Chapter-2

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The occurrence of sudden and heritable changes in the races was first suggested by De Vries (1901) in *Oenothera lamarckiana*. He proposed the use of radiation for the induction of mutations. The first successful use of X-rays was made by Muller (1927) for the discovery of induced mutability and its frequency in *Drosophila*. Later, the successes were achieved by Stadler (1929) in barley and Goodspeed (1929) in Datura and *Nicotiana*. The role of mutations in evolution was emphasized by Baur (1924) and Stubb and Wettstein (1941). Substitution and chromosomal reconstruction clearly demonstrated by Sears (1956) are now valuable tools in planned plant genetics and breeding.

Another phase in the history of induced mutations is the discovery of chemical mutagens during the World War II. So the use of chemical mutagens is comparatively recent in origin. Although the mutagenesis for the first time was tried by Schiemann (1912) by using potassium bichromate on *Aspergillus niger*, but the first successful attempt was made by Auerbach & Robson (1942) by using mustard gas on *Drosophila melanogaster*, whereas Oehlers (1943) concentrated on the chromosomal translocations in *Oenothera* by urethane. Extensive work with chemical mutagens has begun only since 1960 following the introduction of EMS (Heslot, 1964). Rapoport (1947) studied the mutagenic effect of DES in *Drosophila* and postulated that ethylation is a mutation inducing process. Thereafter, DES has been largely used as a plant mutagen. In plants, the chlorophyll mutations with DES were first reported in barley (Heslot & Ferrary, 1958).

Alkylating agents are, by far, the most extensive and important groups of mutagens. However, only a few of the mutagens belonging to the group of alkylating agents such as, ethyl methane sulphonate (EMS), methyl methan sulphonate (MMS), diethyl sulphate (dES), ethyl imine (EI) and N-nitroso-N-methyl urea (NMU) have been reported to be most effective (Rapoport, 1962; Swaminathan, 1966; IAEA, 1970). In general, alkylating agents primarily induce chromatid type aberrations (Revell, 1953; Ockey, 1960; Kihlman, 1961; Evans & Scott, 1964; Heiner, 1971). Induced mutations are considered as
an alternative to naturally occurring genetic variations that serves as the source of germplasm for crop improvement programme and also as an alternative to hybridization and recombination in plant breeding. Mutagens have remarkable potential of improving plants with regard to their qualitative and quantitative characters; and where appropriate selection has been applied, improvement in yield (Brock, 1965., Gregory, 1968), adaptability (Gustafson, 1965), maturity time (Brock, 1970) and numerous other traits (Sigurbjornson and Micke, 1969) have been reported. The extent to which induced mutation provide a useful alternative to the natural variation as a source of germplasm for the improvement of such trait is largely determined by the importance of linked group of genes and the degree to which natural selection has build up linked gene complexes of adaptive significance in the naturally occurring population (Brock, 1971).

3.1 Effect of mutagenic treatment on germination, survival, injury, sterility, seedling height, plant height:

There are many reports to demonstrate the effect of mutagenic treatments on germination, survival, injury, fertility, seedling height, plant height, yield and other morphological characters (Bhatacharjee et al., 1998; Khan, 1999; Mitra and Bhownik, 1999; Khan & Wani, 2005; Jabee et al., 2008; Sharma & Anis, 1995; Omer et al., 2008; Jafri et al., 2011; Choudhary et al, 2012). Increase in pollen sterility and decrease in seed germination with increasing doses of gamma rays in Capsicum annuum was reported by Rao and Laxmi (1980).

The effect of gamma rays and EMS on the seeds of Capsicum annuum L. has been studied by Asha and Nayar (1986) who observed an increase in Pollen sterility with increase in dose, and that gamma rays induced a higher percent of sterility as compared to EMS. The cytomorphology of the spontaneous triploid in Capsicum annuum L. showed delayed growth and prolonged flowering. It showed marked difference in fruit size and in the ratio of healthy and sterile seeds. (Chennaveeraiah and Habib, 1973).

Raghuvanshi and Joshi (1964) observed delayed and extended flowering with larger and varied number of floral parts in the colchiploids of Capsicum frutescense. Total sterility was observed in the fruits of Capsicum annuum produced in the radiation
induced polyploids (Indra and Abraham 1977). Pal et al. (1941) obtained fertile polyploid
with larger fruits in the colchicine induced polyploids of Capsicum annuum L.

Lakshmi and Nalini (1989) isolated tertiary trisomic in Capsicum annuum L. (2n=24)
and found phenotypic variations in the height, internodes and leaves. This trisomic had
2n=25 chromosomes. Sadanandam and Subhash (1985) isolated an aneuploid of
Capsicum annuum L., var. pusa jawala, followed by 40 kR gamma radiation. The variant
was sufficiently vigorous in growth, dark green in colour.

Harini et al. (1990) obtained a chromosomal chimaeral plant with three distinct
primary branches exhibiting diploids, mixoploids (diploid and tetraploidy) and tetraploid
condition. It was recorded for the first time in colchicine treated plants of X235, a local
cultivar of chilli. These three branches showed differences in leaves, flowers, fruits and
 stomatal characteristics. The mixoploid branches displayed intermediate feature of both
diploid and tetraploid branches. Further, fertility and yield were high in chimaeral plant
as compared to those of diploid and tetraploid sibs.

Vandana and Dubey (1988) treated the seeds of Vicia faba L. with different
concentrations of EMS and DES and found that germination, seedling growth, pollen
fertility, time to maturity and survival were adversely affected by both the mutagens.
Plant height, branching, number of leaves, pods and seeds as well as yield/plant showed
varying responses to different concentrations of mutagens. However, DES at all doses
and EMS only at the highest dose had adversely affected these traits whereas the lower
dose of EMS had either no effect or a slight promoting effect.

Gautam et al. (1992) observed a direct relationship of pollen and ovule sterility with
gamma rays and EMS doses in Vigna mungo, the maximum occurring at higher doses.
Increase in pollen sterility and decrease in seed germination with increasing doses of
gamma rays in Capsicum annuum was reported by Rao and Lakmi (1980).

Kumar et al. (1993) treated the seeds of Vicia faba L. with single and combined
application of 0.75% DES and 10kR γ-rays. They recorded reduced germination, seedling
growth, plant height, number of branches, number of pods, number of seeds/pod, test
weight, survival percentage and seed yield in the mutagenic treatments in addition to increased pollen sterility and delayed maturity. Application of γ-rays both singly and in combination with DES induced more severe effects than application of DES alone.

Bhatnagar (1984) reported the adverse effects of combined (EMS and Gamma rays) treatments on germination and survival of plants in chickpea. Reduction in seed germination with the increase in dose of gamma rays in chickpea was reported by Khanna (1991). The EMS treatment was found to cause higher sterility than gamma rays in chickpea (Kharkwal, 1981b).

Singh (2003) studied the effect of gamma rays, EMS and their combination treatments on germination and survival of plants in mungbean (Vigna radiata L. Wilczek) cultivars namely, T44 and PDM11. The germination and plant survival were obtained highest at lowest doses of mutagens and combination treatments. The mutagenic effects were obtained higher at higher doses of both the mutagens individually, while in the combination treatments lower doses showed maximum effects. Varietal preference to the mutagens was also noticed.

Banu et al. (2004) made a comparative study in CO-6 and VBN-1 varieties of cowpea (Vigna unguiculata L. Walp) to study the mutagenic effects of gamma rays and EMS. Physical mutagen recorded higher percentage of reduction than chemical in M1 generation. The mean values of all the characters taken for study i.e. germination, survival, plant height, seed fertility and pollen fertility, decreased as the doses of mutagens increased and there exist a linear relationship between them.

Dhanyanthi and Reddy (2002) treated the seeds of chilli (Capsicum annuum L.) var. Co-9 with gamma rays (10-35kR), EMS (0.5-1%), and MMS(0.5-1%) and studied the effect of these mutagens on seed germination, seedling survival, percent lethality and seedling injury. Lower doses were stimulative, while higher doses had inhibitory effect on these biological parameters. The highest percentage of seed germination and seedling survival were recorded at the lowest dose/concentration of all the mutagens used while the highest percentage of lethality and seedling injury were noticed at the higher dose/concentration of these mutagens. The stimulative effect on seed germination was
more in chemical treated plants than the physical mutagen. There was a proportionate decrease in germination percentage and seedling survival with an increase in concentration of both the chemicals.

Dose dependent decrease in these biological parameters has also been observed by Wani et al. (2004) in lentil and Kumar (2005) in Coriandrum sativum following treatments with EMS, and Kirtane and Dhumal (2004) in onion following treatments with SA, gamma rays and their combination. Mesharam et al. (1981) obtained a plant having spontaneous multiple translocation from normal populations of chilli cultivar CA-960. The plant was very healthy having broad green leaves and big size flowers.

Edwin and Reddy (1993) studied the effect of gamma rays, EMS and their combination treatments in hexaploid triticales. Reduction in germination, seedling survival and seedling height was observed in the treated populations. Combinations treatments were comparatively more effective followed by EMS treatments. An increase in chlorophyll variants, seedling injury, Pollen sterility, abnormal stomata were also observed in almost all mutagenic treatments.

Jayabalan and Rao (1987) studied the effect of gamma rays, ethyl methane sulphonate and nitroso methyle urea on the seeds of tomato cv. Co-2 and found decrease in percentage of seed germination and seedling survival with increasing dose/concentration. Reduction in germination, seedling growth and Pollen fertility following mutagenic treatments has also been reported in Capsicum annum L. (Singh et al., 1988).

Rangaiah et al., (2002) studied the effect of gamma rays in hybrids (F₁M₁) and variety of chilli. A progressive reduction in seed germination, seedling growth and vigour in general with increasing doses was observed. The hybrid and varieties responded differently to gamma radiation, the former was less affected compared to the latter.

Seed germination and survival rate steadily decreased with increasing dose of two organophosphorous pesticides, Ekalux EC 25 and Metasystox on Capsicum annum L. var. X235 and same result was shown by fungicide “Bavistin and Deltan” (Parakash et al., 1988). X-ray treatment of chilli seeds at doses up to 15 kR resulted in reduced
seedling survival, an initial stimulating effect on growth, earlier flowering, morphological abnormalities and chlorophyll mutation. The highest dose also significantly increased pollen sterility (Sahib and Abraham, 1970).

Gradual reduction in germination and survival in *Capsicum annuum* L. was observed when treated with two insecticides “BHC and Nuvacon” and similar results were also obtained as shown by herbicides “Lasso and Basagran” treatment (Reddy and Rao, 1981 and 1982a). Jaheen and Mirza (2002) also reported similar results in *Capsicum annuum* L.

Laxmi and Gupta (1983) studied the response of different concentrations of EMS on various quantitative characters of *Trigonella* in M₂ generation. A significant gradual decrease in plant height, number of branches per plant, number of Pods per plant, number of grains per pod, pod length, and grain yield per plant was noted with an increase in EMS concentration.

Restaino (1983) isolated the pepper brachytic forms (*Capsicum annuum* L.) with different doses of ethyl methane sulphonate and gamma rays. Two recessive brachytic mutants were isolated from M₄, one was compact with reduced internodes, stems, and branches and other was semi compact with a slight reduction in length of the stem and internodes. Rajan et al. (19813) studied the effect of myomycin-C on soaked seeds of chilli. It reduced the percentage of seed germination and seedling survival drastically.

Rao et al. (1989) studied the effect of gamma rays, ethyl methane sulphonate and nitroso methyle urea (NMU) singly and in combination on *Capsicum annuum* L. in M₂ generation and found that the mean values of most of the quantitative characters were lowered as compared to control and frequency of chlorophyll mutations increased following increased mutagenic treatments. Pollen sterility increased with an increase in dose of gamma rays. However, combination treatments were more effective than single doses.

Alkantara et al. (1996) determined the optimal conditions for mutagenesis in *Capsicum annuum* L. Seeds of cultivar Keystone resistant giant no. 3 were treated with
0.5%, 1.0% and 1.5% ethylmethane sulphonate (EMS) and exposed for 3, 6 and 9 hr. at 5°C, 10°C, 15°C, and 20°C. Several unique and interesting mutants were generated. In M1 generation, seed treated with 1.5% EMS at 20°C for 9 hr. had the lowest germination percentage among 36 treatments, but they observed that differences in germination were not significant. They suggested that concentration and duration of seed exposure to EMS could be increased to induce even greater number of mutants.

Vandava and Dube (1988) observed reduction in seedling height and pollen fertility in *Vicia faba* by DES and EMS treatments, whereas lower doses of EMS exhibited slight promoting effect for morphological characters in *Vicia faba*. EMS, MMS and SA also reduced the seedling height in *Vigna radiata* L. (Khan & Wani, 2005). *Solanum melongena* L. (eggplant) showed a linear reduction in seedling growth with increasing doses of chemical mutagens (Hussein & Siddiqui, 1997; Shahab et al., 2007). Reduction in plant height by Gamma rays was observed *Capsicum annum* (Omer et al., 2008). Varshney and Siddiqui (1997) found dose dependent decrease in plant height in bread wheat by the mutagenic action of thiourea. Linear reduction in plant height was also observed in *Oryza sativa* (rice) after the exposure of low UV-B radiations (Mohammed et al., 2007). UV-B radiations reduced vegetative tiller production (25%) and total panicle dry weight (15%) in rice. A plant showing dwarf stature was identified from the progenies of 30 Gy gamma rays exposed population in *Hevea brasiliensis* (Saraswathy Amma et al., 1990), whereas *Trigonella foenum-graecum* showed significant increase for plant height after gamma rays treatment (Yadav et al., 2000). Caffeine showed a stimulatory effect on plant height and yield attributing characters at lower doses in *Capsicum annum* L., while higher doses were found inhibitory (Kumar & Tripathi, 2004).

Reduction in plant survival and pollen fertility with increasing doses of gamma rays was reported in *Vigna mungo* (Sharma et al., 2005), *Helianthus annuus* L. (Khursheed et al., 2008). Survival and root length was inversely affected by increasing doses of gamma rays in *Capsicum annum* L. (Omar et al., 2008).
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*Capsicum annum* L. showed gradual decrease in seedling growth and pollen fertility in MMS treated populations (Sharma & Anis, 1995), whereas lower doses of MMS enhanced the percentage of germination, survival and pollen fertility in *Vigna radiata* L. (Khan et al., 1987). Ethyl methane sulphonate (EMS) and its combinations with gamma rays have induced the seedling growth, pollen fertility and days to maturity in two varieties of *Lathyrus sativus* L. (Kumar & Dubey, 1998b)

Two varieties of *Capsicum annum* L. namely CO-I and Ujwala treated with different concentrations EMS showed a gradual increase in pollen sterility with increasing concentration of EMS. Percentage of sterility was recorded higher in variety Jawala than CO-I (Salam and Thoppil, 2010). The effect of gamma rays and EMS was studied on *Jatropha curcus* L. The germination of treated plants had shown a sharp dose rate relationship, which decreased with increase in the dose/concentration of the mutagenic treatments. A decreasing trend in shoot and root length, seedling survival and vigour index with increasing dose/concentration of the mutagens has also been observed. EMS was more drastic in reducing seedling vigour than gamma rays. Lower doses of gamma rays has shown stimulatory effect on plant height whereas EMS treatments in both lower and higher concentrations showed inhibitory effect as compared to the control (Dhakshanamoorthy et al., 2010).

Mutagenic potential of lead nitrate has also been studied on *Trigonella foenum graecum* L. Seed germination and pollen fertility decreased with increasing concentration of lead nitrate. Lower concentration showed stimulatory effect on plant height and number of pods/plant. However, higher concentration showed inhibitory effect on these two parameters (Chaudhary et al., 2012). Seed germination, pollen fertility and seedling survival showed a dose dependent reduction in *Vicia faba* L. after the treatment of DES and SA. However, DES showed inhibitory effect than SA (Bhat et al., 2007). A reduction in seed germination and seedling survival with increasing dose of gamma rays in faba bean has also been observed (Mabrauk et al., 2012). Reduction in pollen fertility with increasing doses of gamma rays was reported in safflower (Srivastava, 2012). Seeds of *Capsicum annum* L. var. Azad treated with different concentrations of lead nitrate
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showed a decreasing trend in seed germination, plant height and pollen fertility with increasing concentrations (Gupta and Kumar, 2008), and when the same var. treated with 0.5% EMS for 3, 5 and 7 hr duration, the morphological parameter namely plant height, leaf area, no. of nodes and 100 seed weight showed a decreasing trend with increasing duration of treatment, however, days to maturity increased with the increase of the treatment hour (Kumar and Gupta, 2009).

3.2 Mutations Affecting Plant Morphology:

The availability of ample genetic variability is pre-requisite for attempting selection in plant breeding to develop desired plant types in any crop. Several induced morphological mutations have been reported in literature showing alterations in the morphology of various plant parts.

Singh (1988a) isolated 25 types of morphological mutants for plant habit, stem, leaf, height, flower and seed characters in chickpea. Generally, physical mutagens induce more morphological mutations and chemical mutagens induce more chlorophyll mutations (Gaul, 1960; 1964). Contrary to this, Singh (1988a) observed that EMS induced marginally more morphological mutations than gamma rays. Pleiotropic effect of morphological mutations was reported by Deshmukh et al. (1972). According to Blixt (1972) morphological changes are either as a result of pleiotropic gene action or of cryptic chromosomal deletions.

Variation in size, texture, type and modification of leaf parts have been reported by many workers (Patil, 1966; Venkatarajam and Subhash, 1986). Several workers have also reported mutants for plant height, maturity period, branching, seed and pod mutants (Raisinghani and Mahna, 1994 in Vigna mungo; Mary and Jayabalan, 1995 in Sesamum indicum). Singh et al. (1999) isolated several macromutations affecting different morphological character in Vigna mungo L. after treatments with gamma rays and EMS. Gamma rays induced bold seeded mutant was reported in broad bean (Bhat et al., 2006a). The frequency of viable mutations has been found to increase with increase in the dose of EMS, NaNO₃ and their combinations with gamma rays (Thakur and Sethi, 1995). Sharma (1970) reported synergistic effect for viable mutations at lower combination treatments as
against the additive effect observed at the higher doses. He further reported that the combination treatments changed the spectrum by inducing more mutation types that were not observed in the separate treatments. In chickpea, morphological mutants have been isolated for leaf shape (Kharkwal, 1981a), plant habit (Kharkwal, 1981b), growth habit, maturity, seed size (Vanniarajan et al., 1993), Seed weight and total pods per plant (Khanna, 1981) and flowering period (Haq et al. 1989). Mutants have been isolated after seed treatment with physical and chemical mutagens. A wide range of mutants affecting habit, pod distribution, seed size and shape, earliness and resistance to Ascochyta rabiei were also obtained in chickpea due to seed treatment with gamma rays and EMS (Dekov and Radkov, 1982). Subhash et al. (1981) isolated Cluster bud mutants after treatment with EMS in Capsicum annuum L.

Gaikwad and Kotheker (2003) treated the seeds of two lentil cultivars, L-4611 and L-4639 with 3 different concentrations of 2 chemical mutagens, EMS and SA. Nine morphological mutants were isolated in M2 and M3 generations. These morphological mutants were named on the basis of the part of the plant body affected. Among them the early maturity, high yielding and bold seed type mutants have the potential to be incorporated into breeding programs.

Sharma and Kumar (2003) observed EMS induced macromutants in chickpea (Cicer arietinum L.) when treated the seeds with EMS (0.5%) at different durations. The morphological mutants were characterized on the basis of the part of the plant body affected. These mutants can be better fitted in new cropping patterns and with improved agronomic management, their yield ability may even be better. Gamma rays-induced semi-dwarf mutant with semi-dwarfing genes showed positive responses to salt stress in Hordeum vulgare (Forster, 2001).

Rajam and Subhash (1984) treated the seeds of Capsicum annuum L. with mytomycin-C for 30, 60, 90 and 120 min. Several morphological mutants affecting plant height, branching pattern, floral organs, fruit characters and other plant morphological characters were recorded from M2 and M3. Of special interest were the clustered bud, tall, multilocular, spindle fruit, erect fruit and orange fruit mutants.
Singh et al. (2004) treated the seeds of two improved cultivars of urd bean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 with single and combined doses/concentrations of gamma rays, EMS and SA. A number of various types of B morphological macromutations were induced in *M_2* generation. Out of these, 14 mutants from PDU1 and 13 from T9 were identified as true breeding for plant morphology, pod and seed characters and early maturity in *M_2* generation. Many macro mutants showed significant improvement in yield and other yield components as compared to their parents.

Sangsiri et al. (2005) detected gamma rays induced mutations in mung bean (*Vigna radiata* (L.) wilczek) when treated the seeds of two mung bean varieties and their *F_1* and *F_2* with gamma rays Cs-137 source at the dose of 500 Gy. Mutant characters were grouped as chlorophyll, leaf, flower and pod mutants. Chlorophyll mutations included albino, coppery leaf, light green leaf, variegated leaf, waxy leaf, white streak leaf, and xantha leaf. Leaf mutations were lanceolate leaf, narrow-rugose leaf, multiple leaflet, round-cuneate leaf, unifoliate leaf, and wrinkled leaf. The flower mutant was cock’s comb raceme while the pod mutant was a lobed one. All mutants were purified for genetic study and possible uses of the traits.

Solanki (2005) isolated twelve kinds of morphological mutations included changes for growth habit (compact, bushy, prostrate), foliage (narrow, broad, rogue, tendrillar), plant height (tall, dwarf), maturity and flowering behaviour (early, late, sterile) in lentil by EMS and SA treatments. The mutations for growth habit and foliage were induced with higher frequency by EMS, whereas those for plant height and maturity and flowering behaviour were induced with higher frequency by SA on the basis of sterility, SA was found more effective and efficient than EMS.

Kulthe and Kothekar (2006) observed induced morphological mutants in winged bean (*Psophocarpus tetragonolobus* (L.) DC.). The seeds of winged bean var. EC 38955 (A) were treated with three concentrations of two chemical mutagens, namely ethyl methane sulphonate (EMS) and nitrosonethyl urea (NMU). Various morphological mutants were recorded in *M_2* and *M_3* generations. These morphological mutants were named according to their morphology or special characters. Nine different mutants were isolated within
which high yielding, early maturing, dwarf, non-shattering pod mutants have useful potential and are indicative of the genetic improvement of winged bean crop Dhakshanamoorthy et al. (2010).

Kumar and Rao (2003) isolated six autotriploids from the progeny of an M2 line of *Capsicum annuum* L. var. PC1 whose progenitor was an M1 semisterile (induced by 40kR gamma rays). These triploids were characterized by large sized dark green leaves, stomata, pollen, flower and fruit growth besides longer petioles and greater plant spread. Nevertheless, these bear fewer branches, starter internodes, fewer fruits and seeds besides delayed growth and flowering compared to their M1 progenitors and the control.

The frequency and spectrum of morphological mutations were found to be mostly dose dependent in *Capsicum annuum* L. (Kumar et al., 2002). New cultivars having altered fatty acid composition have been released in gamma/X-rays irradiated rapeseed, sunflower, and linseed crops (Bhatia et al., 1999). A light corolla mutant, showing variation in some of the oil constituents with a light purple eye at the base of flower was detected in gamma rays irradiated muskdana (*Abelmoschus moschatus*) [Mishra et al., 2000].

Variation in carpel number and its morphology was observed in local brinjal cultivar treated with gamma rays and EMS (Zeerak, 1998). Two flower colour mutants, viz. red with white stripes and pink with white stripes were isolated in carnation through in vitro application of EMS (Singh, et al., 2000a). The mutants not only performed better in the traits for which they were selected but also found to be important in other quantitative characters.

Gamma rays alone induced several micro and macro-morphological chimaeras in sunflower (Ratnam & Rao, 1994). Mini and deformed flower mutants were isolated in gamma rays irradiated *Beta vulgaris* L. (Chauhan et al., 2006). The anthers in these mutants were both dehiscent and non-dehiscent, exhibiting a variable degree of pollen sterility associated with abnormal behaviour of endothecium and tapetum. Two dwarf mutants and one mutant with yellow pericarp were isolated from heavy ion irradiated sweet pepper (Honda et al. 2006).
Morphological mutants were observed in M2 generation of Black gram (*Vigna Mungo* (L.) Hepper) with the effect of dose/concentration of mutagens (EMS and Gamma rays) and such mutants were, dwarf, tall, tiny leaves, hairy leaves, male sterility, brown seed, early, maturing, long pod, bottom branching, top branching, bushy type, trailing and spreading habit mutants. EMS provided more number of morphological mutants followed by gamma rays (Arunabalachandran and Mullainathan, 2009). A dwarf mutant plant having small leaves and light-orange flower was identified in the M2 generation from gamma ray treated groundnut cultivar VRI 2 (Mothilal and Jayaramachandran, 2012).

Seeds of *Capsicum annuum* L. var. PC1 were exposed to five doses of gamma rays (10, 20, 30, 40 and 50 KR). Sixteen morphological mutants were isolated in M2 and M3 generations. These mutants were classified under four categories i.e. plant height mutant, leaf mutant, maturity mutant and fruit mutant (Kumar et al., 2001). Tall, small broad fruit, dwarf, erect fruit, small pointed fruit, orange long fruit, orange round fruit, purple fruits mutant were isolated in *Capsicum annuum* L. cv. NP 46A, after the treatment with DMS, EMS, MES, HZ, HA and gamma rays (Raghuvanshi and Singh, 1982).

The seeds of 2 varieties of chickpea, Pusa-212 and Pusa-372, were treated with gamma rays EMS and their combination treatments. The M2 population was carefully screened for various viable macromutations. A wide spectrum of viable morphological mutations affecting almost all parts of the plant were isolated in the M2 generation. The most striking mutants isolated included tall, dwarf, broad-leaved, white-flowered, bold-seeded and high-yielding mutants. Differences in varietal response to different mutagens was clearly evident as both of the varieties differed in the spectrum and frequency of mutations induced. Combination treatments were most effective in inducing a wider spectrum and maximum frequency of macro mutations, followed by EMS. Of all the morphological mutations, the frequency of leaf mutations was maximum, followed by plant height and seed mutants. Most of the macro mutants were confirmed to be true-breeding in the M3 generation except for the highly sterile ones. Some mutants were specifically isolated in a particular mutagen type (Wani, 2011).
3.3 Mutations Affecting Yield:

Medium or moderate doses of gamma rays under dry treatment and higher doses under soaked treatment were found more effective in inducing genetic variability for grain yield and its attributing characters in *Vigna mungo* (L.) Hepper (Kumar & Mishra, 2006). Gamma rays, ethyl methane sulphonate (EMS), mitomycin C (MC) and hydroxylamine (HA) induced a proportional increase in the female flowers in *Momordica charantia* L., resulting in a slight shift in sex ratio and yield (Mallaiah & Jafra, 1988).

Thirteen mutants with promising performance for yield components were isolated in M2 generation of gamma rays irradiated *Brassica juncea* Coss. (Javed et al., 2000). Two mutants with significant increase in dry leaf yield per unit area were isolated in gamma rays irradiated *Virginian tobacco* (Ibrahim et al., 2001b). A promising mutant (early maturity and high seed and husk yield) was obtained by combined treatment of gamma rays and ethidium bromide (EB) in *Plantago ovata* (Lal & Sharma, 2002). Several mutants with promising performance for yield and yield components have also been isolated in gamma rays, SA and EMS treated *Ocimum sanctum* L. (Nasare & Choudhry, 2003).

DES, EMS and colchicine increased the fruit yield in *Solanum melongena* L., and DES was found to be more effective in increasing fruit yield (Siddiqui et al., 1988). Remarkable increase in fruit size, weight and number of fruits per plant was found in DES and MMS treated *Solanum melongena* L. (Siddiqui, 1989). NMU induced an increase in mean values for several quantitative characters in *Solanum melongena* L. (Siddiqui, 1993). Successful development of useful mutants with improved early seed maturity, coupled with high seed yield, seed quality and determinate growth habit in *Trigonella foenum-graecum* have been reported by EMS treatments (Basu et al., 2007).

Loss in yield and its attributing characters was observed with increasing doses of gamma rays in different crops (Verma et al., 1999; Pavada & Dhanvel, 2005). Gamma rays have induced variability for various yield attributing characters in *Trigonella foenum-graecum* (Yadav et al., 2000). Waghmare et al. (2001) isolated for the first time a fasciated mutant with less number of primary and secondary branches, reduced pod and seed size, low yield and delayed maturity in gamma rays irradiated *Lathyrus sativus* L.
seed size, low yield and delayed maturity in gamma rays irradiated *Lathyrus sativus* L. (grasspea). Reduction in root weight and shoot dry weight was observed with increasing doses of gamma rays in *Capsicum annuum* L. (Omar et al., 2008). Gamma rays, EMS, streptomycin, acriflavin and ethidium bromide have reduced the biomass production in *Brassica juncea* (Singh et al., 1993). Gamma rays and EMS showed a decreasing trend for the mean values with increasing dosage for five quantitative characters viz. primary branches per plant, clusters per plant, pods per plant, seeds and seed yield per plant in *Vigna unguiculata* (Banu et al., 2005). Remarkable loss in yield has been experienced by chemical mutagens in soybean (Pavadai & Dhanvel, 2004) and bread wheat (Varshney & Siddiqui, 1997). *Gossypium hirsutum* L. (cotton) showed loss in plant height, no. of sympodia and no. of bolls per plant during M1 and M2 generations to the 13h EMS treatment (Sundaravadivelu et al., 2006).

Three hexaploid triticaces showed negative shift in mean for the plant height, tiller number and grain yield and positive shift in mean for spikelet/spike and 100-grain weight due to gamma rays and EMS treatments (Viswanathan et al., 1994). Reduction in yield components was also observed in gamma rays irradiated caraway plants (*Carum carvi* L.), while induced increase in yield components for both *Foeniculum vulgare* (fennel) and *Nigella sativa* (black cumin) (Khalil, 2001).

Colchicine induced autotetraploids in faba bean (*Vicia faba* L.) showed gigantism, bigger leaves and flowers etc. with reduced pollen fertility, number of seeds per pod and number of seeds per plant as compared to diploids (Joshi & Verma, 2004), whereas colchicine-induced autotetraploids have exhibited enhancement in yield attributing components in *Impatiens balsamina* L. (Dikshit & Kumar, 2007).

Four mutants with altered tannin content were screened in gamma rays irradiated winged bean (*Psophocarpus tetragonolobus* L. DC) (Klu et al., 1997). Out of four mutants only one desirable mutant with a level of tannin of about 25% of the wild type and the other mutants having similar or increased tannin levels were recorded.

Ten agronomically desirable mutants were isolated in wheat and triticale after treating with gamma rays and EMS individually and in combination (Viswanathan & Reddy,
Higher concentrations of EMS and its combination with gamma rays were found to be effective in increasing the variability for the fatty acid content in soybean oil (Patil et al., 2007).

Cellulose synthesis was enhanced in green gram seedling by chelating agents viz. EDTA and 2, 2-dipyridyl at low concentration with a corresponding increase in amylase activity and a decrease in sugar content (Rao et al., 1986).

3.4 Chlorophyll Mutations:

The chlorophyll mutation is the clear-cut indication of non directional nature of mutation and possibility of induction of useful mutations. Chlorophyll mutations are considered as one of the most dependable indices for evaluating the genetic effects of different mutagens in several crops (Gustaffson, 1951) and are used as genetic markers in basic and applied research (Reddy and Gupta, 1989). Different types of chlorophyll mutations such as albina, xantha, viridis, maculata, striata, chlorina etc. have reported in several crops by using physical and chemical mutagens (Swaminathan et al., 1962; Reddy and Annadurai, 1991; Das and Kurtagrami, 2000, Singh et al., 2001). EMS has been reported to induce higher proportion of chlorophyll mutations than gamma rays in several crops (Waghmare & Mehra, 2001; Singh & Singh, 2001; Karthika & Lakshmi, 2006). The combined treatments of gamma rays and ethyl methane sulphonate (EMS) produced higher frequency and wider spectrum of chlorophyll mutations followed by single treatment of gamma rays or EMS in mungbean (Vigna radiata L.) (Singh et al., 2005; Sharma et al., 2006), while in Vigna mungo L. Hepper., the gamma rays was more efficient than EMS and their combination in producing chlorophyll mutations (Khan, 1999). Similarly gamma rays induced the higher frequency and wider spectrum of chlorophyll mutations than EMS in urdbean (Sharma et al., 2005; Khan, 1999). Lower doses of gamma rays and EMS showed wider spectrum of chlorophyll mutations in Nigella sativa L. (Mitra & Bhowmik, 1999). Thus physical mutagen was found to be more effective in inducing chlorophyll mutations than chemical mutagen in two cultivars of soybean (Geetha & Vaidyanathan, 2000). Kumar et al. (2001) studied the frequency, spectra and inheritance pattern of chlorophyll mutations by gamma rays in a chili cultivar.
Chlorophyll mutants were albino, chlorina, xantha and viridis. The frequency and spectrum of chlorophyll mutations by gamma rays have been found dose dependent in different crops (Palanivel & Jayabal, 2000; Kumar et al., 2000; Jain et al., 2005). Dry and wet irradiated conditions influenced the rate of chlorophyll mutations in foxtail millet (Ichitani et al., 2003). A xantha mutant (yellow plant) was identified in gamma rays treated cytoplasmic male sterile (CMS) maintainer line 1132 of Oryza sativa L. (Zhou et al., 2006).

Prasad and Das (1980c) observed different types of chlorophyll mutations viz. albina, xantha, albo-xanthalba, alboviridis, virescence, chlorina, trigerna and maculata in six varieties of Lathyrus sativus L. The spectrum of chlorophyll mutations was found to be dependent on the genetic background of the genotype. Moreover, chlorophyll mutation frequency increased with the increase in dose of gamma rays both individually as well as in combination with MES in all the varieties. Contrary to this, Mitra and Bhowmik (1999) reported that lower doses of gamma rays and EMS showed wider spectrum of chlorophyll mutations in Nigella sativa L. Sharma (1970) reported that chlorophyll mutation frequency decreased at higher doses when calculated on segregating M1 families basis. However, on the basis of M2 plants a progressive increase with the increase in EMS doses was observed. Several workers have reported differential varietal response for the induction of chlorophyll mutation (Prasad and Das, 1980c; Singh et al., 1999; Das and Kundagrami, 2000). Sharma and Sharma (1981a) observed no varietal or mutagenic differences with regard to the spectrum and relative proportion of chlorophyll mutations.

Subhash and Venkat Rajam (1983) described the cytological and morphological variations induced in Capsicum cultivar C-5 after X-ray irradiation at 1, 3, 5 and 6kR doses. Chlorophyll mutants namely xantha, albina, straita and viridis were observed. The frequency of chlorophyll mutants increased as the doses of mutagen increased but at higher dose i.e. chlorophyll mutants did not observed.

Singh et al. (1999) observed the mutagenic effects of gamma rays and EMS alone or in combination on frequency and spectrum of chlorophyll and macromutations in two
cultivars, namely PDU, and T-9 of urdbean has been observed. Conclusively, the combination treatments have yielded the higher frequency and spectrum of chlorophyll mutations whereas the various doses of mutagenic agents have independent response towards macromutations in both the cultivars.

Yadav and Padmaja (2004) studied the induced chlorophyll mutations in the two varieties of Cajanus cajan (L) Millspaugh, viz. KPL 93115 and ICPL 93117, following the treatments of gamma rays and EMS. The chlorophyll mutants were quantified on the bases of M3 seedlings and their frequencies were evaluated variety-wise and mutagen-wise for understanding mutagenic effectiveness of mutagens γ-rays and EMS used singly and in combination. Based on the frequency of chlorophyll mutants, ICPL 93117 variety appeared to be more responsive to γ-rays as well as EMS. Further, optimum results generated at 25 kR of γ-rays and 0.2% EMS indicated effectiveness of these doses.

Rajam et al. (1984) treated soaked seeds of Chilli with X-rays and 0.01% EMS separately and in combination. They observed six type of chlorophyll mutants viz., xantha, albina, chlorine, viridis and straita in M2 generation. Occurrence of chlorophyll mutations were in proportion to the dose duration and combined treatments enhanced the frequency of mutation.

Sharma et al. (2006) estimated the spectrum and frequency of chlorophyll mutation by using gamma rays, EMS and their combination on two cultivars, namely, Pant-19 and Pant-30 of urdbean (Vigna mungo L. Hepper). Five different types of chlorophyll mutations viz., albina, xantha, viridis, chlorina and maculata were identified in both the cultivars. Almost all the combination treatments produced maximum frequency and wider spectrum of chlorophyll mutations followed by single treatment of gamma rays or EMS. The frequency of chlorophyll mutations increased with higher doses of mutagens but decrease at highest dose.

Two varieties of Trigonella foenum- graecum L. viz., desi and kasuri methi were treated with different concentrations (0.1, 0.2 and 0.3%) of EMS, MMS and MES. A wide range of chlorophyll mutants were observed in both the varities. The mutants were albina, straita, xiahoalba and chlorina in both the varities. It was observed that the
chlorophyll spectrum with 0.3% MMS in desi methi. The highest chlorophyll mutation frequency was obtained with 0.3% EMS & chlorophyll mutation spectrum with MMS in kasuri methi, but the mutation spectrum was broader in desi methi as compared to kasuri methi (Vasu and Hasan., 2011).

The cultivar IPU-982 of Black gram was treated with different doses of gamma rays, sodium azide and their combined treatment. Six different types of chlorophyll mutants, namely, albino, xantha, dark xantha, chlorina, viridis and striata were induced. Out of these mutants, xantha and dark xantha were most frequent while striata was least frequent. The highest frequency of chlorophyll mutations (8.87%) was reported in the combination of 60kR+0.03%SA. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations whether mutagens were employed singly or in combination (Gaibriyal et al., 2009).

Chilli var. K1 was treated with different doses/concentrations of gamma rays and EMS. Chlorophyll mutants such as albino, chlorine, viridis, virescens and lutescens were observed in M2 generation of treated seeds. Frequency and spectrum of chlorophyll mutations increased as irradiation and chemical mutagen doses increased. Generally, gamma rays induced higher proportion of chlorophyll mutants than EMS. (Sri Devi and Mullainathan., 2011).

Dry and dormant seeds of Capsicum annum cv. PC 1 were exposed to different doses of gamma radiation (5-50 krad) at 5 krad/minute, and subsequently sown in flower pots and then in the field (Andhra Pradesh, India). Six different chlorophyll-deficient mutant types (Albina, Xantha, Chlorina, viridis and Virescense) were isolated from the M2 and M3 progeny lines. The frequency and spectrum of the chlorophyll mutants were dose dependent and the Albina type predominated over the other types. All mutant types were recessive and controlled by a single gene. (Kumar et al., 2000)

3.5 Mutagenic Effectiveness and Efficiency:

The usefulness of any mutagen (chemical or physical) in mutation breeding programmes depends not only on its effectiveness but also on its efficiency. Mutagenic
effectiveness is a measure of frequency of mutations induced by unit mutagen dose, whereas, mutagenic efficiency is the measure of proportion of mutations in relation to undesirable changes like lethality, injury, sterility, mitotic and meiotic chromosomal aberrations etc. In other words, the higher efficiency of a mutagen indicate relatively less biological damage. A highly effective mutagen may not necessarily show high efficiency and vice versa. Synergistic as well as antagonistic effects may occur when various physical and chemical mutagens are used in combination.

A distinction between effectiveness and efficiency of mutagenesis has been a major experimental activity in the past. The purpose of this exercise was to identify criteria by which efficient mutagen and mutagenic doses can be selected on the basis of analysis of M1 parameters (Konzak et al., 1965). Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens were studied in chickpea (Kharkwal, 1998a); *Oryza sativa* L. (Singh et al., 2001); celery, fennel and ajowan (Paul & Datta, 2005). Lower doses of physical and chemical mutagens and their combinations were found to be effective and efficient in several crops by many workers (Prasad, 1972; Sharma & Sharma, 1981; Khan, 1999). It has been reported that among the monofunctional mutagens, methyliating agents are more toxic and thus, need to be used only at lower concentrations (IAEA, 1970) as against ethylating agents that are reported to be less toxic and can be applied at relatively higher concentration to yield more mutations at equimolar concentrations.

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 mutagens have been given to dry as well as soaked seeds, seedling at different developmental stages, different phases of cell cycle at variable temperature and ionic concentrations (Chopra and Pai, 1979). Ramanna and Natrajan (1965) studied the mutagenic efficiency of certain alkylating agents under different treatment conditions of temperature and hydrogen ion (pH) concentration in barley. They
treatment conditions of temperature and hydrogen ion (pH) concentration in barley. They concluded that factors such as concentration and diffusion of the mutagen, rate of hydrolysis and the influence of alkylating and non-alkylating groups of the chemical play a considerable role in determining the mutagenicity of a compound.

According to some authors chemical mutagens have been reported to be more effective in causing mutations as compared to physical mutagens and to their combined treatments with physical mutagens (Raveendran & Jayabal, 1997; Bhattacharjee et al., 1998; Solanki & Sharma, 1999; Kharkwal, 1999, 2001; Shah et al., 2006). MMS was found most effective and efficient than EMS in *Vigna radiate* L. (Wani & Khan, 2005), while EMS has been reported to be more effective and efficient than gamma rays in chickpea (Shah et al., 2006), urdbean (Sharma et al., 2005), *Lathyrus sativus* L. (Waghmare & Mehra, 2001), celery, fennel and ajowan (Paul and Datta, 2005). Sodium azide (SA) and gamma rays show higher effectiveness and efficiency in *Trigonella foemum-graecum* L. (Koli & Ramkrishna, 2002). Lower doses of hydrazine sulphate (HS) were found more effective and efficient, but followed a declining trend with increasing concentrations of HS (Jabee & Ansari, 2005).

The efficiency on the basis of seedling injury has been reported to be generally higher as compared with that based on pollen sterility. The efficiency of individual EMS and DES treatment was found 2 to 3 times higher in comparison to most other mutagenic treatments and the EMS proved itself to be more effective than DES (Kumar & Dubey, 1998b). Moreover the effectiveness and efficiency increased with increasing doses of gamma rays in sunflower (Ratnam & Rao, 1993). The combined treatment of gamma rays and EMS was found to be more effective than individual doses in generating the resistant type of mutants in Indian mustard (Yadav et al., 2001). Similarly, EMS in higher concentrations as well as its combined treatments with gamma radiations was found to be more effective in inducing variability for the fatty acid content in soybean (Patil et al., 2007). Khatod et al. (2002) reported that lower doses of gamma rays were found to be more effective in cotton.
Ethylene imine has been reported to be more effective and efficient than gamma rays (Blixt, 1964). Higher mutagenic effectiveness of MMS was recorded in rice (Rao and Rao, 1983). Dixit and Dubey (1986) observed that NMU treatment was 2-5 times more efficient in comparison to gamma rays, whereas combined treatments showed higher efficiency than respective individual treatments. Higher efficiency of combination treatments has also been reported in barley (Khalatkar and Bhatia, 1975). Khan (1999) studied the effectiveness and efficiency of EMS, gamma rays and their combination in black gram. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens in chickpea has been reported by Kharkwal (1998a). Chemical mutagens have been found to be more efficient in inducing chlorophyll as well as viable and total number of mutations. NMU in particular was found not only to be effective but also efficient than gamma rays and EMS. Rao et al. (1991) observed that gamma rays were found to be more efficient than EMS in Chilli.

Kumar and Dubey (1998c) studied mutagenic effectiveness and efficiency of γ-rays, EMS, DES and their combination in Lathyrus sativus L. and reported an increase in injury with increasing radiation dose in individual as well as in combined treatments (γ-rays + EMS and γ-rays + DES). A substantial amount of sterility was induced in almost all treatments. 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.

Banu et al. (2001) assessed the mutation frequency, effectiveness and efficiency of gamma rays and EMS in cowpea varieties (Co-6 and Vamban 1). Mutagenic effectiveness was higher at lower dosage and lower at higher dosage level. When comparing both the mutagens, EMS was found to be efficient in giving maximum mutations in Co-6, while in VBN 2, gamma ray treatments in general found to be efficient in providing more mutations.

Parveen et al. (2006) studied the efficiency and effectiveness of physical and chemical mutagens in inducing chlorophyll mutations in M2 generation of Trigonella foenum graecum L. A comparative study of the frequency and spectrum of chlorophyll
mutations induced by MMS, EMS and gamma rays in M₂ generation was made in two varieties of *Trigonella foemnum-graecum* viz. Paras-9018 and Krishna-9001. Four different types of chlorophyll mutants viz., Albina, Xantha, Chlorina and Maculata were identified in the treated populations. Frequency of Xantha mutants was highest followed by chlorina and other types. Gamma rays in general proved to be more effective followed by EMS and MMS in inducing maximum frequency of chlorophyll mutations (gamma rays > EMS > MMS).

Kumar *et al.* (2012) Studied the mutagenic efficiency and effectiveness of gamma-rays (10, 15 and 20 kR) and ethyl methane sulfonate (EMS) (0.05, 0.1 and 0.2%) along with control in two varieties of Paprika cv. Bydag Kaddi based on M₁ biological damages (lethality injury) and M₂ viable mutagen frequency. Mutagenic parameters like chlorophyll and total mutation frequency were also assessed in M₂. The results indicated variable response of the variety to gamma rays and EMS. EMS has been found more effective while, gamma irradiations were found efficient for inducing viable mutation.

Shah *et al.* (2008) detected the comparative mutagenic effectiveness and efficiency of gamma rays and EMS in two desi (Pb2000 and C44), one Kabuli (Pb1) and one desi x kabuli introgression line (CH 40/91) of chickpea. The results revealed that EMS was almost seven times more effective and its efficiency was two times higher than that of gamma rays. Mutagenic effectiveness and efficiency were found to depend upon mutagen type and the genotype and both were higher at lower doses of EMS in three genotypes except in desi genotype C44. The introgression line desi x kabuli genotype was found to be most resistant towards mutagenic treatments than desi and kabuli types.

Mutagenic effectiveness and efficiency of gamma rays, EMS and combined treatments was studied in terms of lethality and chlorophyll mutations in two cultivars of soybean (Pusa-16 and PK-1042). In general the frequencies of chlorophyll mutations were high in gamma rays and combined treatments. Four types of mutants viz., albina, xantha, chlorine and viridis were observed in the study. Gamma rays were found to be more effective to induce chlorophyll mutations in both cultivars. PK-1042 cultivar exhibited
higher mutagenic efficiency as compared to Pusa-16 in EMS and gamma rays treatment. (Khan and Tyagi., 2010).

Dube et al., (2011) investigated the mutagenic efficiency and effectiveness of Gamma rays and EMS in alone and combination treatments in Cyamopsis tetragonoloba variety Sharada. The mutagenic efficiency and effectiveness in this plant was decreased with Gamma rays followed by EMS in combination treatments. The mutagenic efficiency recorded on the basis of percent lethality was more in all treatments as compared to it on the basis of percent injury. Gamma rays in alone treatments induced more mutagenic efficiency as compared to that of EMS in alone treatments or Gamma rays followed by EMS in combination treatments. However, the mutagenic effectiveness recorded was more in EMS alone treatments as compared to it in Gamma rays alone treatments or Gamma rays followed by EMS combination treatments.

The seeds of two varieties i.e., Pusa 212 and Pusa 372 of chickpea were treated with gamma rays, EMS and their combination treatments. Mutagenic effectiveness and efficiency was calculated based on biological damage in M1 and chlorophyll mutations in M2. Mutagenic effectiveness increased with the increase in dose/treatment. Combination treatment in general proved to be more effective followed by individual treatment of EMS and gamma rays. Mutagenic efficiency varied depending upon the criteria selected for its estimation. Intermediate treatments in general were found more efficient. The order of efficiency was gamma rays+EMS>EMS>gamma rays. Among the two varieties, var. Pusa 372 proved to be more sensitive to mutagenic treatments than the var. Pusa 212 (Wani., 2009).

Mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments were studied in the genotype of cowpea variety CO-7. Gamma rays, EMS and combined mutagens produced a high frequency as well as a wide spectrum of mutation. The frequency of mutation was more in combined treatments than gamma rays and EMS. The mutagenic effectiveness and efficiency was calculated based on biological damage. In M1 generation based on seed lethality and seedling injury and M2 generation was carefully screened for various chlorophyll and viable mutations. Mutagenic effectiveness

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and efficiency increased with the decreased in dose or concentration. EMS was proved to be more effective and efficient in causing mutations as compared to gamma rays and the combined treatments (Girifa and Dhanavel., 2009).

Cowpea variety CO-6 was treated with EMS, DES and SA to assess the efficiency and effectiveness of these chemical mutagens. The mutagenic effectiveness was found to be the highest at lower concentration with all the mutagenic treatments. EMS was found to be more effective than DES and SA. Mutagenic efficiency varied depending upon the criteria selected for its estimation. Mutagenic effectiveness and efficiency decreased with increase in all mutagenic treatments (Dhanavel et al., 2008).

3.6 Induction of Cytological Aberrations:

Cytological studies during mitosis and meiosis are one of the most dependable index to obtain information regarding the role and the effect of the mutagens. It also provides considerable clue to assess radio-sensitivity of plants to both physical and chemical mutagens. Mutagen induced chromosomal aberrations have been reported by many workers in different plants such as Pea (Kallo, 1972), lentil (Reddy and Annadurai, 1992), fenugreek (Anis and Wani, 1997), Capsicum annum (Anis et al. 2000) and broad bean (Bhat et al., 2007a). Most of these workers observed dose dependent increase in the frequency of chromosomal abnormalities with respect to mutagenic treatments. Ignaczimutu and Sakthivel (1989) observed a significant and positive correlation between chromosomal abnormalities and pollen sterility.

Rao and Laxmi (1980) studied the meiotic abnormalities induced by gamma rays in Capsicum annum L. The meiotic abnormalities included stickiness, clumping of chromosomes, univalent multivalents, unequal separations, laggard, and non orientation of chromosomes.

Rao and Kumar (1983) isolated three desynaptic mutants in a population of local cultivar of chilli. The mutant plant showed reduced chiasma frequency and pollen fertility than normal plants. The desynaptic plants were weak and medium strong types. The mutants showed a monogenic pattern of inheritance.
Sadanandan and Subhash (1984) studied the effect of EMS, DES and SA on chiasma frequency per bivalent and per pollen mother cell of M₁ plants in *Capsicum annum* L. and observed a reduction in chiasma frequency in all mutagenic treatments compared to their respective control. EMS caused greater reduction in chiasma frequency per cell.

Meshram and Patil (1986) studied the cytological effects of dimethyl sulphate (DMS), ethyl methan sulphonate (EMS) and acid juice of mango (AJM) in *Capsicum annum* L. and reported chromosome stickiness, univalents, multivalents, chromatin bridges, fragments and micronuclei.

Prakash et al., (1988) studied the effect of two fungicides (Bavistin and Deltan) on *Capsicum annum* L. var-X235 and reported a dose dependent increase in various chromosomal abnormalities namely univalents, multivalents stickiness, non-orientation of chromosomes, laggards and chromatin bridges. The mean chiasma frequency decreased with increased concentration of mutagens. A decrease in pollen fertility was also recorded with increased concentration of mutagen.

Anis and Sharma (1997) made cytological analysis in treated as well as in control plants of *Capsicum annum* L. treated with EMS, MMS and SA and observed a reduction in chiasma frequency in all mutagenic treatments as compared to their respective control. EMS caused a greater reduction in chiasma frequency than MMS and SA. Various chromosomal aberrations like clumping and stickiness of chromosomes, univalents, multivalents and fragments were observed at metaphase-I. Irregular grouping of chromosomes and laggards were also found at anaphase stages.

Anis et al., (1998) induced autotetraploidy in *Capsicum frutescens* var. suryamukhi by treating apical growing point with colchicine and observed various meiotic abnormalities such as univalents, multivalents, unequal distribution of chromosomes and micronuclei. These irregularities were the major factor for high sterility of pollen grains in induced tetraploid plants of *Capsicum frutescens*. Kumar and Dubey (1998c) studied the effect of gamma rays, EMS and DES on meiosis, pollen and seed sterility and survival percentage in M₁ generation of *Lathyrus sativus*. High frequency of translocations leading to
multivalent associations involving varied number of chromosomes were induced in all the treatments.

Anis et al. (2000) studied the effects of EMS, MMS and SA on various cytological parameter in M₁ generation of Capsicum annum L. and recorded a greater reduction of chiasma frequency caused by EMS. Various meiotic abnormalities in M₂ plants such as univalents, multivalents, fragments, bridges and laggards were recorded. Pollen sterility was found to be increased with increase in concentrations of mutagens.

Kumar and Rao (2003) isolated six autotriploids from the progeny of a M₂ line of Capsicum annum L. Autotriploid showed gigantism in respect of leaf stomata, flower and pollen sizes. However, they had fewer number of flowers, branches, fruits and seeds, besides late flowering as compared to their M₁ progenitor and the control. Univalents, multivalents, unequal separation, micronuclei were the frequent chromosomal anomalies. The mean chiasma frequency, pollen fertility, seed fertility was lower as compared to M₁ progenitor and their control.

Kumar and Rao (2006) isolated fasciated stem mutant in a local cultivar of Capsicum annum L. It was characterized by broad-strap like stem, increased plant height, days to maturity, and pollen sterility. Desynapsis, nondisjunction of chromosomes, chromosome clumping and stickiness, laggard and bridges were found in some of the PMC's of the mutant while the normal plant did not show these irregularities.

Kumar and Gupta (2009) induced karyomorphological variations in three Phenodevants of Capsicum annum L. Seeds were treated with 0.5% solution of EMS for 3, 5, and 7 h durations and genetic segregation was closely observed. Many chromosomal anomalies like stickiness, bridges, and multivalents, secondary associations, laggards, and precocious movement were observed in all the 3 durations of treatment. These anomalies showed a dose dependent increase in frequency. The morphological parameters showed a decreasing trend along with the increasing doses of treatments. However, with the 7 h dose 3 morphological variants were isolated which varied in plant height, number of nodes, leaf area, 100-seed weight (g), vigorousness and days to maturity, from other sib plants and also from control plants.
Subhash and Nizam (1977) reported that increasing dose of X-rays resulted into the formation of increased number of multivalents, fragments, bridges and micronuclei in *Capsicum annuum* L. Subhash and Venkat Rajan (1983) described the cytological and morphological variations induced in *Capsicum* cultivar C-5 after X-ray irradiation at 1, 3, 5 and 6 kR doses. Gross chromosomal anomalies like fragments, bridges, laggards, unequal separation of chromosomes, micronuclei etc were observed at different stages of meiosis.

Katiyar (1978) studied *Capsicum annuum* L. plants grown from gamma irradiated and control seeds for meiotic aberrations and pollen sterility in M₁ and M₂ generations. Chromosomal aberrations included stickiness, altered association, breakage, bridges, unequal segregation, laggards and abnormal microspores and their frequencies were dose-dependent. Pollen sterility showed dose dependent increase. The percentage of chromosomal aberrations were more in M₁ than M₂, which could be due to the operation of recovery mechanisms or elimination of damaged chromosomes in the intervening period. Similar results were also reported by (Rao and Laxmi 1980; Tarar and Dnyansagar, 1980)

Kumar and Rao (1985) isolated one desynaptic plant in a population of *Capsicum frutescens*. Meiotic studies in the desynaptic plant showed reduced chiasma frequency and pollen fertility. Chromosome pairing at pachytene was normal and complete in the control plant, while it was partial in the desynaptic. Twelve bivalents were regularly formed both at diakinesis and metaphase-I in the control plants, while univalents ranging from 12-24 were recorded at the corresponding stages in the desynaptic. At anaphase-I the chromosome segregation was regular (12:12) in the control and it was irregular in the desynaptic. It is presumed that desynapsis in *Capsicum frutescens* may have been due to a spontaneous gene mutation.

Jayaabalan and Rao (1987) irradiated healthy, dry seeds of pusa ruby variety of *Lycopersicon esculentum* Mill. with gamma rays at 10 kR, 20kR, 30kR, 40kR and 50 kR dose levels. Meiotic studies were made in treated as well as in control plants. At metaphase I, meiotic abnormalities like clumping and stickiness of chromosomes,
univalents, multivalents fragments and irregular grouping of chromosomes were observed. At anaphase I, there were laggards and unequal grouping of chromosomes at poles.

Lakshmi et al. (1989) recorded cytomixis, between adjacent PMCs in a sterile plant screened in the population of Sindhur variety of *Capsicum annum* L. In 36.5% of pollen mother cells cytomixis was affected through cytoplasmic bridges resulting in PMCs with variable number of chromosomes ranging from 4-36. Interestingly, the phenomenon of cytomixis was associated with medium strong type of desynapsis. It was also observed that cytomixis has some sort of negative effect on desynapsis resulting in increased pairing in the cells involved in cytomixis.

Nirmala and Kaul (1993) detected desynaptic mutant in DES induced *Pisum sativum* variety Arkel, involving lack or impaired synaptic pairing, confined only to the male sex. This anomaly is controlled by a single nuclear recessive gene msg4, non-allelic to the other msg genes isolated in *Pisum sativum* genome. The synaptic anomaly leads to abnormal male meiosis involving premature chiasmata terminalization, nucleolar multiplication, univalency, unequal and irregular chromosome disjunction at A1 and AII, unequal triads and tetrads and coenocyte formations. This resulted in degenerated microspore formation rendering the mutant total male sterile. The meiotic anomalies exhibited high proportion of variance and the initial anomalies add to the variance of the subsequent abnormalities making male meiosis exceedingly erratic. The major meiotic anomalies are inter-correlated but only some exhibit genetic correlations which unravel the causes and consequences of meiotic anomalies detected in this mutant. The dys gene causing the male sex specific anomalies, does not belong to the gene system, regulating chiasma formation and its terminalization in *Pisum sativum*. Instead, it is a special gene disrupting male meiosis only and is anther specific. Kumar and Rao (2003) isolated six autotriploids from the progeny of an M2 line of *Capsicum annum* L. var. PC1 and the mean chiasma frequencies in the triploids was significantly less than 1.5 times to that of their M1 progenitor and their control.
Bhat et al. (2005a) provided a relative account of cytological and developmental effects of gamma rays, EMS and MMS on meiotic features and pollen fertility in *Vicia faba* L. Studies undertaken in M₁ generation on the variety minor of this species showed that both the physical and chemical mutagens induced various kinds of chromosomal aberrations and reduction in pollen fertility. Such effects were dose dependent and positively correlated with dose/concentration. However, the induction of meiotic aberrations was observed to be higher in MMS treatments followed by gamma rays and EMS, suggesting that MMS could be more effective in inducing genetic variability followed by gamma rays and EMS in this crop.

Bhat et al. (2005b) studied the relative effects of EMS and MMS on meiosis and pollen sterility in *Vicia faba* L. var. major in M₁ generation. Meiotic studies revealed various aberrations like stickiness, laggards, bridges, precocious separations, disturbed polarity, cytomixis and non-synchronisation. Stickiness of chromosomes was the most common aberration followed by bridges and precocious separation. Among the different stages of meiosis the frequency of chromosomal aberrations was maximum at metaphase-I stage and showed a linear increase with increase in concentration of both the mutagens. However, MMS induced maximum frequency of aberrations than EMS. Pollen sterility was the cumulative result of various meiotic aberrations.

Singh and Chaudhary (2005) observed γ-ray induced chromosome in two morphologically distinct varieties of *Capsicum annuum* L. When dormant, dry seeds of two varieties i.e. solitary pendent variety (LCA-335) and a clustering erect variety (RHRC-CE) were irradiated with gamma rays. He reported that radiation induced meiotic abnormalities are directly proportional to the γ-ray doses administered. Altered association and other chromosomal aberrations included stickiness, clumping, bridges, laggards etc. Concomitantly, dose dependent increase in the percent pollen sterility ensued was directly proportional to the meiotic abnormalities. The percent frequency of genetic recovery or elimination of defectives in M₂ generation was greater than M₁. The pendent variety is genetically more stable than the clustering erect variety.
Bhat *et al.* (2006b) reported cytomixis during microsporogenesis in various stages of meiosis in MMS treated populations of *Vicia faba* L. Cytomixis was observed to occur through various methods, i.e. by forming cytoplasmic channels and direct fusion of pollen mother cells. The migration of nuclear content involved all the chromatin/chromosomes or part of it from donor to recipient cell/cells. The occurrence of PMCs with chromosome numbers deviating from diploid number (2n=12) through the process of cytomixis lead to the production of aneuploid cells in all the populations treated with various concentrations of MMS. Increasing concentration of MMS had a positive effect on the percentage of PMCs showing cytomixis. The level of pollen fertility was found to be affected by cytomixis and chromosome stickiness. It seems possible that genetic factors might have also contributed towards pollen sterility.

An aneuploid with the spectrum of anomalies including various associations of chromosomes at diakinesis, lagging chromosomes at anaphase, appearance of micronuclei at telophase-II and microspores with different constitutions of micronuclei was isolated in *Capsicum* by gamma rays (Sadanandam K, Subash, 1985).

Gamma rays-induced six autotriploids were isolated in *Capsicum annum* L. The triploids showed reduced chiasma frequencies and were characterized by large sized dark green leaves, stomata, pollen grains, flowers and fruits, besides longer petiole and greater plant spread (Kumar & Rao, 2003).

Kumar and Verma (2011) treated the seeds of *Vigna unguiculata* (cowpea) with gamma rays and sodium azide. They observed chromosomal aberrations like unorientation, multivalents, laggards, bridges, micronuclei, stickiness and precocious movements etc. Chromosomal aberrations were found to be correlated with the concentrations of both the mutagens individually as well as in combination. The combined treatment proved to be more effective in inducing chromosomal aberrations and sterility as compared to individual treatment sets.

Bhat *et al.* (2007) studied the comparative analysis of meiotic aberrations induced by DES and SA in *Vicia faba* L. Stickiness, stray bivalents, univalents, multivalent, laggards, bridges, cytomixis, micronuclei, disturbed polarity were the main chromosomal

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aberrations and these aberrations increased with the increase in concentrations of each mutagen. The DES was more effective than SA as it induced more chromosomal aberrations.

Seeds of *Capsicum annuum* L. varieties CO-1 and jwala were treated with potent chemical mutagen, Ethyl methane sulphonate. Various types of meiotic chromosomal aberrations such as multivalents, stickiness, clumping, bridges, laggards, micronuclei, tripolar orientation, pentad, non synchronous separation etc., and a dose dependant decrease in pollen fertility were observed in M1 generation. The frequencies of chromosomal abnormalities increased with the increase in mutagenic concentrations. Varietal response to the chromosomal aberrations was very pronounced, i.e. the variety jwala was more sensitive and the frequency of aberrations was comparatively high at all the mutagenic concentrations (Salam and Thoppil., 2010).

Seeds of *Capsicum annuum* L. var. G4 were subjected to different concentrations of methyl methane sulphonate (MMS) and diethyl sulphate (DES). Various types of meiotic aberrations such as univalents, multivalents, stickiness, bridges, laggards, cytomixis etc. were observed in all the treatments. However, the MMS treatments proved to be more effective in inducing meiotic aberrations as compared to DES. The frequency of meiotic aberrations was maximum at metaphase followed by anaphase and telophase stages. As the concentrations increased, reduction in chiasma frequency and pollen fertility was observed in all the treatments and, MMS again was found to be more effective than DES treatments (Gulfishan *et al.*, 2012).

In the light of above summarized literature, it may be concluded that a great deal of work has been done on the mutagenic properties of different mutagens in several plants. At present methyl methane sulphonate (MMS) and diethyl sulphate (DES) has been employed to assess their mutagenicity and cytogenetic assay in chilli (*Capsicum annuum* L.) in M1, M2 and M3 generations to induce genetic/morphological variability for the selection of mutants which may be better than the existing strains.