1996) in winged bean mutants and moth bean mutants by Kothekar and Kothekar (Unpublished). Recurrent mutagenesis has been found to be highly useful in reducing the
trypsin inhibitor content in winged bean by Khandelwal (1997). In winged bean low trypsin and chymotrypsin inhibitor mutants have been identified and isolated from M5 generation. 3-5 TI and CTI isoinhibitors were absent in different mutants as compared with 9 isoinhibitors in control, indicating mutations in the respective gene loci (Kothekar et al, 1996).

It was further demonstrated that no correlation could be established between lower/higher levels of TI, CTI and morphology of mutants. The mutability of TI and CTI genes has been found to be different in the same study. In case of winged bean the chymotrypsin locus has been found to be more prone to mutations. Some of the double headed trypsin-chymotrypsin inhibitors demonstrated loss of CTI activity and retention of TI activity (Kothekar et al, 1996).

Bale (1999) reported lowered levels of trypsin inhibitor mutants induced by EMS and NMU mutagens. The recovery of low TI plant type in lentil through mutational approach has comprised a feature of major accomplishment of the present work. Such lentil mutants carrying lowered levels of trypsin inhibitor are likely to assume significant importance and immense economic value in regard to nutritional status.

The detailed understanding of the genetics of inhibitors and other antinutritional components present in lentil would be immensely helpful to the breeders in planning their breeding programmes directed towards qualitative improvement. The detailed investigations of the presently obtained low TI lentil mutants in regard to other antinutritional components like lectins and polyphenols would indicate further the all round qualitative improvement of the pertinent lentil mutants.
Effect of heat treatment on trypsin inhibitor of lentil:

Since early 1940s, the plant inhibitors have been extensively studied as antinutritional factors due to the potential adverse effects they produce on human and animal intestinal tract (Laskowski and Kato 1980; Liener 1979 and Prabhu et al 1984). The legumes have provided large amount of proteinase in the human diet, however, their use as sole source of proteins has been subdued due to the presence of the antinutritional factors, in particular, the proteinase inhibitors. Nevertheless, there has been a remarkable improvement in the nutritional quality of legume food due to various treatment methods (Friedman and Gumbman 1986 and Rackis et al 1986).

Devaraj and Manjunath (1995) noted that the nature of cooking medium and duration of cooking had profound effect on the protease inhibitor activity in *Dolichos lablab* bean. They further noticed that the dry fried seeds lost their PIA (Protease inhibitor) activity very rapidly. Seeds cooked in slightly alkaline medium lost their PIA quickly compared to those cooked in acidic and neutral pH. Some of the legumes are consumed as food after processing that includes some traditional practices such as soaking, sprouting, fermentation, boiling, roasting, parching and steaming. These practices reduced the level of antinutritional factors (Singh 1988).

In the present investigation, the TI activity revealed a gradual-decreasing feature as the heat treatment duration increased in L-4611 variety of lentil. In the present case, however, after one hour of heating of the seed extract in boiling water bath, the TI activity became totally indetectable.

Batra et al (1986) reported that autoclaving and dry heat treatments significantly reduced the TI activity in lentil and pigeonpea. Similar types of results were reported by

The thermolabile nature of legume protease inhibitors has been known (Liener 1969). Trypsin inhibitor activity of chickpea was inactivated by moist heat at 121⁰c for 30 minutes but not by dry heat (Conteras and Tagel 1974).

Singh and Eggum (1984) demonstrated that the heat treatment partially destroyed trypsin inhibitors in pigeonpea. An increase in protein efficiency ratio was attributed to the destruction of trypsin inhibitors as a result of germination in some legumes (Jaya et al 1975). Considering the effect of heat treatment a remarkable reduction in protease inhibitor activity can be achieved by heating. But excessive heating reduces the nutritive value of legume proteins. Methionine, the most limiting essential amino acid of legume, has been reported to undergo nutritional damage when heated (Shemer and Perkins 1975). Therefore it is important to establish the optimum heat conditions to realize the maximum nutritional advantages of cooking the pulses in respect of their protease inhibitors.

The heat stability of trypsin and chymotrypsin inhibitors was investigated in soybean, mungbean, rye and Triticale by Saini (1989). It was found that both trypsin and chymotrypsin inhibitors were resistant to dry heat but got destroyed by autoclaving at 121⁰c for 5 minutes.

Trypsin inhibitors during seed germination:


Majority of the studies have emphasized dry mature seeds of pulses and the multiplicity of products derived from them. However, development of food products from germinated lentil may be another way to further increase the versatility and utility of lentil. Legume sprout constitutes a good portion of total consumption of food legumes. Germinated lentils are receiving attention because of the probability that flavor and nutritional qualities may be important.

In the present study, it has been found that the TI activity could get retained up to 10th day after germination (DAG) and after this the activity got reduced in L-4611 variety of lentil. This finding received support from the electrophoretic profiles and the assay values demonstrated by the same variety during the present studies. Parallel results have been obtained earlier by Keleque et al (1985) in chickpea. Batra and Dhindsa (1992) in lentil noticed retention of trypsin inhibitor activity during seed germination up to 6 days, followed by its subsequent reduction. Similar types of results were also recorded by el-Mahedy et al (1985) in lentil. The TI activity has been reported to be reduced during seed germination in different legumes and cereals e.g., Somac and Storey (1981) in jojoba seeds, Wilson and Tan Wilson (1983) in mungbean, Babar et al, (1988) in jackbean, Sharma and Sehgal (1992) in faba bean, Mulimani and Paramjyothi, (1992) and Godbole et al, (1994 b) in redgram, Savelkoul et al, (1994) in white kidney bean and Jimenez et al, (1985) and Bale (1999) in soybean.

The germinated lentil could be developed as an effective ingredient of protein in the diets against the protein malnutrition of developing countries. Development of food products from germinated lentil may be a promising way to increase the versatility and utility of lentil.
To date, the advantages and disadvantages of this process have not been thoroughly established for wider utilization of germinated lentil seedlings in human food for specific dietary or even therapeutic purposes.

**Lectin content in lentil germplasm:**

As lectin activity in lentil was first reported by Landsteiner and Raubitshek (1907), in the present investigation, 49 lentil germplasm were analyzed. The lowest lectin content (3.478) was observable in L-345 germplasm while in case of L-4611 germplasm the lectin activity could be seen as the highest one (23.188). In rest of the germplasm, lectin activity ranged between 3.478-23.188.

The varied low lectin content germplasm could be used for nutritional purpose and also can be used in breeding programme to develop improved variety containing reduced amount of lectin content.

Puszati et al (1979) recorded varietal differences in kidney bean. They analyzed 13 cultivars of kidney bean and found that 11 cultivars were highly toxic for rats in raw state and contained high concentration of hemagglutinin lectin over 10% of the total protein, while two cultivars showed non-toxic feature towards rats.

**Lectin content in lentil mutants:**

As there is no preexisting report on lectin analysis of lentil mutants, the present study of lentil mutants was undertaken which revealed EM6 mutant of L-4611 and EM7 mutant of L-4639 cultivar with reduced lectin activity as compared with their controls. The same mutants also had reduced level of TI content and phenol content in them.

In case of the viable mutants especially like the early maturing mutant reduced lectin activity could be evidently seen while the tall mutant showed highest lectin activity as
compared with control L-4611. The high yielding mutant showing reduced level of lectin content and bold seed mutant revealing slightly increased lectin content as compared with control L-4639 cultivar, comprised yet another feature of interest of the present studies.

The lectin activity can be destroyed/reduced by heat treatment or by germination of seeds. In this regard Bansal et al (1988), in chickpea observed that after 10 minutes of boiling and one hour of heating of seed, the activity of lectin got destroyed while it was destroyed after 6-8 days of germination.

William and Swanson (1992) observed in Phaseolus vulgaris that 10 minutes of heating at 97.8°C of presoaked seeds, destroyed lectin activity, while the unsoaked seeds required 20 minutes of heating at 97.8°C. According to some researchers like Janzen et al (1976) and Gatehouse et al (1984), the seed lectins have a protective role against various predators. Similar type of results were reported earlier by Alexander and Caldwell (1987) in tubers of winged bean.

It is concluded from these results that further investigations besides systematic, and comprehensive studies are required for testing seed safely for animal feeding experiments and their consequent acceptability by the panelist for sensory evaluation.

**Phenol content in lentil germplasm:**

The polyphenols inhibit the digestive enzymes and interfere with the biological value of grains, but the phenolic compounds are beneficial to plants against bird depredation, insect attack and disease caused by fungi, bacteria and viruses.

In the present study 49-lentil germplasm were analyzed for phenol content. It was observed that phenol content in lentil germplasm ranged from 159.0-409.3 mg/100 gm of seed powder. L-4642 germplasm showed lowest phenol content (159.0mg/100 gm of seed powder)
where as IC-208326 germplasm showed highest phenol content (409.3 mg/100 gm of seed powder).

Similar types of varietal differences in regard to phenol content were reported by Jood et al (1987) in chickpea and black gram. Singh and Jambunathan (1981b) compared desi and kabuli chickpea cultivars and revealed that kabuli chickpea cultivar contained low amount of phenol content as compared with desi chickpea cultivar. Bandyopadhyay et al (1990) succeeded in recording varietal differences in polyphenol content of five pepper varieties.

**Phenol content in lentil mutants:**

In this case also there was no earlier report in regard to polyphenol content in induced mutants. In present study it was observed that some lentil mutants showed significant reduction in phenol content as compared with control, besides exhibiting reduced TI and lectin activity as well in them.

With regard to viable mutants, the high yielding mutant showed reduced phenol content (193.1mg/100gm) while the spotted seed mutant showed increased phenol content (318.4mg/100gm) as compared with control L-4611. In case of L-4639 cultivar, the early maturing mutant revealed reduction in phenol content (189.9mg/100gm) while the black seed mutant showed increased phenol content (398.1mg/100gm) as compared with control.

The above variability in regard to different biochemical features carried by some of the induced mutants (like, high yielding and early maturing) would stand immense scope in development of nutritionally desirable varieties of lentil, by involving them in conventional breeding programmes.