Chapter-2
REVIEW OF LITERATURE

2.1 Induced mutagenesis

2.1.1 General

Induction of mutations through physical and chemical mutagens is considered as an alternative source to naturally occurring variability for crop improvement programmes. Conventional methods of breeding have been extensively used for crop improvement but lack of genetic variability limits the scope of improvement through selection or hybridization. Induced mutagenesis plays a very important role in enhancing new genetic variability. Induced mutations are random, poly-directional and quantitative in nature and bring about heritable changes in polygenic system (Ignacimuthu and Babu, 1993).

The idea of inducing mutations and utilizing them for improving cultivated plants is more than eighty years old. For the first time when Muller (1927) succeeded in inducing mutations in a fruitfly *Drosophila melanogaster* by X-rays, it marked the beginning of a new era in genetics and induced mutations became the focus of genetic studies. Pioneer studies on induced mutations in plants were undertaken by stadler (1928). Scientists working in different facts of mutagenesis have since then being able to accomplish a significant breakthrough in understanding the mechanism of mutagenesis and also its applied value for the benefit of mankind. Indication about the possibility of induction of mutations by the use of chemical mutagens started appearing within a decade after discovery of
the phenomenon. However, the first elaborate report was presented by Auerbach and Robson (1942) who showed that mustard gas could induce mutations as well as chromosome breaks in *Drosophila*.

Mutagens have remarkable possibilities of improving plants with regard to their qualitative and quantitative characters and where appropriate selections has been applied, improvement in yield (Brock, 1965; Gregory, 1968), adaptability (Gustafsson, 1965), maturity time (Brock, 1970), disease resistance (Yamabaki and Kawai, 1968) and numerous other traits (Sigurbjornson and Micke, 1969) have been reported. The generation of genetic variability through induced mutagenesis provides a base for strengthening plant improvement programmes. Various classes of chemical and physical mutagens differ in their efficiency in inducing mutations and spectrum of mutations induced. Eversince, the discovery that mutations could be produced artificially, one of the aims of studies on mutations has been to find the treatment combinations of the mutagens that could induce higher magnitude of useful mutations. Our knowledge in the fundamental aspects of mutational processes and the mechanism of action of various physical and chemical mutagens have been fairly widened with the reports of Blixt and Gottschalk (1975), Gottschalk and Wolf (1983) and Sharma (1985). Though there are several unanswered questions regarding the classification and mechanism of actions of mutagens, yet a more comprehensive account of them was given by Sharma (1985).
2.1.2 Mutagens

Sodium azide (SA), a respiration inhibitor is metabolized in vivo to a powerful chemical mutagen in many plant species including barley, rice, maize and soybean (Owais and Kleinhofs, 1988). Sodium azide (SA) mutagenecity was first observed by Wyss et al. (1948) in their studies on the role of peroxides in radiation induced mutagenesis. Later, sodium azide, an inhibitor of catalase and peroxidase enzymes was found to be very effective mutagen under certain treatment conditions (Kleinhofs et al., 1974); it made possible to obtain high mutation frequency, mostly gene mutations, with negligible frequency of chromosomal aberrations. Sodium azide as such does not possess radiomimetic activity but has also synergistic effect with radiations, methyl nitroso urea (NMU), ethylmethane sulphonate (EMS) resulting in increased frequency of chromosomal aberrations (Kihlman, 1959; Sideris et al., 1969), seedling growth injury (Choudhary and Kaul, 1976) and chlorophyll mutation frequency (Khalatkar et al., 1979; Singh and Olejniczak, 1983).

Sodium azide appears to be highly efficient mutagen in barley because it does not induce chromosome breaks (Nilan et al., 1973). In pea, leaf aberrations were not observed on sodium azide treated plants, which is an indication that sodium azide did not cause chromosome damage (Sander and Muehlbauer, 1977). Sander et al. (1978) reported that sodium azide may only act on replicating DNA as a point mutation mutagen. Pearson (1973) and Pearson et al. (1975) studied the effect of sodium azide on barley cell cycle and concluded that the principle effect of sodium azide was to delay in the initiation of metabolism.
following germination. This results in a uniform delay in mitotic activity, seedling growth and ATP and DNA synthesis and this delay was interpreted as being due to an ATP deficiency.

In barley, azide induces reduction in seed germination and M₁ seedling growth (Nilan et al., 1976, Sander et al., 1978). High mutational yield of azide was reported in rice by Hasegawa and Inone (1980). El-Den (1993) developed a mutant line, E3559 in barley (Hordeum vulgare var ER/APAM) as a result of induced mutagenesis with sodium azide. The mutant line was consistently taller and produced more straw than the parental variety. In Sesame (Sesamum indicum), Kang and Zanten (1996) reported latest variety developed from seeds treated with 2mM sodium azide, for 3 hrs. The latest variety has not only the higher yield than its control variety but also improved oil quality. They also reported a semidwarf mutant, Suwon 128 obtained after treatment with sodium azide which has excellent lodging resistance and a good yield potential when planted in high density. Misra (1995) also reported induced mutants of blackgram with single and combined treatment of sodium azide. Rachovska et al. (1995) reported the production of winter bread wheat lines with high grain quality by means of induced mutagenesis with gamma rays and sodium azide.

Maleic hydrazide (MH) has not been studied extensively for its ability to induce point mutation either in procaryotes or eucaryotes. However, chromosome breaking effects of MH in plants were first described by Darlington and McLeeish in 1951. It is known to induced localized chromosome breakages and mitotic suppression in Vicia faba root meristem. It also resulted in
inhibition of spindle formation and chromosome breakage during mitosis in root tips of onion and barley (Kaul and Choudhary, 1975).

Cytological observations of MH treated plants have been studied by many workers and it has been reported that MH induces chromosomal damage and also different types of chromosomal aberrations (Meschini et al., 1988). Various chromosomal/cytological abnormalities have been reported with maleic hydrazide in *Vicia faba* (De Marco et al., 1995). Misra (1995) reported fifty four induced mutants of black gram (*Vigna mungo*) line T-9, which were developed by single and combined treatment with chemical mutagens, ethylmethane sulphonate, sodium azide, N-methyl-N-nitroso-guanidine and maleic hydrazide. Genetic variability has been reported to be increased in quantitative characters as a result of MH treatment in green gram (Khan et al., 1998).

Maleic hydrazide (MH) has been recognized as plant growth inhibitor. Structurally it is an isomer of uracil (a pyrimidine compound of RNA). The mode of action of MH is possibly through its interference with synthesis of uracil or becoming incorporated into RNA molecule replacing the uracil or it reacts with sulphhydryl groups of nucleic acids. The final result in any case is presumably a weakness in the structure of chromosome leading to chromosome breakage (Grant and Harney 1960, Hestot, 1977). Cortes et al. (1987) are of opinion that the cytogenetic action of MH resembles to the bi-functional alkylating agent in many respects. Its action includes the reaction with phosphate group of DNA or purine base especially the guanine of DNA.
Alkylating agents are by far the most extensive and important group of mutagens. These compounds bear one or more reactive alkyl groups capable of being transferred to other molecules at positions where the electron density is sufficiently high. They cause alkylation of phosphate groups of DNA as well as the bases. The most frequent event being the formation of 7-alkyl guanine.

Alkylating agents carry one, two or more alkyl groups in a reactive form. These are called mono, bi or poly-functional alkylating agents such as EMS, MMS, dES and DMS. EMS and dES adversely affected the germination, seedling growth, pollen fertility, branching, number of leaves, pods and seeds per plant in *Vicia faba* (Vandana and Dubey, 1988). Vandana (1991, 1992) observed mutations affecting days to flowering, size, colour, and number of flowers/node, shape and size of cotyledonary leaves, plant height, branching and texture of plant surface in *Vicia faba* with EMS and dES. According to her, dES induced much higher frequencies of chlorophyll and sterile mutants than those of EMS. EMS has been reported to induce dwarfism in *V. faba* (Filippetti, 1988).

2.1.3 Dose effect

The dose required for high mutation efficiency of a chemical mutagen depends on the properties of the agent, the solvent medium and the biological system in question. In general, the dose of a chemical mutagenic treatment comprises several parameters, of which the most important are concentration, duration of treatment and temperature during treatment.
With a view to enhance the mutation rate and also to alter the spectrum of mutations, many variations in treatment methodology have been used by different workers. Treatments with chemical or physical mutagens have been given to dry as well as soaked seeds, seedling at different developmental stages, different phases of life cycle, at variable temperature and ionic concentrations of chemical mutagens (Chopra and Pai, 1979).

Dose linked effectiveness of NMU was noted in *Vigna radiata* in terms of germination loss, reduction in pollen fertility and seedling height (Singh and Chaturvedi, 1980). Handique and Sarma (1989) reported in *Catharanthus roseus* that increase in concentration and duration of treatment resulted in reductions in percentage of germination, seedling growth and seedling viability. Similar results have been reported by Ping et al., (1996) in Sunflower with sodium azide. Gautam et al., (1992) reported a progressive increase in the frequency of chlorophyll and viable mutations with an increase in gamma rays and EMS doses whereas, Vanniarajan et al. (1993) reported that the frequency of viable mutations was higher in medium dose of EMS and gamma rays and it decreased in higher doses in black gram. Reddy and Revathi (1992) also reported an increase in mutation frequency with duration and concentration of mutagenic treatment in wheat, barley and triticale. Solanki and Sharma (1999b) reported that the medium dose of chemicals and the highest dose of gamma rays induced maximum variability resulting in highest number of promising progenies for almost all the eight polygenic traits in lentil.
2.2 Biological damage

There are many reports to demonstrate the effects of treatment on germination percentage and survival of plants (Goud, 1965; Kempanna et al., 1969; Khan, 1979). Reduction in the percent germination following gamma irradiation has been noticed by several workers viz., Salim et al. (1974) in peas, Rao (1980) in Guar and Okra, Deshmukh (1981) in Anethum sowa, Rao (1988) in Cicer arietinum.

Krishnaswamy et al. (1977) reported a linear relationship with regard to an increase in dose for reduction in survival and mean height of green gram in M₁ as a result of treatment with physical mutagens. Grover and Tejpaul (1979) observed a reduction in the percentage of germination and survival with an increased dose of gamma rays, maleic hydrazide and their combined treatments in green gram. Similar results have been reported by Rao and Rao (1982) as a result of X-ray irradiation in Cyamopsis tetragonoloba.

Filippetti and De Pace (1983) concluded that the higher dose of gamma rays induced lower survival and higher sterility in Vicia faba. Filippetti and De Pace (1986) reported reduced emergence, survival and fertility in M₁ and M₂ with EMS and gamma rays in the same plant. Mehetre et al. (1990) observed a significant reduction in germination percentage in mungbean with high doses of gamma rays. Higher concentration of sodium azide (SA) significantly reduced emergence, plant height and survival in Phaseolus vulgaris (Silva et al., 1994).
Kumar and Dubey (1994) studied the effect of chemical mutagens (DES and EMS) in yellow sarson \((\text{Brassica sp.})\) and observed that germination and seedling growth was promoted by mutagens at low concentrations and retarded at high concentrations whereas pollen sterility increased with an increase in the concentration of mutagens.

Adamska et al. (1995) observed the effect of chemical mutagens in winter swede rape and concluded that high concentrations of NMU (N-methyl-N-nitrosourea) reduced the plant height and root length in \(M_1\) whereas, sodium azide considerably reduced germination percentage. Wang et al. (1994) reported that germination percentage, emergence percentage and height of seedlings decreased with an increased concentration of sodium azide in \(\text{Helianthus annuus} \ L\).

In Pigeon pea, effect of chemical mutagens (Streptomycin sulphate and sodium azide) have been reported by Pandey et al. (1996) and they concluded that germination percentage decreased with an increase in mutagenic dose of both the chemicals. Boncheol and Maluszynski (1996) reported that higher doses of N-methyl-N-nitrosourea, sodium azide and gamma rays resulted in a higher rate of growth reduction in \(M_1\) seedlings of barley whereas, seed germination rate, growth rate of coleoptiles and roots were better in the double treatments than in single treatments.

A reduction in germination, survival and seedling height and an increase in seedling injury, stomatal frequency, abnormal stomata and pollen sterility with EMS, gamma rays and their combination were observed by Arumugam et al. (1997) in barley.
Khan et al. (1998) reported a reduction in germination and pollen fertility in green gram with maleic hydrazide (MH).

2.3 Meiotic abnormalities

Mutagen induced structural anomaly of the chromosome is the primary basis of genetic change and therefore, investigations on the mechanism of chromosome breakage, type of aberration and their genetic consequence form an integral part of most of the mutation studies. Further, cytological abnormalities either in mitosis or meiosis and their magnitude are most convenient for evaluating the effect of mutagen. It also provides the considerable clue to assess radio-sensitivity of plants for both physical and chemical mutagens.

Various meiotic abnormalities like stickiness, clumping of chromosomes, fragments of chromosomes, univalents, disturbed bivalent association, multiavalents, micronuclei, lagging chromosomes, bridges with or without fragments have been reported by Sudhakaran (1971) in Vinca rosea with various doses of gamma rays.

Shaikh and Godward (1972) reported heavy fragmentation of the nucleolus at diakinesis, univalents, tetravalents, multivalents, fragments, complex interchanges, clumping and unusual configuration at metaphase I, abnormal separation of the homologues, single and double bridges with or without fragments, laggards, unequal separation of chromosomes at anaphase I, single bridge with or without laggards at anaphase II, unequal volume of chromatin material in daughter nuclei and unequal pollen grains in M₁ generation of Lathyrus sativus and Vicia ervilia irradiated with various doses of gamma rays.
Katiyar (1978) observed abnormal chromosomes which included stickiness, clumping, altered association, breakage, bridges, unequal segregation, laggards and abnormal microspores in *Capsicum annuum* with various doses of gamma rays and the frequencies of meiotic aberrations were dose dependent.

Rao and Lakshmi (1980) reported gamma rays induced meiotic abnormalities in *Capsicum annuum* L. such as stickiness or clumping of chromosomes, univalents and multivalents in late prophase and metaphase, non-orientation of chromosomes at metaphase, disturbed spindle organization leading to unequal groups of chromosomes, aberrant microspores, abnormal karyokinesis and/or cytokinesis and high pollen sterility.

Tarar and Dnyansagar (1980) reported that as a result of gamma rays and EMS treatment, *Turnera ulmifolia* Linn. showed varying degree of meiotic irregularity. This irregularity increased with the higher exposure to gamma rays and higher concentration and duration of EMS treatment. The various types of abnormalities observed in different stages of M1 generation included stickiness of chromosome, chromosome fragments, disturbed bivalents associations, multivalent formation, precocious movement, laggards, bridges with or without fragments and formation of micronuclei at meiosis I and II. The frequency of meiotic abnormalities was recorded less in EMS as compared to gamma irradiation. Similar types of meiotic abnormalities have been reported in *Lycopersicon esculentum* Mill. var. Pusa Ruby with gamma irradiation (Jayabalan and Rao, 1987b). Zeerak (1992a) reported a dose dependent increase in meiotic irregularities and pollen sterility as a result of individual and
combined effect of EMS and gamma rays in *Lycopersicon esculentum*.

Extreme meiotic abnormalities in chromosome number and behaviour were reported by Haque and Ghoshal (1980) in three species of *Salvia* viz., *S. farinacea*, *S. glutinosa* and *S. taraxacifolia*. The maximum number of chromosomal aberrations were found in *S. farinacea* which was directly correlated with maximum percentage of pollen sterility.

Reddy *et al.* (1992) reported a number of meiotic aberrations such as quadrivalent, trivalent, ring and rod bivalents, unoriented chromosomes, bridges/fragments, micronuclei in M₁ generation of lentil (*Lens culinaris* Medik) induced by gamma radiation, EMS and sodium azide. Reddy and Annadurai (1992) studied the effect of gamma rays, EMS, sodium azide and their combination on various cytological parameters in M₂ generation of Lentil var. PL-639. They reported that the mean value of quadrivalent, rod bivalents, univalents, fragments/bridges, cytological abnormal cells and pollen sterility were increased in mutagen treated population. Combined treatments showed additive effects. EMS produced slightly more abnormalities over sodium azide.

Mitra and Bhowmik (1996) assessed the radio-sensitivity in 2 cultivars of black cumin (*Nigella sativa* L.) based on cytological abnormalities induced by gamma rays and EMS. Both mitotic and meiotic abnormalities showed increasing trend with mutagen dose. Abnormalities like uni, multivalents, stickiness, uneven separation of chromosomes, chromosome bridges with or without fragments etc. were observed in meiotic studies.
Chromosomal aberrations like clumping and stickiness among bivalents, formation of multivalents, univalents, fragments at Metaphase-I and irregular grouping of chromosomes and laggards at anaphase I were observed by Anis and Sharma (1997) and Anis et al. (2000) in Capsicum annuum in response to chemical mutagens.

2.4 Mutagenic sensitivity

It is well known that the same mutagen dose can cause different degrees of effect in different species. Varied mutagenic sensitivity of different genotypes was first reported by Gregory (1955) in groundnut and by Lamprecht (1956) in peas.

Bykovets and Vasykir (1971) conducted mutation studies on leguminous crops like soybean, french bean, peas and Lathyrus with three mutagens NEU, NMU and dES. It was found that all crops are not mutable to the same extent and that the maximum mutagenic effect appeared in peas followed by soybean and Lathyrus. Singh and Chaturvedi (1980) found distinct varietal differences in Vigna radiata using NMU and gamma rays.

Filippetti and De Pace (1986) studied the effectiveness of gamma rays in inducing mutations in one major (cv. Aguadulce) and one minor (cv. Manfredini) Vicia faba cultivar and observed that the minor cv. Manfredini was more sensitive to the lower dosage of gamma rays than the major cv. Aguadulce. Handique and Sarma (1989) studied the differential toxicity of chemical mutagens in Catharanthus roseus and Nicotiana rustica and reported that the two species exhibited different degrees of tolerance to EMS and DMS with regard to germination, but C. roseus was more tolerant with regard to seedling growth.
Pandey et al. (1996) observed the effect of chemical mutagens in different varieties of Pigeonpea and reported that the var. DA II was more sensitive to SA and showed no germination at all except for the lowest concentrations whereas, var. Bahar showed the highest seed germination following treatment with both mutagens. Khan et al. (1998) studied the mutagenic effect of maleic hydrazide (MH) in two different varieties of green gram and found var. PS-16 to be more sensitive than var. K-851.

2.5 Mutation frequency and spectrum

In the mutation induction programme, the choice of an effective and efficient mutagen will certainly increase the possibility of recovering desired mutations in M2 generation. Konzak et al. (1962) reported that mutagen may show differences in mutation spectrum. Auerbach (1967) has suggested that spectrum changes could possibly be induced more by controlling the recovery pattern rather than the primary process of induction of mutations.

Chemical mutagens especially alkylating agents, in contrast to radiations, produce a wider spectrum of mutations (Swaminathan et al., 1962). Nilan et al. (1973) reported that sodium azide (SA) induced higher rates of chlorophyll mutations as compared to EMS. There are strong indications that the total mutation frequency and spectrum are associated with dose of mutagen (Hussein et al., 1974). Filippetti and De Pace (1986) reported that EMS was two to four times more effective than gamma rays in inducing mutations in Vicia faba whereas, Vanniarajan et al. (1993) reported that gamma rays were more
efficient than EMS in inducing chlorophyll mutations in black gram.

Nascimento et al. (1994) reported that sodium azide was more efficient as compared to gamma rays in inducing micromutations in Oats. Solanki and Sharma (1999b) reported that NEU induced maximum variability, highest frequencies of mutated progenies, promising progenies, with multiple characters in both $M_2$ and $M_3$ generations followed by EI and gamma rays in lentil.

2.6 Mutagenic effectiveness and efficiency

Mutagenic effectiveness is a measure of the mutations induced per unit dose of a mutagen, while mutagenic efficiency is a measure of proportion of mutations in relation to undesirable changes like lethality, sterility, injury, chromosomal aberrations etc. The usefulness of any mutagen in plant breeding depends not only on the mutagenic effectiveness but also on the mutagenic efficiency which measures the mutation rate in relation to the biological damage. The methods of calculating mutagenic efficiency and effectiveness were suggested by Konzak et al. (1965) and Walther (1969).

In Sorghum, Sree Ramulu (1970) noted the highest mutagenic effectiveness at lower doses of EMS, NMU and X-rays and reported that mutagenic efficiency of EMS was higher than that of NEU. Prasad (1972) found NMU to be more effective and efficient mutagen under low concentrations. Sharma (1977) noticed a decline in the effectiveness and efficiency of the mutagen with increased doses of NMU and gamma rays.
Nerkar (1977) studied the effectiveness and efficiency of gamma rays, NMU and EMS in *Lathyrus* and found that based on their effectiveness, the three mutagens stood in the declining order of NMU > EMS > γ-rays. However, on the basis of their efficiency, the sequence was γ-rays > EMS > NMU. Both mutagenic effectiveness and efficiency were higher at lower doses of mutagens. Relative efficiency, effectiveness, and factor of effectiveness for treated and control population of the two rice varieties, *Tella hamsa* and IR.24, have been reported by Rao and Rao (1983). The highest mutagenic efficiency was recorded with NMU followed by MMS and HA (hydroxyl amine). The effectiveness was remarkably high in chemical mutagen treatments and markedly low in gamma rays treatments.

Reddy and Smith (1984) studied the mutagenic effectiveness and efficiency of hydrazine (HZ) and ethylmethane sulphonate with a local *Sorghum* variety of Texas, Tx-414, based on seedling injury, sterility and chlorophyll and morphological mutations in M₂ generation. Based on low seedling injury, sterility and seed sterility, they found HZ to be a more efficient mutagen than EMS.

Gautam *et al.* (1992) assessed the mutation frequency, effectiveness and efficiency of gamma rays, ethylmethane sulphonate (EMS) and synergistic effect of their combination treatments in T-9, an improved variety of black gram and concluded that EMS was 2-2.5 times more efficient than gamma rays.

Thakur and Sethi (1995) studied the comparative mutagenicity of gamma rays, EMS and NaN₃ in barley cultivars,
HBL 98, HBL 175, EB-1482 and reported that mutagenic efficiency increased with an increase in the dose of EMS and NaN₃ and their combination with gamma rays in HBL 98, EB 1482 but not in HBL 175. Of the three mutagens, NaN₃ was found to be the most effective and efficient mutagen in HBL 98, EB 1482, whereas it was not so in HBL 175.

Mutagenic effectiveness and efficiency of gamma rays, EMS, dES and their combinations were studied in Khesari (Lathyrus sativus L.) by Kumar and Dubey (1998b) and reported an increase in injury with the increasing radiation dose in individual as well as combined treatments. A substantial amount of sterility was induced by almost all the mutagenic treatments. The efficiency on the basis of seedling injury was generally higher as compared with that based on sterility. The efficiency of individual EMS and dES treatments was 2 to 3 times higher in comparison to most other mutagenic treatments. EMS proved to be more effective than dES.

2.7 Chlorophyll mutations

Chlorophyll mutations although not useful for plant breeding purpose may be used to assess the efficiency and effectiveness of the mutagens in order to select suitable or appropriate dose/concentration for their use in applied mutagenesis programme. Several workers reported the occurrence of different types of chlorophyll mutations in different crops, such as albina, xantha, chlorina, maculata, striata etc., in the M₂ generation with various mutagenic agents e.g. in mung bean (Dahiya, 1973), in chilli (Venkatrajam et al., 1984), in barley (Sharma, 1969), in rice (Rao and Gopal Ayengar, 1964) in wheat (Chopra and
Swaminathan, 1966) and in green gram (Krishnaswami et al., 1977; Grover and Tejpaul, 1979).

Prasad and Das (1980c) reported albina, xantha, albo-xantha, albo-viridis, virescence, chlorina, albescence, tigrina and maculata type of chlorophyll mutations in 6 varieties of *Lathyrus sativus* in M$_2$ generation after gamma rays and MMS treatments. Filippetti and De Pace (1983) noticed chlorophyll mutations in *Vicia faba* with gamma irradiation. Khan (1981) reported chlorophyll mutations with gamma rays and concluded that the frequency and spectrum of chlorophyll mutations in M$_2$ generation does not show a consistent increase with dose.

A wide spectrum of chlorophyll mutations has been reported by Dhaliwal et al. (1987) in *Triticum monococcum* following seed treatment with ethylmethane sulphonate (EMS). The mutagenic effects of gamma rays, EMS and NMU singly as well as in combination were studied in rice (Satyanarayan et al., 1993) and it was concluded that the frequency of chlorophyll mutations increased with the increase in dose of the mutagens.

Vanniarajan et al. (1993) reported multiple chlorophyll mutants like albina, xantha, chlorina, viridis and albomaculata (white dots on green leaves) in two varieties of black gram treated with EMS and gamma rays.

El-Den (1993) observed chlorophyll mutations in barley with sodium azide. Boncheol and Maluszynski (1996) reported a higher rate of chlorophyll mutants following double treatment with chemical mutagens than with a single treatment in barley.
2.8 Macromutations/morphological mutations

Macromutants like leaf mutants, branchless mutants and grain colour mutants have been scored in M₂ generation of greengram as a result of X-ray and gamma rays treatment (Krishnaswamy et al., 1977). As a result of action of MH, gamma rays and combined treatments on the seeds of green gram (*Phaseolus aureus* Roxb.), a number of macromutants has been reported by Grover and Tejpaul (1979). Filippetti and De Pace (1983) reported a broad range of useful and new morphological variants in *Vicia faba* following gamma irradiation treatment.

Numerous viable mutants such as male sterile, dwarf, early maturing, compact, free threshing etc. were induced in *Triticum monococcum* following seed treatment with ethylmethane sulphonate (Dhaliwal et al., 1987). Gamma rays and EMS induced morphological mutants for plant height and leaf size have been reported by Filippetti and De Pace (1986) in *Vicia faba*.

Certain morphological variations affecting root, stem, leaves and flowers were also noticed in M₁ generation of *Cicer arietinum* as a result of gamma irradiation (Rao, 1988). Several viable mutations affecting growth, habit, leaf and flower characteristics have been isolated by Zeerak (1992) in tomato as a result of gamma rays treatment.

Satyanarayan et al. (1993) reported a number of macromutants such as extreme dwarf and tall plants, early and late flowering, high yielding with high spikelet fertility in M₃ generation of rice as a result of gamma rays, NMU and EMS.
treatments. Vanniarajan et al. (1993) reported different types of viable mutants produced by gamma rays and EMS with respect to growth habit, leaf size, seed size, and sterility in Black gram. Ansari and Siddiqui (1995) isolated recessive mutants for dwarfness, erect and semi-erect stem creeping branches and dissected leaves in M2 generation of *Ammi majus* as a result of EMS treatments.

Induced mutations for disease resistance in beans (*Phaseolus vulgaris* L.) have been successfully developed in Brazil (Tulmann et al., 1977, 1981, 1993) using EMS or gamma ray treatment. 'NEP-2', a white seeded bush bean mutant was induced from the black seeded variety 'San Fernando' through mutagenesis. It was found resistant to common mosaic virus, with good canning quality and good agronomic traits.

Induced variability in morphological characters of Sesame treated with EMS has been reported by Mary and Jayabalan (1995). They obtained height mutants, branching mutants, leaf mutants, plant colour and texture mutants, mutants for maturity period, floral and sterile mutants, capsule mutants and seed mutants.

Adamska et al. (1995) studied the effect of N-methyl-N-nitrosourea (NMU) and sodium azide (SA) in winter swede rape and reported morphological mutations which involved many traits as earliness, dwarfness, flower colour and size and compactness.

### 2.9 Quantitative traits

Improvement of cultivated plants largely depends on the extent of genetic variability available within the species.
Mutagenesis has provided a handy tool to enhance natural mutational rate and thereby enlarge the genetic variability and increase the scope for obtaining the desired selections. The exploration of the possibility of crop improvement by induction of mutation and the factors governing the inheritance of quantitative traits has been recommended by several breeders and it is now quite clear that the polygenic mutations result in the induction of considerable variability in mutagen treated population.

The significance of small mutations in evolution was first recognized and emphasized by Baur (1924) and later it has been studied by many workers in different crop plants. Gaul (1965) has emphasized the significance of micro-mutations in plant breeding by saying that "there appears to be no doubt that micromutations may affect virtually all morphological and physiological characters as do large mutations and they might have higher mutation rate than the macromutations".

Several workers have so far reported encouraging results about the induction of useful quantitative variability in different crop plants viz. Gregory (1955, 1956) in peanuts, Gustafsson (1963) in barley, Borojevic (1965) and Goud (1967a) in wheat, Sree Ramulu (1974) in Sorghum and Khan (1990) in mung bean. There is much difference of opinion on the relative incidence of induced polygenic variation and shift of the mean in the negative or positive direction in M2 and later generations.

Sree Rangaswamy et al. (1973) reported short stunted mutants of mung bean having more pods per plant and higher grain yield than the parent. Rajput (1974) reported increased
variability in \( M_2 \) population after irradiation of mungbean with gamma rays. Rubaihayo (1975) obtained several gamma rays induced mutants in white haricot bean, which exhibited higher yielding capacity than the parental line. Krishnaswamy et al. (1977) studied the phenotypic responses of green gram (\textit{Phaseolus aureus} Roxb.) to X-rays and gamma rays and reported an increase in variance for all the economic traits in \( M_2 \), offering good scope for selection.

Khan (1979) reported induced quantitative variability in mung bean as a result of gamma rays treatments. A shift in mean values of irradiated material for all the polygenic traits occurred towards positive direction except for mean days to flower and plant height where there was no particular trend. Sharma and Sharma (1982) studied mutagenic effects of gamma rays and NMU on four quantitative traits in \( M_1 \)- \( M_3 \) generations of the lentil variety L-235 and concluded that gamma rays and NMU treatment decreased the mean values and increased the coefficient of variation of all the characters in \( M_1 \) generation except 1000 seed weight.

Rao and Rao (1982) studied the effect of X-irradiation on \textit{Cyamopsis tetragonoloba} (L.) Taub. and reported that the yield attributes like number of pods per plant, number of seeds per pod and 100 seed weight increased at lower doses and decreased at higher doses. Gamma rays treatments in mungbean (\textit{Phaseolus aureus}) shifted the mean value and range of variability of all the characters in positive direction in \( M_2 \) and \( M_3 \) generation (Khan, 1983).
A higher variance over the control at all doses in the M3 generation has been reported by Verma and Singh (1984) in green gram. Filippetti and De Pace (1986) observed reduction in number of pods per plant, seeds per plant and seeds per pod in M₁ generation as a result of EMS and gamma rays treatment. Wani (2000) reported increase in mean values of various quantitative characters at lower treatments of gamma rays and EMS in *Cicer arietinum* L. Several high yielding mutants showing improvement in yield and yield contributing traits were also isolated in M₃ generation. Khan (1988) conducted a study in mung bean (*Vigna radiata* L.) to evaluate the three quantitative characters i.e. number of pods, 100 seed weight and plant yield after treatments with gamma rays and ethylmethane sulphonate (EMS) and reported that mean of all the three characters increased in the treated population as compared to control with increased phenotypic variability.

Jayabalan and Rao (1989) reported a reduction in fruit yield, in number and weight per plant in M₁ generation with an increase in dose/concentration of EMS and NMU in tomato. Sinha and Bharti (1990) reported an increase in mean value for pods per plant in the mutant population in urdbean. Range and coefficient of variation widened in mutant population for all the characters which was maximum for pods per plant and minimum for pod length.

Ignacimuthu and Babu (1992) used different concentrations of EMS and gamma rays, separately and in combination and reported a broad spectrum of induced variability in most yield traits in urd and mung beans. Marked reduction in plant height,
branching, number of siliqua, test weight and seed yield per plant has been observed by Kumar and Dubey (1994) in yellow sarson (*Brassica campestris*) as a result of DES and EMS treatments.

Khan *et al.* (1998) studied the response of green gram (*Vigna radiata*) to maleic hydrazide and observed an increase in genetic variability in all the three qualitative characters i.e. plant height, days to flowering and days to maturity but the different characters responded differently to the mutagenic treatment.

Solanki and Sharma (1999b) reported an increase in variability for different polygenic characters in M2 and M3 generations in lentil (*Lens culinaris* Medik). Similarly, Tickoo and Chandra (1999) reported induced polygenic variability in mungbean (*Vigna radiata* L. Wilczek). Gamma rays and EMS induced genetic variability for different quantitative traits in urdbean (*vigna mungo* L.) was reported by Singh *et al.* (2000).