Discussion
Controlled induction of desired mutations is a challenging task before the Plant Breeders. This challenge can not be met without a proper understanding of the mechanism of mutagenesis. Conventional methods of plant breeding, adopted ever since the start of a scientific approach to agriculture, were solely dependent on genetic variability arising spontaneously. Discovery of various mutagens and their usefulness in plants, opened the way for mutation breeding. Early attempts in this field focussed attention mainly on mutants of easily identifiable qualitative characters i.e. macromutations, but soon it was realised that there was only a limited scope for evolving macromutants suitable for direct cultivation. The present day mutation breeders focus their attention mainly to the increase in variability for certain quantitative traits, combined effect of which ultimately determines the yield (Swaminathan, 1963, 69a, b). The results obtained from the investigations carried out in lentil (Lens culinaris ned.) var. K-333 through genetic manipulation brought about by exposure to gamma irradiation and EMS separately as well as simultaneously are discussed here in the light of earlier work in this field.
Radio sensitivity in M1 generation

Percentage of seed germination following mutagenic treatments has often been regarded as a criterion for judging the effectiveness of mutagenic agents. Mutagens generally reduce the germinability of treated seeds although some reports of promoting effects of mutagens, when applied at low doses, are also available (Sax, 1963; Singh et al., 1978; Venkateswarlu et al. 1978; Trivedi and Dubey, 1982 and Vandana and Dubey, 1988). In the present experiment reduced field germination was induced by all the mutagenic treatments. Besides reduction in germination percentage, the process of germination was also delayed. Reduced and delayed germination following mutagenic treatments has earlier been recorded by a number of workers in various crops (Eherenberg, 1955; Blixit, 1960; Blixit et al., 1963; Shivaraj et al., 1962; Ashraj et al., 1965; Ojomoo and Raji, 1978; Haque et al. 1981 and Dixit and Dubey, 1981a).

Early growth of seedlings was retarded by the higher doses of gamma irradiation while the lowest irradiation dose as well as the EMS treatments promoted growth of seedlings. Among treatments involving combined application of the two mutagens, increased seedling growth was recorded only where lowest irradiation dose of 5kr was applied. A dose dependent retardation in seedling height is a common feature induced by ionising radiations (Caldecott, 1954, 61; Sharma, 1968; Conger et al. 1973; Singh, R.M. et al., 1977 and Singh, R.B.
et al., 1978). However, promoting effects on seedling growth by low doses of mutagens as recorded in the present study have also been reported (Sax, 1963; Singh et al., 1978; Venkateswarlu et al., 1978; Trivedi and Dubey, 1982 and Vandana and Dubey, 1988). Retardation in seedling growth has been attributed to growth inhibition due to destruction of apical meristem or partial failure of internode elongation (Gunkel, 1965). Radiation induced depression in seedling height may be caused due to auxin destruction (Smith and Karstem, 1942), inhibition of auxin synthesis (Gorden and Webber, 1950), chromosomal damage or mitotic inhibition (Sparrow et al., 1952).

Decreased survival, plant height, branching, fruiting and yield, delayed maturity and increased pollen sterility were the other features of various mutagenic treatments applied during the present study. These results are in conformity with the findings of (Caldecott, 1954; Wolf and Sicard, 1961; Sharma, 1968; Mekhalova, 1974; Bottino et al., 1975; Chandra, 1979; Dixit and Dubey, 1981, 86a; Vandana and Dubey, 1988 and Kumar et al., 1993). Interestingly test weight showed a significant increase in many mutagenic treatments which indicated a possibility of isolating bolder seeds in later generations.

Alterations in the form of chromosomal breakage and their reunion in later stages, induced after effects of physical and chemical mutagens have been of practical interest. Differential sensitivity and variations in
chromosome organisation can be studied through difference in both quantity and kind of chromosomal alterations. Present investigations reveal that minimum percentage of mitotic anomalies were recorded at telephase stage. Highest dose of gamma irradiation individually as well as in combination with EMS induced more frequent interphasic anomalous cells. Various anomalies induced at interphase, metaphase, anaphase and telophase are directly correlated with the dosage of mutagens. Individually EMS proved to be more effective in inducing mitotic anomalies than gamma ray treatments. Additive effect of the two mutagens was not observed in the combined treatments.

Partial or complete failure of spindle mechanism and induced chromosomal aberrations lead to the mitotic anomalies. Non-orientation of certain chromosomes on equatorial plate indicates partial failure of spindle mechanism while polyploid cells show its complete failure. Chromosomal stickiness and clumping may also be responsible for failure of spindle mechanism. According to Sudhakaran (1972) in Vinca rosea, the stickiness and clumping of chromosomes are the outcome of physiological effects. Alterations in the surface property of the chromosomes result in their stickiness. Stickiness indicates an induced imbalance in the physiology of the cells even in metabolic stage (Khilman, 1952; Dolchar, 1960; Hussein and Hakim, 1961; Jain and Singh, 1967; Dixit and Dubey, 1984).
According to Skipper (1951) alkylating agents are mutagenic to cells and damage the rapidly dividing cells. He also suggested that these biological effects may be the result of chemical reaction between alkylating agents and molecular constituents of the cells which in turn cause dire metabolic disturbances such as stickiness, clumping and chromosome breaks. Giant interphase nuclei might be polyploid in nature while the binucleate condition apparently is the result of failure of cytokinesis.

Chromosomal aberrations lead to the anaphasic anomalies like chromatin bridges, lagging chromosomes or fragments and unequal distribution of chromosomes at the two poles. Chromosomal exchanges lead to chromatin bridges and their number depends upon the number of chromosomes taking part in the exchange but all the exchanges induced between chromosomes may not form bridges at anaphase (Conger, 1965). According to Sudhakaran (1972), the structural changes include chromatid and chromosome breaks at different portions on the chromosome, leading to various structural rearrangements by union of broken ends. Lagging chromosomes and fragments are observed as a result of formation of acentric chromosomes during exchange or due to chromosomal breaks. Existence of such breaks has been reported in many plants treated with chemical as well as physical mutagens (Gile, 1941; La Gour, 1952; Lovelace, 195 and Levan, 1951).

In later stages lagging chromosomes lead to the formation of micronuclei at telophase. Formation of micronuclei at
telophase has earlier been induced as a result of gamma irradiation of *Vicia faba* seeds (Neary and Williamson, 1959 and Evans, 1962) and lentil root tips (Sinha and Godward, 1968).

Chromosome breaks *per se* do not constitute mutation as they usually lead to death of the cell, itself or its early progeny, owing to loss of some vital information. However, in most of the cells, broken chromosomes can heal either by restitution or by exchange of two broken chromosome portions, that happen to be close enough in space and time. As a result of such an exchange, mutations can arise, indeed all known chromosome breaking agents, also produce mutations. Disturbed spindle formation leads to the multipolar distribution of chromosomes while abnormal alignment of chromosomes at equatorial plate brings unequal distribution.

Among meiotic anomalies, those recorded at diplotene/diakinesis were more frequent than at other stages of heterotypic and homotypic divisions. Metaphase, anaphase and telophase of heterotypic division were the other stages showing higher incidence of anomalous pollen mother cells. Percentage of anomalies increased with the increasing dose of mutagens in individual as well as combined treatments. Superiority of either gamma rays or EMS could not be made out in the present study in bringing about meiotic anomalies. However, Tarar (1980) from his studies on *Turnera ulmifolia* has concluded that ionising radiations induced much more meiotic aberrations than the chemical mutagens.
Translocations or inversions lead to irregular pairing which in turn give rise to multivalent associations including rings or chains of 8, 10, 12 or all the 14 chromosomes. Increasing multivalent associations presumably reflect the number of translocations induced. According to Prasad (1965) and Sinha (1967) the complex interchanges might occur due to many breaks and reunions. Breaks in the nucleolar organising region of the nucleolar organising chromosomes may lead to the nucleolar fragmentation as suggested by Shaikh and Godward (1972). Failure of spindle mechanism may lead to non orientation or precocious separation in some bivalents and unequal distribution of chromosomes observed at anaphase I.

Chromosome breakage and types of aberrations play a role in inducing sterility which in turn influences the recovery of mutations. A definite localisation of breaks induced in root tip chromosomes due to alkylating agents like EMS has been noted by Natarajan and Upadhyay (1964) and Ward (1966). It has also been pointed out that alkylating agents particularly EMS exceptionally attack the chromosomal region rich in guanine-cytosine base pairs. Evidence of DNA alkylation by monofunctional agents such as EMS for preferential attack at N-7 atom of guanine has been put forward by a number of workers. Work on this aspect has been reviewed by Lawley (1966). Scalera and Ward (1971) have clearly demonstrated that in-vitro EMS alkylation of Vicia faba root tip DNA is predominately a guanine ethylation, approximately one half of the guanine present, produces 7
ethyl guanine. One third of the total 7-ethyl guanine produced was released from DNA by depurinations. This depurination would be responsible for base deletions or transitions and are responsible for mispairing with thymine instead of cytosine.

At anaphase I and II bridges were recorded while laggards were common both in anaphase I and telophase I. The single and double bridges at anaphase I might have resulted from dicentric chromatids and chromosomes. According to Mc Clintock (1931), the origin of dicentric bridges and acentric fragments in anaphase I and II has been ascribed to a consequence of crossing over between relatively inverted segments. Breakage and reunion of chromatids during meiotic prophase lead to the formation of dicentric bridges and/or acentric fragments (Haga, 1953; Lewes and John, 1966 and Newman, 1966). Jones (1968) has described these two contrasting modes of origin of bridges and fragments in simple terms i.e. one depends on crossing over between relatively inverted segments, the other depends on inverted crossing over between non inverted segments. He has, however, drawn these conclusions on observations based on spontaneous breakage of chromosomes.

According to Bhattacharjee (1953) interlocking results in the formation of bridges at anaphase I in lentil. Unequal distribution in anaphase I/II resulted in the formation of pollen grains with chromosome number greater or lesser than
Sinha and Godward (1972b) suggested that the paracentric inversions may lead to the formation of chromatin bridges at anaphase I/II or telophase I/II. Presence of bridge without fragments can be attributed to their loss during squashing. Ring and translocated chain formation lead to unequal number of chromosomes to poles and lagging chromosomes which in turn result in the formation of unequal pollen grains and micronuclei. Accumulated acentric fragments or laggards may also result in the formation of micronuclei at telophase I/II and that of polyads. According to Bhattacharjee (1953) in lentil irregular distribution of chromosomes results in size variation and abortion of pollen grains.

Percentage of pollen and ovule sterility and percentage of plant survival are important considerations for determining the effectiveness of mutagens. In the present study pollen sterility showed a dose dependent increase both in case of gamma rays and EMS. Dosage of gamma rays inducing a higher level of sterility than that of EMS. The level of pollen sterility in the combined treatments of the two mutagens also induced an increasing level of pollen sterility with an increase in the dose of either mutagen. Trend of ovular sterility and plant survival was also similar. Ovular sterility showing an increase and plant survival corresponding decrease with the increasing mutagen dosage. The only exception was the middle irradiation dose of 10 kr which was more drastic than the two marginal irradiation doses. The combined treatments with the two mutagens did not
show an additive effect in increasing pollen or overlar sterility or retarding survival percentage. These results are in contradiction to the findings of Sharma (1970) in barley, Singh, R.B. et al. (1978) in pearl millet and Premsikar (1981) in pigeon pea, as they concluded that combined treatment with gamma rays and EMS had a synergistic action on pollen sterility. Sinha and Godward (1972a), Sharma et al. (1976) and Dixit and Dubey (1981) in lentil concluded that sterility increased with increasing radiation dose in M₁ generation. According to Behra and Patnaik (1979) pollen and ovule sterility in Amaranthus increased uniformly with increasing concentration of three mutagens viz. EMS, DES and gamma rays. According to Sato and Gaul (1967) pollen sterility induced by chemical mutagens could be ascribed to gene mutation, invisible deficiencies or both. In gamma ray treatments the sterility could be mainly due to chromosome breakage, translocations or other cytological anomalies. Laxmi et al. (1965a & b), Das and Kundu (1973), Ghafoor (1974) and Khalikar (1975) have recorded that increase in the chromosomal aberrations is always accompanied by a decrease in yield due to pollen and ovule sterility.

To sum up the studies of different parameters of radio/chemo sensitively in M₁ generation, it may be concluded that mutagenic treatments brought about a reduction in germinability of seeds and growth and survival of seedlings. Vegetative and reproductive growth of the plants was adversely affected leading to reduced seed yield. This was
coupled with an increase in the extent of mitotic abnormalities in root tip cells; meiotic aberrations in PMC's and pollen as well as ovular sterility. Promoting effect of low doses of mutagens on germination and early seedling growth in sand culture was also recorded. Simultaneous application of the two mutagens proved to be more effective than individual application of either mutagen, for almost all the parameters. Although the combined treatments did not show an exact addition of the separate effects of the two mutagens, a dose dependent increase in various mutagen sensitivity parameters was induced by both the mutagens separately as well as in combined treatments. However, on parameters like field germination plant survival, pod number, seed number and seed yield, middle irradiation dose of 10 kr proved to be more effective than the marginal doses of 5kr and 15kr. This indicates that the 10kr may be the optimum dose of gamma irradiation for the cultivar K-333 of lentil.

Relationship between chromosomal aberrations and pollen and ovule sterility

The present results indicate that chromosomal aberrations scored at mitosis or meiosis, under different mutagenic treatments, have a positive association with pollen and ovule sterility. Among the various doses of gamma rays, the relationship between two is approximately linear and so is the case among the two doses of EMS. Combined treatments showed a higher occurrence of chromosomal aberrations as well
as pollen or ovule sterility than the respective single doses of either mutagen. These results indicate that pollen and ovule sterility are affected by chromosomal aberrations. In addition gene mutations have profound effect on pollen and ovule sterility. Singh, M.P. et al. (1972) suggested that induced cryptic and gross chromosomal changes are responsible for manipulating the genetic material and thereby affecting fertility. A similarity in the pattern of chromosomal aberrations and sterility induced by different mutagenic treatments has earlier been recorded in barley (Chandra, 1979), lentil (Dixit, 1985) and fababean (Vandana, 1990).

**Frequency and spectrum of mutations in M₂ generations**

As pointed out by a number of workers (Gustafsson, 1941, 47, 55; Aurobach, 1967 and Swaminathan, 1966, 69), induction in a predictable manner of a broad spectrum of mutations, with increased frequency, is an important goal for mutation breeders as it leads to increased chances of achieving desirable plant characteristics. In the present investigations considerably high frequency of mutations, was induced in different mutagenic treatments. Among the three gamma ray doses as well as the two doses of EMS, mutation frequency increased with the increase in dose. Mutation frequencies were further enhanced when gamma irradiated seeds received a soaking treatment in EMS solution. A dose dependent increase in mutation frequency was recorded both in terms of families segregating for mutations and number of mutants per thousand M₂ plants.
Mutation frequency has been partitioned for three types of mutations viz., chlorophyll, vital and sterile. The rate of vital mutations was much higher than that of chlorophyll mutations or sterile mutations. Fillipetti and Depace (1986), El Showny and El Hosary, (1985a,b) and Vandana (1990) also recorded a higher frequency of vital mutations than chlorophyll or sterile mutations following varying mutagenic treatments in Vicia faba. However, Chandra (1979) in barley found that rate of chlorophyll mutations induced by gamma rays was much higher than that of vital mutations. Dixit (1985) in lentil observed that NMU induced a higher frequency of vital mutations than the chlorophyll mutations where as varying doses of gamma rays induced a higher frequency of chlorophyll mutations, concluding there by that chemical mutagens appeared to be less effective in inducing chlorophyll mutations than the physical mutagens. However, this conclusion is not supported by the present study.

Chlorophyll mutations induced in the present investigations included Xantha, Viridis and Virido-xanthe types. Barren tip seedlings characterised by no leaf formation after 3 or 4 leaf stage and a polyembryonic seedling apparently consisting of a haploid and diploid twin were also included in the same category. No albina type mutant characterised by total lack of pigments was recovered in our study. Spectrum of vital mutations are most important from the point of view of isolating desirable mutant types. Seed yield is the ultimate goal in leguminous crops but yield
being a composite character can be manipulated through a number of component characteristics. The basic requirement is the genotype capable of utilizing the inputs fully and to withstand attacks by pests and diseases. Such a manipulation of plant's structural components so as to induce desirable alterations in the component characteristics of yield will provide invaluable material for the breeders. Several of the mutants from among a wide range of vital mutations induced in the present study, may prove useful from breeders point of view.

Mutations affecting plant height induced during the present study included tall, dwarfs and stunted. Fertility and yield levels in first two of these mutants was not adversely affected. Similar mutants have earlier been induced in lentil through gamma rays, NMU, EMS and DES by Sharma and Sharma (1977) and Dixit and Dubey (1983 & 84). The dwarf mutants induced by Sharma and Sharma (1977a) were semisterile or sterile. It is not yet established in case of lentil that the dwarf types will have higher yielding ability as in the case of cereals. Jana and Tholia (1975) compared the performance of eleven promising lentil varieties and concluded that the tall genotypes were superior to the dwarf types in their yielding ability.

The bushy, spreading and large leaflet mutants induced in the present study are of considerable value as increased branching ultimately leads to increased flower and fruit
bearing capacity i.e. "the sink potential" while increased leaf surface area leads to increased photosynthesizing ability or "the source". Importance of tetraflororate and pentaflororate mutants hardly needs any emphasis as the increased sink size in them will have direct bearing on yielding ability. Similar multiflorate mutants have earlier been recorded by Sharma and Sharma (1977g and 1980) and Dixit and Dubey (1983). Some genes increasing the number of flowers in the inflorescence are known in pea and french and broad beans. But these mutants had reduced seed production or the plants were unable to support the increased seed number, resulting in the same level of seed production as the parental strain (Blixit and Gottschalk, 1975). The fertile mutants of lentil bearing 4 or 5 flowers per inflorescence, instead of normal 2 or 3, isolated presently, may prove valuable for the improvement of this crop. Early maturing mutants, some of which flowered as early as eleven days earlier than the control also hold promise from breeders point of view.

To sum up, it may be mentioned that a wide spectrum of vital mutations including several desirable features like enhanced branching, increased leaf area, increased number of flowers and fruits and early maturity, were induced in the present study and the population provides invaluable material for selecting types with ideal combination of desirable characters.
Besides desirable mutations discussed above a number of mutants of academic, interest such as stunted, unbranched, shiny, hairy, yellow tip, pale green and late maturing were also induced. Further cytogenetical analysis of these mutants is expected to throw new light on the genetics of the traits involved in these mutations.

**Polygenic variability**

Mutagens enhance variability for polygenic traits in the form of micromutations. Micromutations might occur much more frequently than macromutations and unlike macromutations, they often do not affect vitality adversely as the minute changes have physiological behaviour and are less drastic, therefore, they are comparatively more important than macromutations (Gaul, 1965).

Induction of micromutations in a population results in a shift in the mean value for the particular polygenic trait. Such a shift in mean values following mutagenic treatments has been recorded in a number of crops such as wheat (Bhatia and Swaminathan, 1962; Sharma et al., 1982 and Kar and Yadav, 1986); rice (Nayer and Ninan, 1979); mung bean (Rajput, 1974; Chaturvedi and Singh, 1980); Safflower (Sahu et al., 1980), fenugreek (Laxmi and Gupta, 1983), barley (Vasudevan et al. 1969; Chandra, 1979), lentil (Dixit and Dubey, 1984) and faba bean (Vandana and Dubey, 1990).
Among the eight polygenic traits in the present study, branch number, pod number, seed number, seeds per pod and seed yield showed a shift in negative direction in all the treatments applied. This negative shift was more pronounced for pod number, seed number and seed yield. In case of remaining traits i.e. plant height, days taken to flower and test weight also, general trend of shift in mean values was in negative direction, although in two mutagenic treatments for plant height, only one for days to flower and four treatments for test weight a shift in positive direction was recorded. Shifting of mean values in the negative direction indicates that in the mutagen treated M2 populations the negative mutations out weighed the positive ones. In case of days to flower negative mutants i.e. those with lesser number of days to flower are a desirable feature.

Increased interfamily and intrafamily variance regarding plant height, days to flower, seeds per pod and test weight was recorded while pod number, seed number and seed yield showed decreased variance at both inter and intrafamily levels. Branch number showed decreased interfamily variance and increased intrafamily variance. Increased interfamily as well as intrafamily variance was induced by EMS for plant height and test weight only. Synergestic effect of the two mutagens was not recorded in the present study on inter or intrafamily variance.
Increased coefficient of variability at interfamily level for plant height, days to flower, branch number and test weight and at intrafamily level for branch number, pod number, seed number, seed yield and test weight was observed in the present study. Generally the intrafamily coefficients of variability were higher than the interfamily ones, indicating a better scope for selection within the families than between the families. Among earlier workers, Sharma and Sharma (1970) have induced considerable variability for pod and seed size in lentil. Dixit and Dubey (1984) in their study on cultivar T-36 have reported higher values of CV at interfamily level than at intrafamily level following gamma rays and NMU treatment.

Khamankar (1981) from his studies on bread wheat, concluded that the most effective mutagen in terms of induced variability for polygenic traits is ionizing radiations. He has raised doubts regarding suitability of chemical mutagens in mutation breeding programmes. Induction of greater variability by higher EMS dose for seed number, pod number, seed yield and test weight than any of the gamma ray doses coupled with genetic variability of the same order as the gamma ray doses for the remaining five traits shows that this chemical can serve as an effective mutagen for inducing micromutations in lentil. Dixit and Dubey (1984) have also found that NMU induced greater genetic variability for a number of polygenic traits. As pointed out by Khamankar (1981) it is possible that chemical mutagens may be more...
efficient in case of diploid organisms than the polyploids like wheat, which have a number of genes for a character at corresponding loci in the different genomes or in the different sets of homologous chromosomes.

Relationship between parameters of mutagen sensitivity in M1 and mutation frequency in M2-

(i) Mutation frequency vs field germination - The damage caused by the mutagens, whether genetical or physiological results in declining germination percentage. In the present study reduced field germination was recorded under various mutagenic treatments and it was coupled with high frequency of induced mutations. Both germination percentage and mutation frequency showed a dose dependent increase in separate as well as combined treatments of the two mutagens. Thus the amount of damage produced by the mutagens can be correlated with their mutational efficiency. However, this conclusion will be valid for those mutagens only which induced genetial rather than physiological damage and may not apply for those mutagens which induce more physiological damage resulting in lethality but little genetic alterations.

(ii) Mutation frequency vs plant survival - In the present investigation the degree of reduction in survival percentage of the treated plants was found to be correlated with the mutation frequencies induced by different treatments. This indicates that the lethality induced by various mutagenic
treatments was coupled with genetic alterations leading to mutational events. Similar relationship between plant survival and mutation frequency has been reported in barley by Chandra (1979), in lentil by Dixit (1985) and in faba bean by Vandana (1990).

(iii) **Mutation frequency vs cytological anomalies** - A linear relationship between mutation frequencies and mitotic and meiotic anomalies was noted among various mutagenic treatments. Thus the percentage of cytological anomalies induced in M1 may be taken as an index of mutation frequency to be expected in M2. Vandana (1990) has drawn a similar conclusion from her studies on EMS and DES treatments of faba bean. Eherenberg (1956), Gaul (1958), Heiner et al. (1960) and Ramanna and Nattarajan (1965) from their studies in barley concluded that chromosomal breaks and gene mutation are independent of each other. Nattarajan and Ramanna (1965) using different mutagens suggested that the phenomenon of chromosomal breakage and mutation frequencies are not the same but two parallel processes. Dixit (1985) supported this idea on the basis of the findings regarding relative capacity of gamma rays and NMU in inducing mutation frequency and chromosomal breaks.

(iv) **Mutation frequency vs pollen and ovule sterility** - Mutagen induced male and female sterility has often been ascribed to chromosomal breaks and other cryptic chromosomal alterations. Eherenberg et al. (1961), Rao and Nattarajan (1965), Ramamna and Nattarajan (1965), Sharma (1968), Chandra
(1979), Dixit (1985) and Vandana (1990) have observed a correlated response to mutagen doses for mutation frequency in M₂ and pollen sterility in M₁. In the present study also increasing doses of both gamma irradiation and EMS brought about an increase in the mutation frequency as well as pollen and ovule sterility.

Mutagenic efficiency of the treatments:

There is an obvious difficulty in comparing the effectiveness of radiation and chemical mutagens because of the difference in unit of dose and treatment methods. However, their efficiency can be compared in terms of ratios between chloromutation frequency on one hand and lethality or sterility induced on the other hand as suggested by Konzak et al. (1965). During the present study mutagenic efficiency based on injury was found to be higher than that based on sterility in most of the treatments. However, negative values of injury based efficiency were recorded in some treatments due to promotion of seedling growth under these treatments. Radiation at 10kr proved to be more efficient than 5kr or 15kr individually as well as in combination of EMS. The lower dose of EMS was found to be more efficient than the higher dose in individual as well as combined treatments. Combined treatments did not prove to be more efficient than the respective individual treatments of either mutagen. Thus no synergestic response regarding mutagenic efficiency was recorded in combined treatments. However, Sharma and Sharma
(1981) and Dixit and Dubey (1986) in lentil found that combined treatments showed a higher efficiency than the respective individual gamma ray treatments.

**Variability studies in twenty one elite M₃ mutants:**

Twenty one elite mutants were selected for variability studies in M₃ generation. Most of the mutants were either superior to the control or had no significant difference from it for all the traits studied. More than two thirds of the mutants showed significant difference from control for plant height and days to flower, for rest of the traits number of such mutants ranged from one to five. Only one to three mutants were found to be inferior to the control for days to flower, pod number and seeds per pod while for the remaining traits none of the mutants was significantly inferior to the control. Variability results are being discussed here in the following paragraphs.

**Variance** - Highest values of phenotypic, genotypic as well as environmental variances were recorded for seed number which were followed closely by those for pod number, while variances for other traits were comparatively smaller. Lowest values of variance at all the three levels were recorded for seeds for pod. Environmental variance was higher than the genotypic variance for all the traits studied except for days to flower. This indicates that plant height, branch number, pod number, seed number, seeds per pod, test weight and yield are more susceptible to the environment and for these traits
environment plays an important role in inducing variability while in case of days to flower most of the variability is due to inherent reasons. Dixit and Dubey (1986) in their study on 40 elite mutants of lentil found that differences between phenotypic and genotypic variance for days to flower and seeds per pod were very small. They concluded that these traits were less influenced by environment than other traits studied by them.

Similar to the phenotypic variance, highest values of phenotypic coefficient of variability (PCV) were recorded for seed number. Pod number and yield stood next to this trait in order of PCV values. These results are in conformity to the findings of Nanda et al. (1988) and Vandana (1990) in faba bean. Branch number had fairly high value of PCV while medium values of PCV were recorded for test weight, plant height and seeds per pod while lowest PCV was recorded for days to flower.

PCV values followed the same trend as the ECV values while GCV showed a different trend except for days taken to flower. This confirms that in the present study environment played an important role in inducing phenotypic variability. These observations are contradictory to the findings of Vandana (1990) in faba bean where she found higher GCV values than ECV for most of the traits under study. However, Nanda et al. (1988) in faba bean reported that GCV values for all traits were very low as compared to the PCV values. In the present study PCV values were higher than GCV or ECV. Similar findings were also recorded by Nandan and Pandya (1980).
Heritability - Study of transmission of vitally significant genetic traits in following up the breeding programmes deserves special merit in discussion on the subject. In the present study comparatively higher heritability was recorded for days taken to flower, seed yield, number of seeds, number of pods and plant height which indicates higher genetic variability or lesser susceptibility to environmental effects. However, lower values of heritability recorded for seeds per pod, test weight and branch number indicates low variability and greater susceptibility to environment for these traits. High estimates of heritability for plant height, maturity period and test weight in lentil are in agreement with the observations of Singh and Singh (1975) and Sindhu and Mishra (1982). High estimates of heritability for plant height and days to maturity were also recorded by Singh and Dixit (1970) and Chaudhary et al. (1978) respectively. Singh, K.B. and Singh (1969), Singh and Singh (1975) and Singh, P. et al. (1977) have reported high heritability values for pod number and seed number. Dixit and Dubey (1985) also reported high heritability values in lentil for days to maturity, plant height, 200 seed weight, grain yield and pod number. High heritability for seed yield and traits like test weight, seeds per plant, seeds per pod, pods per plant etc. have been reported by Bakheit and Mahady (1988b) and Nanda et al. (1988) in fava bean. Vandana (1990) also recorded high heritability values for seeds per plant, seed yield, plant height and days to flower in fava bean.
Selection for traits for which higher heritability values were recorded can be made on phenotypic basis. However, good response will not be obtained from selection for less heritable traits because such traits are more susceptible to the environment.

**Genetic advance and net genetic gain** - The genetic advance is the change of population means, brought about by selection, depending upon heritability of the character and intensity of selection as measured by selection differential. Higher estimates of genetic advance were obtained for pod number and seed number. Days to flower showed moderate genetic advance. When genetic advance was expressed as percent of mean, higher values were recorded for pod number, seed number and seed yield while plant height, days to flower, branch number, seeds per pod and test weight showed medium genetic advance as percent of mean.

Actual plant improvement was expressed as percent of control which represents the difference between the parent population and the general mean of mutants. High degree of actual improvement was recorded for plant height and test weight. In case of days to flower, branch number, pod number, seed number and seeds per pod, negative values were recorded.

Net genetic gain recorded was very high for pod number and seed number while moderate values of net genetic gain were obtained for days to flower and plant height. In the present study higher genetic advance was not found to be
coupled with higher actual improvement values. Hence these results are contradictory to their findings of Vandana (1990) in faba bean. Moderate heritability was found associated with higher genetic advance for pod number and seed number. Nanda et al. (1988) have observed higher value of genetic advance coupled with high heritability value for seeds per plant. High values of genetic advance for pod number were also recorded by Chaudhary et al. (1978) and Sindhu and Mishra (1982). From the present study conclusion can be derived that weightage on vigorous selection breeding should be placed for pod number and seed number.

Correlation:­

Correlation studies among different traits are important assets to the breeder, since in any crop breeding procedure, the potential progress expected in fulfilling the breeding objectives, depends on the magnitude and relationship of the genetic variance and covariances for different characters. Yield is the multiplicative end product of many factors which jointly or singly influence the grain yield (Whitehouse et al., 1958 and Grafius, 1951). For better yield, selection has to be made for yield contributing characters simultaneously. Association of different traits with yield thus, assumes a special importance as a basis of selection for desirable attributes. The observed correlations can be partitioned into a portion that is due to genotypic causes and one due to factors outside the genotype. The
relative contribution of the two is also of interest to the breeder in selecting for superior genotypes. Therefore, in this study correlations existing at the phenotypic, genotypic and environmental levels were determined.

**Phenotypic correlation:** Grain yield showed strong and positive correlation with plant height, number of branches, pod number, seed number and seeds per pod. Days taken to flower and test weight were the only two traits which had a negative correlation with yield. When correlation between component characters were taken into account, most of the values between plant height, branch number, seed number and pod number were positive and significant. However, a considerable number of correlations involving days to flower, seeds per pod and test weight were negative and insignificant. In the study, pod number and seed number appear to contribute substantially to yield as stronger correlations were recorded with yield.

The results regarding strong positive correlation of pod number and seed number with yield noted in the present study agree with those of Oraon et al. (1977) in chickpea, Sinha et al. (1977) in Yambean, Singh, T.P. and Singh (1973) in green gram and Singh (1977) and Dixit and Dubey (1985a) in lentil. Akinola and Whiteman (1974) estimated that in pigeon pea, yield was influenced by the number of sites available for pod production as was supported by strong positive correlation between yield and number of pod bearing branches.
Genotypic correlation: - Grain yield showed positive and significant correlation with plant height, branch number, pod number, seed number and seeds per pod. Significant negative correlations of yield were recorded with days taken to flower and test weight. Positive and significant correlations were recorded between component characters like plant height, branch number, pod number and seed number. More than half the correlations of days to flower, seeds per pod and test weight were found to be negative. Like phenotypic correlations pod number and seed number appear to contribute substantially to yield at genotypic level also as stronger correlations of yield were recorded with these traits. Findings regarding strong positive genotypic correlation of yield with plant height, branch number, pod number and seed number in lentil are in agreement with those of Jaihini et al. (1977), Tikka et al. (1977), Nandan and Pandya (1980), Chauhan and Sinha (1982), Singh et al. (1970a), Sarwar et al. (1982), Kumar et al. (1983) and Dixit and Dubey (1985). Positive association of cluster and pod number with yield has also been reported in other grain legumes such as mung bean (Singh and Malhotra, 1970 a&b), urdbean (Singh et al., 1970b, Bakhiet and Mahady, 1988 a&b) and faba bean (Vandana, 1990). In almost all cases genotypic correlations were higher than phenotypic correlations. This is in agreement with the results obtained by Singh, K.B. and Singh (1969), Singh and Dixit (1970), Chauhan and Sinha (1982) and Dixit and Dubey (1985) in lentil; Singh et al. (1968) in mung bean; Johnson et al.
(1955) in soybean and Povalities (1965) in tobacco. This indicates that though there is a strong inherent association between the characters studied, the phenotypic expression is reduced under the influence of environment. Webber and Moorthy (1952), Johnson et al. (1955) and Kaw and Manon (1977) also made similar observations.

**Environmental correlations:** Seed yield showed significant positive correlation with seed number, pod number, branch number and plant height at environmental level also. Among components of yield, significant positive correlation of plant height and branch number was recorded with pod number and seed number. The other positive correlations noted were those between pod number and seed number and between seed number and seeds per pod. Test weight was negatively correlated with seed number and pod number while days to flower was not correlated with any of the traits studied.

It is well known that the pairs of characters, in which genotypic correlations are closely associated with phenotypic correlations, are least influenced by the environment.

**Path coefficient analysis:** The number of component characters effecting yield are so many and their interrelationships and interactions are so complex that considerations of correlation between these yield contributing characters and yield itself does not very much clarify the situation. This type of knowledge requires multiple regression or path coefficient analysis as suggested
by Wright (1921). The importance of path coefficient analysis in understanding this complex relationship between yield and its component characters has already been emphasized under "Review of Literature". In the present study path coefficients at phenotypic, genotypic and environmental levels were worked out with seed yield per plant as the dependent variable. The data on the total correlations with seed yield as well as the result of partitioning of these correlations into direct effects and indirect contributions through other variables has been presented under "Observations". A comparison of the path values with the total correlations show that at all the three levels i.e. phenotypic, genotypic and environmental, the more subtle indirect effects were also important and have often served to mask the direct effects. Some characters such as days to flower at genotypic level, plant height, days to flower and branch number at phenotypic and plant height and branch number at environmental level have a very low direct effect on seed yield but through indirect effects via other variables such as pod number and seed number, these characters had a significant correlation with grain yield.

The diagram of path coefficient analysis greatly facilitates the understanding of the nature of the cause and effect system. These diagrams presented under observations reveal that at all the three levels i.e. phenotypic, genotypic and environmental pod number and the seed number were the two traits having maximum direct effect on grain
yield. But there was a difference in the effects of these two traits at the three levels at which path analysis was made. At genotypic level pod number and seed number effected yield in opposite directions as their direct effects, as well as, indirect effects via other traits, were opposite to each other i.e. if one was positive the other was negative. However, such an opposite relation between effects of pod number and seed number was not noted at phenotypic or environmental levels. Thus it appears that the effect of the genotype is modified by the environment so that phenotypic path for yield resembles the environmental path and not the genotypic one.

In an earlier study on 40 elite mutants of lentil Dixit and Dubey (1984) have also pointed out that pod number was the most important yield component with maximum direct and indirect effect on seed yield. Singh, P. et al. (1977), Nandan and Pandya (1980) and Singh et al. (1970a) also reported the direct and indirect effects of pods per plant as the largest contributor towards grain yield in lentil. The path coefficient analysis gives a somewhat different picture of the situation than does simple correlation analysis and it is obvious that in many cases the information obtained from this analysis is more reliable than that of the results of the correlation analysis. Dewey and Lu (1959) pointed out that the apparent conflict between the two analyses arises largely from the fact that the two methods are measuring different things. Whereas correlation simply
measures mutual association without regard to causation, the path coefficient analysis specifies the causes and measures their relative importance. Path analysis is thus more useful when conditions permit its application.

In the present study estimates of a number of genetic parameters have been obtained with regard to the components of yield in lentil. Summing up the results it may be mentioned, that considerable scope for selection exists with regard to the yield contributing characters particularly pods per plant, seeds per plant, plant height and branch number. Correlation studies and path coefficient analysis have confirmed the importance of these characters as contributors to yield.

CLASSIFICATORY ANALYSIS

To study the pattern of morphological variations and quantify the genetic divergence in the induced mutants, Anderson's index scoring as described by Singh and Chaudhary (1977) and multivariate analysis using Mahalanobis's $D^2$ statistics (Mahalanobis, 1978) were applied.

$D^2$ analysis - A quantitative estimate of the genetic divergence between the elite mutants and the control variety emerge from $D^2$ analysis. When Toucher's method (Rao, 1952) of cluster formation was applied to the $D^2$ estimates obtained from multivariate analysis, the 22 genotypes could be grouped into six clear cut genetically distinct clusters. The pattern of cluster formation revealed that there was a wide genetic
diversity with regard to yield and its components in the genotypes isolated on the basis of macromutations.

Largest number of genotypes i.e. 15 out of 22 fell in cluster I and this cluster also had maximum intracluster distance. Cluster II and III with two genotypes each had less pronounced genetic diversity within the clusters. The remaining three clusters were monogenotypic. Values of intercluster genetic divergence revealed that the genetic distance between the cluster pairs varied. Cluster VI generally showed greater intercluster distance with all other clusters. Relative importance of plant height, branching, pod number and seed number besides the seed yield, in contributing towards genetic divergence was established when intracluster group means were compared. This study has clearly brought out in quantitative terms the wide genetic, divergence induced in the mutants isolated from the same parental genotype through mutagenic treatments in lentil.

Dixit and Dubey (1988) in lentil and Vandana (1990) in faba bean have applied D² analysis and found that their respective induced mutants fell into several genetically distinct groups based on variations in yield and it's components.

**Metroglyph and Index Score analysis:**

Index scoring of mutants and control variety brought forward wide genetic diversity among these genotypes. The
index score of twenty one genotypes ranged from nine to twenty one out of the possible maximum score of twenty four. Mutant LM6 with a total score of twenty one was the best performer for almost all characters and thus was quite distinct among all genotypes. Six other mutants with index scores ranging from 17-20 were the other good performers. Ten genotypes including the control variety with index scores ranging from 13-16 could be considered as medium performers while the remaining five mutants in which the index scores ranged from 9-12 were comparatively poor performers. Dixit and Dubey (1988) in lentil and Vandana (1990) in faba bean have also demonstrated the pattern of morphological variation in induced mutants using index score method.