2.1 Mutagenesis for crop improvement

Mutations in plants are powerful tools, not only for clarifying physiological mechanisms in plants but also for developing new plant varieties in practical breeding programs. Mutation methods such as gamma ray irradiation, Fast neutron bombardment and chemical treatments have been studied, developed and used to induce mutations (Gottschalk and Wolf, 1983). The term mutation was first introduced by Hugo de vries in 1901. Mutations were known to occur in animals and plants much before this time. Mutagenic action of X-rays was discovered by Muller in 1927 on Drosophila and in 1928 by Stadler in barley (*Hordium vulgare*) and maize (*Zea mays*).

Muller was awarded the Noble prize in 1946 recognition for this work. In 1946, Auerbach and Robson reported that nitrogen-mustard produced mutations in Drosophila. Subsequently, a number of chemicals with mutagenic action were described. Immediately after Muller’s discovery in 1927, Mutation breeding programmes were initiated in Sweden, U.S.S.R and Germany. Mutation breeding attracted considerable attention during 1950’s
and 1960’s and several countries took up research project in mutation breeding. Intensive mutation breeding was commenced in 1930 by Gustafsson and mutation research in wheat was started in U.S.S.R by Delaunay (1931).

Mutation induction is one approach for creating genetic variation in crop plants. The technology of mutation induction has become an established tool in plant breeding in order to supplement existing germplasm and to improve cultivars in specific traits. Improved varieties of many crops have been released to forms as a result of induced mutations which have been used directly as new cultivars or in cross breeding programs (Gottschalk and Wolf, 1983 and Micke et al., 1987).

2.2 Crop improvement

The crop improvement programe is dynamic and continual in order to meet the changing trends in production and utilization through mutation, either spontaneous or artificially induced. The development of improved cultivars, mutation techniques has also been used as a simple tool. The wide use of model plant mutations, in studies of molecular organization of genomes or metabolic pathway has stimulated interest to utilize these techniques in crop improvement by conventional or molecular genetics methods. The traditional methods of employing mutagenic chemicals and radiation have been supplemented by the use of in vitro culture and
transposable elements. Van Harten and Broertjes (1989) which have been useful methods complementary to conventional breeding of crop plants can significantly accelerate mutation techniques. Mutation breeding, a much heralded short cut breeding technique, mainly based on conventional breeding approach, brings novel and high yielding genotypes through heritable changes in genotype and phenotype of a particular trait. Induced mutagenesis has been employed to create desired genetic variability, the base of crop improvement.

Mutations are theoretically changes which occur in DNA sequence and result in changes in the genetic code. A gene mutation or point mutation is the group of all heritable changes which occur within the limits of a single gene. The majority of gene mutations show recessive inheritance but dominant gene mutations occur at a very low frequency (Micke, 1999). Mutation is considered as one of the easy, rapid and effective tool of crop improvement. Spontaneous mutation cannot be expected to serve the cause of crop improvement effectively due to very low. Induced mutation may be induced using treatment with certain physical (Gamma rays) or chemical mutagens selection of macro mutation for different contrasting traits can be used as a variety or a parent for the bringing of desirable traits in to the otherwise well adapted
cultivars. Induction of the useful macro mutations for increasing genetic diversity and utilization of such trait specific genetic stock for further crop improvement would certainly be useful (Kumar et al., 2009).

Mutations are the tools used to study the nature and function of genes which are the building block and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007). Induced mutations have great potentials and serve as a complementary approach in genetic improvement in crops (Mahandjiev et al., 2001).

Uhl et al., (2003) reported that mutations occurring at the gene level comprise base substitution mutations, which lead to changes of individual amino acids (or a termination of the amino acid chain) a frame shift mutations (base insertions and deletions).

Arulbalachandran and Mullainathan (2009) reported gamma rays indicate improve high quantitative traits.

### 2.3 Mutation breeding

Mutation breeding plays a key role in self- fertilized crop with limited variability. In this context, it was to be noted that may varieties like Kalika Uma, Usha in Sesame, (Sharma, 1993); Aruna in Castor (Ankinneedu et al., 1968); Shaobatica sonira in Wheat
(Swaminathan, 1969); MH2, TG1 (Trombay Groundnut 1), TG3,BG1 (Bissa Ground 1) and BG2 were developed by mutation breeding using irradiation and CO₂ (Coimbatore-2) by using chemical mutagens, have been developed through induced mutagenesis.

Interestingly the authors have pointed out demethylation as one of reason of mutations and hence restoration of gene expression. However, these epigenetic mutations have proved to be less stable than normal DNA sequence alterations. Hence conventional methods of mutation breeding remain much acceptable means of crop improvement. As such basic advantage of traditional methods of mutagenesis is that it can give raise to various modifications in the traits and also can give raise to different mutant alleles, which should help in an unbiased natural selection (Chopra, 2005).

The mutation breeding has been recognized since the beginning of this century as one of the driving force of evolution, besides selection and evolution. The heritable change occurs through modifications in the DNA of an organisms. They are random can occur in any gene and are recurrent nature, which mean they can occur again. Most induced mutation does show pleiotropy, which may be due to heritable changes in closely linked genes. Hence, it is important to use large populations preferable more than 10,000 for the selection of best possible mutants.
Random modification in DNA will certainly change the function of
the gene or the number of genes resulting significant heritable
changes through mutations are generally recessive in nature and
are harmful/ lethal to the organisms, mutations can be dominant
as well. Some of the effects of mutations can be beneficial as well.
However, out of all possible mutations, the useless mutations for
outnumber than those having significant and beneficial effects. In
some cases, useless changes in the DNA may have a significant
outcome as well. However, genome of most living beings especially
plants has the self- repairing phenomenon, which actually corrects
these harmful mutations during the process of DNA replications.
Deletion of damage in the DNA template or incorrect base pairing or
copying is few reasons of unwanted mutations and can be detected
through this mechanism (Tah, 2006).

Mutation breeding is one of the conventional breeding
methods in plant breeding. It is relevant with various fields like,
morphology, cytogenetics, biotechnology and molecular biology etc.
Mutation breeding has become increasingly popular in recent times
as an effective tool for crop improvement (Acharya et al., 2007).

Akhilesh et al., (2010) reported that mutation breeding can be
used to induce a number of desirable traits in garden pea, which
may contributed for the development of high yielding genotypes.
The mutants isolated by become a gene pool for pea improvement.
Environmental stress due to application of mutagens may activate the transposes which should be the reason of acquired genetic diversity (Zhang et al., 2002). These transposable elements have the capacity of modifying major gene functions, variation in gene size and major changes in gene or genome structure. Contradictions have also arisen from differences in generation of morphological mutants in the laboratory and those related to mutants in natural populations mainly because of the environmental variation and different breeding strategies leading to problems in limitation in natural selection (Cubas et al., 1999).

### 2.4 Induced mutagenesis (Choice of mutagens)

Induced mutagenesis by means of both physical and chemical mutagens has been playing crucial role in creating genetic variability there by improve the characteristics of plant and animals. Variability in the population creates the change of selection for desirable improvement.

Induced mutation in sugarcane effects of physical and chemical mutagens proved effective for quality improvement in sugarcane (Khairwal et al., 1984).

Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutations are
highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee et al., 2002).

Induced mutagenesis can be used to create variability, as the rate of spontaneous mutation is very low. The use of induced mutation has been widely accepted by plant breeders as a tool in crop improvement. The induction of mutation in plant materials can be achieved either through physical or chemical mutagens (Karim et al., 2008).

Chemical mutagens are ideal for inducing dominant mutant alleles, while physical mutagens are ideal for recessive mutations. Recurrent mutagenic treatments can increase mutation frequency, and consequently may introduce new beneficial mutations (Shu, 2009).

Induced mutations have played a great role in increasing world food security, since new food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production reported by (Sonu Goyal and Samiullah Khan, 2010) in urdbean.

Sharifi- Sirchi et al., (2012) reported physical and chemical dose of mutagens and for its trait to have the best breeding program by mutagenesis.
Induced mutagenesis is best approach for creation of genetic variability but most of interest is paid towards crop plants with only a few exceptions. The all efforts of mutation breeding which were made for the improvement of *Catharanthus roseus*. (Ashutosh Kumar Verma *et al.*, 2013).

Mutagenesis is a powerful tool that has been used to create genetic materials for studying functional genomics, breeding, and for understanding the molecular basis of disease resistance. Approximately 100 000 putative mutants of rice (*Oryza sativa* L.) have been generated by mutagens (WANG Zhong- hua *et al.*, 2014).

**PHYSICAL MUTAGENS**

### 2.5 Gamma rays

Certain abnormalities in millets induced by X-rays, irradiation with X-rays has been much resorted to by many workers in genetics and plant breeding in order to induce genetic variability. It has been the common experience of almost all the workers that in the first generation are produced a number of mutations, which are usually recessives and at the same time many of them may take the shape of abnormalities rarely met with in natural populations (Krishnaswamy and Rangaswami Ayyangar, 1942).
Effect of Gamma irradiation on some morphological characters of three wheat varieties, parameters *viz.*, days taken to earing started, days taken to earing completion, number of tillers, number of leaves per plant and spike length increased gradually as compare to control (Abdus Saboor Khan and Muhammad Ashraf Malik, 1999) in wheat.

Ionizing radiations have been effectively utilized in inducing genetic variability in pearl millet (Smith, 1972). Arulbalachandran *et al.*, (2010) in black gram reported gamma irradiation proved to variation in genetic level and enhance the quantitative traits at 60kR compared to control plants.

Radiation sensitivities also differ among plant organs. This difference is thought to be due DNA content, water content and so on. Cells in ‘S’ phase of the cell cycle are the most sensitive to radiation because in this stage, the DNA content increases and the chromosomal DNA molecules are unpacked, leading to a cell status that is readily attacked by radiation and the secondary radical products. Radicals such as hydroxyl radicals are a major cause of DNA damage. It is well known that these radicals are generated by reactions between water and radiation. Therefore, plant material such as dry seeds, in which the water content is very low; tend to slow high resistance to radiations. Irradiation dose should be carefully determined according to the kinds of ion species and energies, plant species, plant varieties, plant state of materials such as cell cycle and water content (Margori *et al.*, 2010).
Physical and chemical mutagenic treatments of gamma rays were found to be greater compared to EMS treatments (Dhakshanamoorthy et al., 2010) in *Jatropha curcas*.

The effect of gamma rays on morphological characters and yield parameters in bhendi was studied M₁ generation different doses of gamma rays (10, 20, 30, 40, 50 and 60 KR). The germination percentage, days of first flower, root length, shoot length, seedling survival, number of fruits per plant, fruit length, seed yield per plant, fresh weight per plant, dry weight per plant, 100 seed weight, decreased with increasing level of gamma rays treatment and days to first flower increased with increase in dose of gamma rays (Jagajanantham et al., 2012).

Sahoo et al., (2013) reported effects of different mutagens in rice plant have immense potential for the genetic variation leading to the crop improvement and agricultural sustainability. UV mutagens in particular have huge importance in upgrading the genetic lines providing strengthened platform for the enhancement of crop productivity and yield.

**CHEMICAL MUTAGENS**

**2.6 Ethyl Methane Sulfonate (EMS)**

Ethyl methane sulfonate (EMS) has recently received much effective. The use of induced mutation has been widely accepted by plant breeders as a tool in crop improvement.
Studies on chemical mutagens received greater importance with the pioneering work of Auerbach and Robson (1946) with sulphur and nitrogen mustard gas in Drosophila. During the same period, Oehlkers (1943) demonstrated the use of ethyl urethane and potassium chloride mixture to induced mutations in Oenothera. Loveless and Howrah (1959) were first to use ethylmethane sulphonate for inducing mutations in plants. Konzak et al., (1965) have reported that ethyl methane suphonate had higher mutagenic efficiency than radiation of all the chemical mutagens, ethyl methane sulphonate (EMS) is the most widely used one in crop plants, Since it appears to be the most powerful mutagen for the induction of chlorophyll mutation in higher plants (Gaul and Asstveit, 1966). Later Auerbach (1967), Auerbach and Kilby (1971), Rahman and Sorian (1972) and Dubinin, (1976), used it. Among the numerous known chemicals, the alkylating agents have been found to be very useful for practical purposes of mutation induction in crop plants (Heslot, 1970). Swaminathan (1969) and Ashri (1988) stated mutagens like EMS and sodium azide were commonly applied for inducing mutations.

When compared with irradiation mutagenesis, EMS induced relatively few strand breaks that lead to inversion or deletion mutations (Koornneef et al., 1982). Alkalyting agents such as ethyl methane sulphonate (EMS) induced chemical modification of
nucleotides, which results in mispairing and base changes. Strong, biased alkylation of guanine (G) residues results, forming 6-ethylguanine, can pair with thymine (T) but not with cytosine (C).

Chemical mutagens are known to produce a higher rate of gene mutations generally preferred. However, chemical mutagens present particular problems such as uncertain penetration to the relevant target cells, poor reproducibility and persistence of the mutagen or its metabolites in the treated material and finally the risk of safe handling (Singh et al., 1978).

Chemical mutagenesis can be used not only to search for loss or gain of functional mutants but also to understand the role of specific aminoacid residues in protein function. Results of many studies suggest that use of chemical induced mutants can also provide useful information for understanding the functions of essential genes by generating weak non lethal alleles. In addition, EMS mutagenesis can be used for generating breeding lines (Lee et al., 2003).

Dhole et al., (2003) reported the EMS treatment in two Soybean varieties namely, JS-80-21 and Brogy, the increased variability most of the quantitative characters viz., Chlorophyll, Late flowering, tall, dwarf, densely foliate, branched, single pod in M₃ generations.
Generally, alkylating agents like EMS add ethyl, methyl and more complicated alkyl group attach to nucleic acid bases, which changes their chemical nature (Chougule et al., 2004).

Chemical mutagens have become important tool in crop improved. The mutagens are being used to produce resistance in various susceptible crops to improve their yield and quality traits to resist against harmful pathogens. There are several mutagens available for crop improvement and each mutagen has its important role producing a positive or negative effect on crops (Al- Quarainy and Khan, 2009).

A comparison of the effects of physical and chemical mutagens in sesame, EMS was the most effective treatment for inducing mutations (Tamina Begum and Tapash Dasgupta 2010).

EMS mutagenesis and this mutant resource will be highly useful in dissecting the mechanism underlying mutant phenotype (Reddaiah et al., 2014)

Elangovan and Pavadai (2015) reported that the effects of EMS and DES treatment in M₁ generation of Bhendi gradually reduced in all parameters except days to first flower to increase concentration of treatment. In M₂ populations, the significant increase of grain yields and yield component. The highest mean value for all parameters was recorded in 0.5% of EMS treatment.
2.7 Germination

All the mutagenic treatments brought reduction in seed germination, pollen fertility and survival at maturity. Such reductions, with an exception of survival, were found to be dose dependent (Samiullah Khan et al., 2004). Khan et al., (2005) and Wani (2007) reported dose related reduction in seed germination and pollen fertility by both Gamma rays and EMS.

Dhanavel et al., (2008) in cowpea and Kavithamani et al., (2008) in soybean reported the germination and survival percentage decreased with increasing dose/concentration and a field condition was observed in M₁ generation.

Growth and yield of pearl millet (Pennisetum glaucum) Downey mildew and smut diseases in Kabuga area the local variety, INMV 55 was found to be the most tolerant (Mustapha and Mustapha., 2008).

The germination in M₁ populations was less than over the control population and it was varied from 96.98% in population derived from 10kR gamma rays treatment to 10.95% in population derived due to 40kR gamma rays treatment (Singh and Balyan 2009) in Bread Wheat.

Sensitivity of rice varieties to gamma irradiation, the germination percentages of all six rice varieties were decreased after gamma irradiation treatment. But the decrease was neither
proportional to the increase in dosage nor definite pattern was found in all the six rice varieties (Sasikala and Kalaiyarasi 2010).

The alkylating agent EMS induced variability in two economically important mutants of *Vicia faba* the percentage of seed germination gradually decreased with increasing concentration of mutagens (Rubina perveen *et al.*, 2012).

Seed germination and survival of plants at maturity decreased with increasing concentration/dose of the mutagens, while seedling injury, Pollen sterility and lethality were found increased with increasing concentration/dose of the mutagens in urdbean (Bhosale *et al.*, 2013).

Avijeet Chatterjee *et al.*, (2014) in Poppy reported ethyl methane sulphonate and combined treatments in their higher doses were most effective mutagens incorporating maximum reduction in germination percentage.

### 2.8 Estimation of LD50 value

Vijendra Das (1978) irradiated dry seeds of two genotypes, HB3 (an F1 hybrid) and MS 7625, with 40, 50, 60, 70, 80 and 90kR of x-rays (50 kVp) and 10, 20, 30 and 40kR of 60Co gamma rays. The approximate M1 LD50 was 60kR for X-rays and 20kR for gamma rays.
Sasi (2004) reported LD$_{50}$ value for bhendi was 0.8 percent of EMS and 0.6 percent of DES with soaking duration of 12 hours. Jayamala (2004) observed the LD$_{50}$ value for seed germination and survival of induced mutations in green gram and observed optimum doses was found to be 0.07 percent of both EMS and DES.

Lydia et al., 2010 reported Pearl millet Okashana2 variety responded to gamma rays with a decreasing percentage survival rate when the gamma ray doses were increased. The LD$_{50}$ of 599,12 Gy dosages and LD$_{30}$ at 346,27 Gy seem to be the maximum for the pearl millet and thus the ideal doses for Okashana2 were estimated to be between 300 Gy and 350 Gy while for Kangara between 400 Gy and 500 Gy.

Thilagavathi and Mullainathan (2011) in Black gram reported LD$_{50}$ value was observed in 15mM of EMS and 25mM of DES and 60kR of gamma rays.

Ali Benjavad Talebi et al., (2012) reported the LD$_{25}$ and LD$_{50}$ values observed based on the growth reduction of seedlings after treatment that was 0.25% and 0.50% in the EMS mutagenesis, Also the LD$_{25}$ and LD$_{50}$ values occurred during 250 and 450GY of exposure for the variety *Oriza sativa* L. spp. Indica cv. MR219.
Gamma irradiation has been widely used as a breeding technique to obtain new cultivars in ornamental species such as Alstroemeria, where several cultivars have been obtained through rhizome radiation. The optimum dosage for an appropriate induction of mutation must be considered for breeding purposes and it depends mainly on plant susceptibility (Danilo Aros et al., 2012).

Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses, the optimum doses at LD$_{50}$ for genotypes Nakare and Shindimba are at 150 and 200 Gy (Horn and Shimelis 2013).

Determination of Lethal dose for Gamma rays and Ethyl methane sulphonate induced mutagenesis in Cassava, the increase in concentration of EMS, a decrease in survival, shoots length, leaf length and width. The LD$_{50}$ doses/ concentration for gamma rays and EMS were 27.5 Gy and 122mM. These optimum mutagen doses determined for the cassava genotype could be useful while formulating cassava mutation breeding programme for improvement of specific traits in cassava (Kangarasu et al., 2014).

2.9 Useful mutant types

Plant geneticists and breeders are greatly concerned with viable mutations such correlations, which are based on the chlorophyll mutations, would hardly be of practical value (Singh, 1980).
A wealth of new traits or combinations of traits is readily obtainable in mutagenized populations. Cultivars developed using induced mutants may carry improvements in a wide variety of characteristics. Mutation induction has become an established tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. Developmental studies on mutants provide useful data on the stage and time of expression of particular mutation; the response of mutant in a particular environment and the characters associated with it and also help in proper understanding of architecture of the concerned crop species (Kumar and Ramesh, 2004).

2.10 Chlorophyll mutation (Albina, Xantha, chlorine and Viridis)

Biological damage and morphological mutations in pearl millet inbreds were studies using gamma rays and EMS. EMS was more effective than gamma rays in reducing germination, seedling height, seedling survival and pollen fertility. Combination treatments showed synergistic action in the case of seedling survival and pollen fertility. Albina, chlorina, xantha and viridis were recorded in $M_2$. The frequency of chlorophyll mutations expressed as the percent of $M_1$ families segregating increased with the dose, but when expressed as percent of $M_2$ plants it decreased. Lower frequency of mutations were observed in EMS than in gamma rays which may be due to poor plant survival in the former (Singh et al., 1978).
Raveendran and Jayabalan (1997) reported in cowpea four types of chlorophyll mutants *viz.*, albino, Xantha, Chlorina and viridis were observed in M2 seedlings. Albina occurs at 60kR, while Xantha and chlorina occurred in all the treatments. Viridis found in 50kR and 60kR only. The pooled segregation showed an inconsistent trend with dosage of treatments. Viable mutants were recorded from early seedling stage to complete maturity stage. The highest chlorophyll mutation frequency was noticed in gamma ray treatment.

Geeta and Vaidyanathan (2000) reported chlorophyll mutations are easily detectable as they have been extensively used to find out sensitivity of crop plants to mutagens.

In M2 generation that EMS was more pronounced in inducing chlorophyll mutations than gamma rays and among the spectrum, the viridis (less drastic mutation) was more than that of albino (extreme mutation) as categorized by westergaard (Shah *et al.*, 2006; Deepalakshmi and Anandakumar, 2003; Singh and Mohapatra, 2004; Solanki, 2005).

Hemavathy and Ravindran (2005) reported that the occurrence of albina in the urdbean was less than the other type, when treated with different doses of gamma rays. Maximum frequency of chlorina and xantha was recorded at higher doses of gamma rays.
Arulbalachandran et al., (2007) reported gamma rays and EMS the optimum dose of mutagens gives higher chlorophyll content in black gram.

Some of the chlorophyll mutants was observed in the different dose/ concentrations of gamma rays they were chlorina, albina, xantha and viridis. Among the mutagens, morphological mutants in M$_2$ generation with effect of dose/ concentration of mutagens and such mutants were, dwarf, tall, tiny leaves, hairy leaves, male sterility, brown seed, early maturing, long pod, bottom branching, bushy type, trailing and spreading habit mutants were observed in M$_2$ generation. Mutants and its derivatives when used in cross breeding have found to be more productive in the development of improved varieties of black gram. EMS provided more number of chlorophyll and morphological mutants (Arulbalachandran and Mullainathan 2009).

Ashok Kumar et al., (2010) reported effect of physical mutagen on expression of characters in arid legume pulse cowpea gamma rays induced higher proportion of chlorophyll mutations.

Khan and Tyagi (2010) reported four types of chlorophyll mutants viz., albina, xantha, chlorine and viridis in gamma rays and gamma rays combined EMS treated population of soybean. Gamma rays were found to be more effective to induced chlorophyll mutations.
Firdose Kolar et al., (2011) reported the phenotypic response of Delphinium malabaricum to chemical mutagens (EMS and SA) and physical mutagen (gamma rays) were studied. It was observed that Delphinium malabaricum manifested specific reaction to the treatments with EMS, SA and gamma rays. Different mutation frequencies and width of mutation spectra were induced under the action of different concentrations of the applied mutagens. Eleven different types of chlorophyll mutations namely albino, albino-green, xantha, aurea, chlorine, viridis, yellow viridis, trigrina, striata, maculate and variegated types were identified. The highest frequency of chlorophyll mutations (9.74%) was reported in the 0.25% EMS. Quantitative estimation of chlorophyll pigments was also done in different kinds of chlorophyll mutants and chlorophyll content was found in the following decreasing order: chlorine > maculata > variegated > striata > trigrina > viridis > yellow viridis > albino green > aurea > xantha > albino.

Induced mutagenic frequency and spectrum of chlorophyll mutants in French bean, varieties Varun and Waghya, different chlorophyll mutation such as albina, xantha, chlorina and viridis revealed diversity in their frequencies in both the varieties. The spectrum of chlorophyll mutants in both the varieties indicated the dominance of chlorina and viridis mutants as compared to albino and xantha (Mahamune and Kothekar 2012).
The induced mutational studies in M1 generations of pigeon pea different types of chlorophyll chimeras *viz.*, yellow, light green, yellowish green and dull green (Sangle and Kothekar, 2013).

Sanjai Gandhi *et al.*, (2014) in green gram observed four types of chlorophyll mutation *viz.*, albino, xantha, chlorina and viridis. The mutation frequency increased with increase in the dose/concentration of mutagen. The chlorophyll mutant was higher in EMS than the gamma rays treated plants.

Pegah Ramezani and More (2014) in grasspea observed three types of chlorophyll mutations such as, Albino, Xantha and Viridis were induced with effect of mutagens. The highest frequency of chlorophyll mutations were observed in the combination of EMS and gamma rays. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations whether mutagens were employed singly or in combination.

Ram Narayan Ahirwar *et al.*, (2014) in lentil observed three types of mutants *viz.*, albina, xantha, viridis. The induction of albino (2) mutants was obtained only at 0.3% EMS, whereas xantha and viridis types of mutants were recorded at all the doses, maximum being at 0.3% of EMS mutagens. For induction of chlorophyll mutation 0.3% EMS alone mutagens proved to be the best option. Thus, it may be concluded that the mild doses of mutagens may be more useful to induce desirable type of chlorophyll mutants.
2.11 Viable mutation

Tall and dwarf mutants were observed in different mutagenic treatments of dose or concentration dependent. In gamma rays maximum number of mutants screened (Ramesh and Seetharami Reddi, (2002) in rice and Yadava et al., (2003) in kodo millet.

The leaf mutant, long leaf was observed in different mutagenic treatments. Among the dose or concentration maximum number of leaf mutants was recorded at 50mM of EMS treatments (Sengupta and Datta., 2005).

Sonu Goyal and Samiullah Khan (2010) in blackgram observed mutagenic treatments with ethyl methane sulphonate and sodium azide, which induced morphological mutants in the M$_2$ generation viz., dwarf, bushy and narrow leaf.

2.12 Carbohydrate

Maneemegalai and Nandakumar (2011) in *Pennisetum typhoides* reported carbohydrate content of the germinated seeds was significantly (P<0.001) decreased with the increase in the days of germination.

The values of carbohydrate obtained by Anthrone method were high in treated experimental plant parts compared to control plants (Parikh Punita and Vyas Payal 2015).
2.13 Frequency of mutation

Mutagen induced damages such as plant injury and lethality, as observable in first generation \( (M_1) \) has been showed as due to physiological, chromosomal and factor mutations. Cald cott (1961) showed a close relationship between decreases in \( M_1 \) seedlings height and chlorophyll frequency in \( M_2 \) generation.

The effects of different doses of gamma rays and EMS on formation of chlorophyll mutations in Durum Wheat, the spectrum and frequency of mutations were varied with treatments of mutagen and cultivars (Mehmet Ali Sakin and Ozer Sencar, 2002).

Khan et al., (2004) reported the frequency and spectrum of morphological mutants was relatively wide with EMS treatments followed by HZ and SA in chickpea. Venkateswarlu et al., (1988) resulted on chlorophyll mutation in \( M_2 \) generation following treatment with gamma rays and EMS individually and in combination in which results showed that the chlorophyll mutation frequency increased with increase in the dose of gamma rays and duration of chemical treatment.

Solanki et al., (2004) reported the EMS treatments induced much higher mutation frequency was increased with the increasing doses of both mutagenic treatments.
The chlorophyll mutation frequency increased increased with increasing dose up to certain limit, beyond which it exhibited a decline. This shows that a saturation point was reached at higher dose level to the rigor diplontic and haplontic selection in the irradiated materials (Kumar et al., 2007).

Gamma rays and ethyl methane sulphonate (EMS) the frequency of mutations was more in EMS than the gamma rays (Velu et al., 2007).

Frequency and spectrum of morphological mutants in Cluster bean, the mutation frequency was high in M1 plants than M2 (Velu et al., 2008).

The frequency of chlorophyll mutations increased with an increased in the concentration of the mutagens except at 20mM EMS concentrations (Tambe et al., 2010) in soybean.

2.14 Effectiveness

The effectiveness, which gives the frequency of mutations induced by a unit of dose of mutagen was found higher at higher duration of EMS either alone or in combination supporting that chemical mutagens are more effective than gamma rays (Reddy et al., 1993).

Sheeba et al., (2003) studied induced chlorophyll mutation effectiveness in sesame and noted that 40 and 60kR doses of gamma rays were found to be the most effective.
In crop improvement programme through induced mutation where treated with larger number of population are taken to estimate the plant injury and pollen sterility in M1 generation. It may guide the breeders for identifying effective treated populations in M1 generation to reduce unnecessary load of in effective populations in subsequent generation (Singh and Mohapatra, 2004).

A number of chemicals have been found to be equally and even many times more effective and efficient mutagens (Ganapathy et al., 2008, Dhanavel et al., 2008, Basu et al., 2008, Rekha and Langer, 2007 and Solanki, 2005).

Radiation induced chlorophyll mutation in sesame; the highest mutagenic effectiveness was estimated at 10kR (Ghulam Sawar and Haq, 2005).

Dose linked effectiveness of EMS and Gamma rays were noted in chickpea in terms of germination, reduction in pollen fertility, chlorophyll mutations and seedling height (Parveen 2006).

Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate (EMS), the EMS treatment was more effective than gamma rays in M2 generations (Velu et al., 2007).

Dhanavel et al., (2008) reported that the effectiveness decreased with increase in concentration of EMS, DES and SA in cowpea.
Girija and Dhanavel (2009) reported mutagenic effectiveness and efficiency increased with the decreased in dose or concentration. In EMS was proving to be more effective and efficient in causing mutations as compared to gamma rays and combined treatment.

Mutagenic effectiveness was found to be dependent upon dose and genotype concerned (Larik et al., 2009).

Kumar and Yadav (2010) in sesame reported the EMS mutagenic effectiveness increased with the increase in the dose/treatment.

Thilagavathi and Mullainathan, (2011) reported physical and chemical mutagens, among these two mutagens results showed EMS was found to be more effective and improved the yield characters compared to gamma rays treatment.

2.15 Efficiency

Efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as, injury, lethality and sterility induced by mutagen. Besides, the efficiency is a parameter, which gives highest mutation rate due to mutagenecity (Ehrenberg, 1960).

The usefulness of a mutagen in mutation breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. The response of plant genotype different physical or
chemical appears as modification in phenotypes. For the plant species these modification may be neutral or beneficial, other may be differential and may cause lethality to the organism. Therefore, effectiveness and efficiency of the mutagen and the utility of induced variability depend upon a large extent on induced lethality. For any induced mutation experiment, the selected mutagen must have specificity to act on genus, so, that high mutation rate could achieve (Reddy and Annadurai, 1991).

EMS was highly efficient than other mutagens in lentil concluded by Reddy and Annadurai, 1991. Gautam et al., 1992 reported that in general EMS treatments were found to be more efficient than gamma rays in Phaseolus vulgaris.

Pavadai and Dhanavel (2004) in soybean and Velu et al., (2008) in cluster bean reported EMS, DES and COH growth and other quantitative traits were proportionally decreased with increasing concentration of chemical mutagens in number of branches, number of seeds per plant, fresh weight, dry weight of plants, single plant yield and 1000 seed weight.

Mutagenic efficiency with gamma rays can be increased through irradiating seeds at extremely low temperature. Moreover, many findings proved that the mutation frequency obtained by the various ways can be positively influenced by specific kinds of pre or post treatment. With regard to the mutagenic efficiency, these chemicals are comparable to the physical mutagens used or better (Tah, 2006).
Efficient mutagenesis results in production of the maximum number of desirable changes accompanied by the least possible amount of undesirable change (Kumar et al., 2007).

The mutagenic efficiency was higher in gamma rays followed by EMS concentration in mustard and in lentil (Singh et al., 2007).

Thilagavathi and Mullainathan (2009) reported isolation of macromutants and mutagenic effectiveness, efficiency in black gram the EMS treatments were found more efficient in causing less biological damage and inducing maximum amount of mutations.

Higher efficiency of EMS in inducing chlorophyll mutations and higher frequency of viridis mutant was observed by Khan and Tyagi (2009) in Soybean.

2.16 Mutagenic effectiveness and efficiency

Chemical mutagen (EMS) was more effective and efficient than physical mutagen (Gamma rays) in inducing mutations. This may be due to the fact that chemical mutagens are less drastic in their effects than ionizing radiations, producing more gene mutations and fewer chromosome disruptions, secondly action of chemical mutagens may be delayed. So, that the mutation does not appear until several cell divisions have occurred. EMS has been also reported as the most effective and efficient mutagen in comparison to DES and gamma rays in rice (Kaul and Bhan, 1977).
Singh (2007) reported mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in mungbean and found that treatments of the mutagens suggesting the direct relationship with the dose dependent increase.

The effectiveness and efficiency of chemical mutagens in cowpea plant Ethyl methane sulphonate (EMS), Diethyl sulphate (DES) and Sodium azide (SA). EMS treatments were found highly effective than the other chemicals. Mutagenic effectiveness and efficiency decreased with increased in all mutagenic treatments (Dhanavel et al., 2008).

A number of chemical mutagens have been found to be equally and even many times more effective and efficient mutagen reported by Nirmalakumari et al., (2008) in Little millet and Basu et al., (2008) in Fenugreek.

The frequency and spectrum of macromutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays in two little millet varieties (CO-3 and CO(Samai)4). In both genotypes, no chlorophyll mutations were observed in the control population. The two treated genotypes behaved differently in the frequency of occurrence of chlorophyll mutations. A 500 Gray registered the highest mutation rate both on M1 panicle family basis and on M2 seedlings basis in both varieties. The spectrums of chlorophyll mutations (albina, xantha, chlorina, viridis and striata)
were observed and grouped. Except striata, remaining four kinds of mutations *viz.*, xantha, chlorine, viridis and albino were more frequently. The overall mutation spectrum for both the varieties showed that xantha (2.55%) occurred with the highest frequency, followed by chlorina (1.88%), viridis (0.84%) and albino (0.59%). The mutagenic effectiveness decreased with the increase in dose of mutagen in both genotypes, indicating that negative relationship between effectiveness and dose of mutagen. Mutagenic efficiency (mutation rate in relation to M₁ damage) in both varieties, highest at the lowest dose and it decreased with the increase in dose (Ganapathy *et al.*, 2008).

Mutagenic effectiveness and efficiency generally increased with increasing dose of EMS in chilli (Sri Devi and Mullainathan, 2011).

The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. Studies on mutagenic effectiveness and mutagenic efficiency of physical mutagen (gamma rays) and chemical mutagen (EMS) on two varieties of pea, namely, DDR-53 and DMR-55. The treatments included three doses of gamma rays (5kR, 7kR, and 10kR) and three concentrations of EMS 0.05%, 0.10% and 0.15% (Dhulgande *et al.*, 2011).
Ganesh and Umesh (2013) reported effect of mutagen on chlorophyll mutation in horse gram, the EMS treatments indicates more effective and efficient mutagen than SA and NMU.

Mutagenic effectiveness 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 treatment (Mangaiyarkarasi et al., 2014) in Catharanthus roseus.

Kaushik Kumar Panigrahi et al., (2015) reported mutagenic efficiency and effectiveness of gamma rays, Ethyl methane sulphonate (EMS), Nitrosoguanidine (NG) and their synergistic effect for different polygenic traits in black gram (Vigna mungo (L.) Hepper) through induced mutagenesis. The gamma rays and combination treatments were more desirable, low plant damage and higher genetic effects.

2.17 Genetic variability

Lower doses of Gamma rays and EMS were ineffective for creation of desired variability for yield and yield components in lentil (Singh et al., 2006)

Arulbalachandran and Mullainathan, (2009) in black gram reported genetic variability was improving quantitative traits of M$_2$ generation induced by EMS.
The generation of genetic variability by induced mutagenesis provides a base for strengthening plant improvement programs. Various classes of physical and chemical mutagens differ in their efficiency in inducing mutations and in the spectrum of mutation induced. Combination of different mutagens, if their mutagen induction process is independent and capable of interaction, should increase the mutation frequency and alter the mutation spectrum. While, ionization radiations still remain the most suitable agents for inducing genetic variability (Tah, 2006, Sangsiri et al., 2005, Joseph et al., 2004, Irfaq and Nawab, 2003, Bhatia et al., 2001).

Rafiq et al., (2010) reported that quantitative characters such as grains per row, 100 grain weight, grain row per year, earlength and eardiameter of genetic variability and nature of interrelationships should be used as target traits to improve maize grain yield.

Effect of mutagens on quantitative characters in $M_2$ and $M_3$ generations of horse gram, Both the mutagens proved to be very effective to induce variability in quantitative traits like plant height, primary branches per plant, number of days required for first flowering and first pod maturity, number of pods per plant, pod length, number of seeds per pod, 1000 seed weight and yield per plant (Bolbhat Sadashiv and Dhumat Kondiram, 2012).
Induction of variability through mutagenesis in opium poppy, broadening the genetic base through induced mutation is a supplementary tool that can lead to the development of genetic variability (Avijeet Chatterjee et al., 2012).

The mutagenic treatment with 20kR irradiated and 0.5 percent EMS treated GPBD-4 and CTMG-1 populations and $F_2M_2$ population have resulted in creation of higher genetic variations and hence serve as an excellent source material for variety development responding a more effective source of genetic variability than gene pools conserved by nature (Shanthala et al., 2013).

The success of any breeding program depends upon the genetic variation in the materials at hand. The greater the genetic variability, the higher would be the heritability. Hence the better the chances of success to be achieved through selection (Sami et al., 2013).

Sathya et al., (2013) in pearl millet studied morpho-physiological traits viz., days to 50% flowering, Plant height(cm), number of tillers, earhead length(cm), earhead breadth(cm), root length(cm), shoot length length(cm), root-shoot ratio and grain yield (g/plant).

Mutagenic sensitivity of Gamma rays, EMS and Sodium azide in fenugreek, the lower treatments of these mutagens used in the present study could be successfully enhancing genetic variability in this crop plant (Shagufta Bashir et al., 2013).
The spectrum of mutation and induced variability for various quantitative traits was observed in M₁ generation such as germination percentage, Plant height, Primary and secondary branches per plant, Days to first flowering, Fruit length (cm), Fruit girth (cm), Total number of fruits per plant, Number of seeds per fruits, Seed weights per fruit(g), 100 seed weight(g) and Pericarp (Sanjai Gandhi et al., 2014).

2.18 Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

The success of breeder in selecting genotypes possessing higher yield and growth traits depends largely on the existence and exploitation of genetic variability. Although, range can provide a preliminary idea about the variability but coefficient of variability is reliable as it is independent of unit measurement. The extent of variability as measured by PCV and GCV also gave information regarding the relative amount of variation in different populations.

Sengupta and Detta (2004a) observed that induced genetic variability has been important yield traits at M₂ and M₃ in Sesame flowering treatments with EMS and DES at 25% and 50% Concentration for 2 and 4 hrs durations, the mutagenic treatments have induced wide magnitude of genetic variability than control and same M₄ populations have shown it in positive direction.
Deepalakshmi and Anandakumar (2004) recorded high PCV and GCV value in plant height, number of primary branches per plant, number of clusters per plant, pods per plant, pod length per plant and seed yield per plant in black gram with effect of gamma rays and EMS.

The variability analysis such as phenotypic and genotypic coefficient of variation, heritability and genetic advance showed at 60 kR than 40 and 80 kR of gamma ray treatment. It could be regarded as an indication of additive gene action due to gamma ray treatment (Sreelathakumary and Rajamony, 2008).

The PCV and GCV expressed in terms of percent points were comparatively high at 60 kR gamma rays for plant height, branches/plant, leaves/ plant, clusters/plant, pods/plant, seeds/pod, yield/plant (g) and 100 seed weight (g). This closer magnitude suggested that greater role of variability due to the induction of gamma rays at genetic level. The PCV and GCV were high at all the quantitative traits including yield in sorghum bicolor and these characters having possessed better potential for crop improvement (Unche et al., 2008).

The maximum GCV was present for the grain yield/plant indicates that simple selection for yield may be advantageous as compared to its components under study. Several characters viz., number of pods/plant, number of seeds/pod and 100 grains weight showed high degree of GCV recorded in pea (Kumar, 2008).
The PCV and GCV estimates were found to be high for seed yield potential followed by green fodder yield potential, panicle length, spike density, thousand seed height and total number of tillers, which suggests that there is enough scope for selection based on these characters. The high heritability combined with high genetic advance was observed for plant height, total number of tillers, panicle length, panicle thickness, spike density, 1000 seed weight, green fodder yield potential and seed yield potential which showed these characters were controlled by additive gene effects and phenotypic selection for these characters were likely to effective (Kumari Vinodhana et al., 2013) in Pearl millet.

2.19 Heritability (h²) and Genetic advance (GA)

Satish et al., (2003) noted that high heritability for all the characters such as, days to 50% flowering, Plant height, Panicle number per plant, Panicle length, number spikelets per panicle, number of grains per panicle, 1000 grains weight, grains length and grains yield per plant in rice cultivars. Similarly, genetic advance in percent of mean was high for number of grains per panicle, number of spikelets per panicle and grain yield per plant in rice.

Deepalakshmi and Anandakumar (2004) observed high heritability and genetic advance as percentage of mean in plant height, number of primary branches per plant, number of clusters per plant, number of pods per plant, pod length and seed yield per plant in black gram with different dose/concentrations of EMS.
High heritability estimates obtained for days to first flower, duration of reproduction phase, 100- seed weight, days to maturity and harvest index suggests that these characters are highly heritable and therefore the traits can be easily transferred parent to offspring. Secondary traits are very valuable in selection for improved cowpea grain yield (Omoiguil et al., 2006).

Kalpande et al., (2008) reported high heritability for plant height, seed cotton yield per plant, number of bills per plant, number of sympodia per plant and average boll weight in F$_3$ generation of cotton. It indicated that predominance of additive gene action.

Samiullah Khan and Sonu Goyal (2009) reported heritability along with genetic advance would be helpful in assessing the nature of gene action. High genetic advance, as percentage of mean, was noticed for fertile branches per plant and pod per plant. Both these traits also had high heritability, which indicates that expression of these traits is governed by attentive gene action and as a result, there is scope of improving these traits through selection procedure. Low genetic advance with moderate heritability was observed for total plant yield. It shows that this trait is most probably governed by non additive gene action.

The wide range of variability for different traits coupled with high heritability and high genetic advance for important yield traits hence selection is effective for these traits. The gamma rays played a key role in crop breeding through mutation (Arulbalachandran et al., 2010).
Sanjai Gandhi et al., (2012) in black gram reported the high values of heritability and genetic advance indicate the possibility of inducing desirable mutation for polygenic traits accompanied by effective selection. It indicated that genotypic coefficient of variation and phenotypic coefficient of variation and heritability were significantly high for different characters i.e. Plant height, number of branches per plant, days taken for first flowering, Clusters per plant, Pod per plant, Seed per pod, Seed yield per plant, 100 seed weight, protein and amino acid content.

2.20 Cytology

The physical mutagens have been most effective in breaking chromosomes and producing various chromosomal aberrations. Chromosomal rearrangements have been induced in pearl millet with a number of chemical and physical mutagens (Pantulu (1967), Burton and Powell (1966), Hanna and Young (1974), Jauhar (1974, 1981), Tyagi (1976) and Lal and Srinivasachar (1979).

Viswanathan (1996) worked out on wheat and Triticale with reference to cytogenetics and induced mutation. Biological effects of laser and $^{60}$CO gamma rays on $M_1$ plants of groundnut by Huang-Bozhani et al., (1997) showed chromosomal aberrations in root tip cells, the frequency increased with treatment dosage. Laser treatment induced chromosome adhesion and gamma radiation induced chromosome rings.
Anis et al., (2000) observed the effect of EMS, SA and MMS on various cytological parameters in $M_2$ generation in Capsicum annum, EMS caused a greater reduction in chiasma frequency. The mean value of rod bivalent, Univalent, fragments/bridges, cytological abnormal cells and pollen sterility were found to be increase in mutagen treated population.

Fragments at metaphase may due to the failure of broken chromosomes to recombine. Fragment might have arisen due to the stickiness of the chromosomes and the consequent failure of the arrival of chromatids at the poles. Fragments may also be acentric chromosomes formed as a result of inversion (Agarval and Ansari, 2001).

Arslan et al., 2001 has reported that induced mitotic abnormalities by gamma range in sunflower, variety EKIZ1. They also noted decreased in the mitotic index of the $M_0$, $M_1$ and $M_2$ generations, increased in the total abnormality percentage in the $M_0$, $M_1$ and $M_2$ generations with the increased dose of radiation. They reported mitotic abnormalities, lagging chromosomes, ring, chain and rod chromosomes. Kumar and Sharma (2001) observed that the seeds of Cicer arietinum with treatment of 0.5% aqueous solution of EMS for 4, 6 and 8 hrs during the study of meiosis, 2-desynaptic mutants were isolated at 8 hrs of treatments.
The thick sticky bridges may be due to the stickiness chromosomes. This stickiness interfered in the normal arrangement of chromosomes at metaphase and further led to their inability to separate, thus leading to sticky bridges. When the spindle fibres pulled the chromosomes towards the poles these bridges were broken in to fragments, which either moved towards the poles or formed laggards and micronuclei (Kumar and Singh, 2002).

Chromosome and chromatid type aberrations can be detected microscopically in metaphase cells. Most compound that cause gene mutations also induced chromosome aberrations. In plants, mainly meristematic root tip cells are used for chromosome aberration experiments. The analysis of anaphase cells provides additional information on the origin of chromosome aberrations-breaks; laggards and bridges can be identified at this stage of the cell cycle. The evolution of chromosome aberration is quite time consuming and is facilitated by the use of indicator species that have a small number of large chromosomes. Species, which fulfils these criteria include Tradescantia sp., *Crepis capillaries*, *Vicia faba* and *Allium cepa* (Uhl *et al.*, 2003).

Sharma and Kumar (2004) observed that meiotic abnormalities increased along with the increase concentration in EMS treatment for two cultivars of *Cicer arietinum*. 
Chromosome cluster, fragments, laggard, chromatin bridges and micronuclei were observed as the effects of physical and chemical mutagens (Kumar and Singh, 2004).

Cytological and developmental effects of gamma rays, EMS and MMS on meiotic features and pollen fertility in *Vicia faba* L. was studied by (Bhat *et al.*, 2005).

A wide spectrum of mutations has been induced in maize by a number of different sources in addition to chemicals, namely gamma- laser and ultraviolet radiation, $^{40}\text{Ar}$, $^{56}\text{Fe}$, $^{20}\text{Ne}$, HZE particles, fast and thermal neutrons, tissue culture and X-rays (Grant and Owens, 2006). They have been used to chemical and physical agents in maize for studying most of the cytological end points in both somatic and meiotic cells. These included lagging chromosomes, acentric chromosomes, bridges, fragments, inversions, translocations, spindle disturbances, micronuclei and multipolar anaphases.

Zaied *et al.*, (2007) reported that mutation is any heritable alteration in genetic material that includes diverse phenomena as change in the number of chromosomes, changes in the structure of chromosomes and changes within the genes themselves. The changes in the number and structure of the chromosomes are of considerable importance to the evolutionary geneticist and to the plant breeder. Point mutation always occurs within a gene and it
should be the smallest possible changes in structure of the genetic material that is detectable as a mutation. Point mutation involving a change in a single nucleotide base pair (the smallest possible change) and a large change involving a small number of adjacent nucleotides.

Bhat et al., (2007) reported that chromosomal bridges may also be due to the chromosomal stickiness and subsequent failure of anaphasic separation or may also be attributed to unequal translocation or in origin of chromosomal fragments. Lagging chromosomes may be explained on the basis of abnormal spindle formation and failure of chromosome movement. Mutagen may have caused chromosomal breakage by binding to DNA at GC rich regions and making the DNA unstable and hence formation of fragments and laggards. Bridges and laggards with (or) without fragments were found both at anaphase and telophase, bridges without fragments were found at higher concentrations of the mutagens, both single and double bridges were found but the multiple bridges were not also rare. Multiple bridges were mostly found at anaphase and the single bridges and telophase.

Velu et al., (2007) reported the effect of gamma rays, ethyl methane sulphonate (EMS) and sodium azide (SA) on chromosomes during meiosis in Vigna radiata showed aberrations like univalent, trivalents, multivalent, ring chromosomes and laggards. The frequency of cells showing chromosomes aberrations gave a linear increase with dose. EMS produced the highest chromosomal abnormalities.
Girjesh Kumar and Kumar Rai (2007) reported the EMS treatment, many chromosomal abnormalities, namely precious movements, Stickiness, Univalents, Bridges, Laggards, Multivalents etc., were induced in all the inbred lines of maize. Higher frequencies of chromosomal abnormalities were observed maximum dose (7h) of treatment in all the inbred lines of maize.

Sonu Goyal and Samiullah Khan (2010) in blackgram mutagenic treatments with ethyl methane sulphonate and sodium azide chromosomal abnormalities, such as univalents and stickiness at metaphase-I, bridges and laggards at anaphase-I and cytomixis was noticed at telophase-II. The induction of meiotic aberrations was higher in ethyl methane sulphonate than sodium azide treatments.

Sri Devi and Mullainathan (2011) reported frequency of chromosomal aberrations increased linearly with EMS test concentration between 10 and 50mM and decreased above 50mM which showed significant difference between control and 50mM EMS or higher test concentration.

Alka et al., (2012) studied different types of meiotic abnormalities as laggards, bridges, chromosome stickiness, stray chromosomes, precocious movement of chromosomes and multinucleate conditions. The meiotic abnormalities increased along with the increased in concentration in mutagens. The maximum frequency of abnormalities both structural and behavioural was induced by EMS in Linum usitatissimum.
Cytological effect of Ethyl methane sulphonate and Sodium azide in *Linum usitatissimum*, the induction of meiotic aberrations was observed to be higher in EMS and SA treatments. The EMS could be more effective in inducing additional variability than SA. The meiotic abnormalities increased along with the increase in concentration in mutagens (Ambreen Akhtar, 2012).

Gamma rays and ethyl methane sulphonate chromosomal aberrations are precious movement, Stickiness, bridges, fragments, laggards etc. As increased in the concentration, the frequency of cells showing chromosomal aberrations shows a linear increase up to certain level. Compared to gamma rays, EMS produced the highest chromosomalous aberrations (Sanjai Gandhi *et al.*, 2014)

### 2.21 RAPD (Random Amplified Polymorphic DNA)

Chowdari *et al.*, (1998) reported Pearl millet, the cultivars were developed from a narrow gene pool. Wild Pennisetum species offer diverse germplasms that can be used to improve pearl millet, and useful characteristics for this purpose are disease and insect resistance, genes for fertility restoration of A1 cytoplasm, cytoplasmic diversity, QTLs for yield, apomixis, maturity and many inflorescence and plant-morphological characteristics. At present, no serious attempt has been made to study the primary, secondary and tertiary gene pools in Pennisetum using DNA markers. (GATA)4 and RAPDs could be of great value both for exploiting the wild
germplasm during introgression breeding programs and in the identification of varieties having good combining abilities, without the evaluation of \( F_1 \) hybrids in the field, in order to predict heterotic combinations.

Erdem and Oldacay (2004) reported that radiation is one of the best known physical mutagens. It dissociates the atoms of water molecules and causes the generation of hydroxyl radicals that are the most reactive. They react with most of the biomolecules including DNA and scavenge the electrons from them. The oxidation of biomolecules by the radicals damages the DNA structure and biological activity. Hence, genetic alterations occur on the DNA molecules, which is the cause of mutations depends on radiation.

Sghaier ZIDANI et al., (2005) studied genomic DNA extraction method from pearl millet (\textit{Pennisetum glaucum}) leaves using CTAB method.

Govindaraj et al., (2009) reported 20 pearl millet genotypes randomly selected from germplasm collection were used for this study. 30 oligonucleotides were screened to identify polymorphism markers, out of which 12 oligonucleotides have clear and reproducible banding patterns. To assess the reproducibility of the profiles the same template DNA was amplified in 3 different amplification reactions using the same primer. The results were highly comparable. Only strong and reproducible bands were
considered for further analyses. The 12 primers produced 99 polymorphic bands with an average of 8.25 polymorphic bands per primer polymorphic bands generated by primers OPE 04 and OPE 18 respectively.

Molecular characterisation of the 15 early duration finger millet (*Eleusine coracana* G.) genotypes was used RAPD markers. Twenty-five decamer primers were used for initial screening. Of these nine primers (OPA4, OPA13, OPA16, OPC12, OPC18, OPD8, OPN7, OPN15 and OPN16) showed polymorphic banding pattern for the genotypes. Total number of bands produced ranged from 2 to 9 bands per primer. The nine primers produced 60 amplification products, of which 9 were monomorphic and 51 were polymorphic. The primers OPA4, OPC18 and OPN15 produced a greater number of polymorphic bands than OPA16 and OPN16. Polymorphism ranged from 50 to 100%. Primers OPA13, OPC18 and OPN15 revealed 100% polymorphism, whereas OPA16 and OPN16 showed 50% (Das *et al.*, 2009).

Babaei (2010) reported that Nemat rice seeds were exposed to different doses of gamma radiation and 15 selected genotypes in M2 generation along with check were calculated for genetic diversity using RAPD molecular marker. The number of fragments produced by various primers ranged from 200- 2100bp. The number of fragments produced by various primers ranged from 3- 15 with an
average of 6.5 fragments per primer. Maximum 15 bands were amplified with primer OPH20 and minimum 3 bands were amplified with primer OPH14 and the best primer was OPH11. On the basis of RAPD and dendrogram resulted, M₂ genotypes were classified in 5 groups regarding their similarities and distinctness.

The genetic variation was evaluated with 20 random primers, generated total 202 fragments scored with 58 polymorphic alleles, and the average was 10.1 alleles per locus and a range of 1-9 alleles. The average polymorphic rates were 38.37 among the mutants and parents through the 20 primers. Primers OPA-14 and OPI-04 revealed 35% of DNA polymorphism. The genetic distance (GD) among the genotypes was 0.19 suggesting a significant degree of genetic diversity. The five genotypes were used to construct a dendrogram based on the similarity matrix, revealing a genetic distance varying from 0.600 to 0.725. This variation was due to the mutation induced by gamma rays and Ethyl methane sulphonate (Arulbalachandran et al., 2010).

Fahriye Sumer Ercan et al., (2012) reported the RAPD technique could be used to improve identification and to better understand the genetic polymorphism of Trichogramma spp.

Ganapathi et al., (2008) studied the effect of gamma irradiation on banana using RAPD-DNA analysis. They observed changes in the DNA bands, where the main changes in the RAPD
profiles of the present investigation were the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages. Thus, the variation in band intensity and disappearance of some bands may correlate with the level of photoproducts in DNA template after radiation which can reduce the number of binding sites for Taq polymerase. Appearance of new bands is usually result from different DNA structural changes (Breaks, transpositions, deletion etc) (Danylchenko and Sorochnsky, 2005).

Wendt et al., (2001) who used the RAPD markers to study the effect of gamma radiation on potato cultivar Macaca concluded that treatment with 500 Gy promoted the highest variation in genetic distances. RAPD-DNA analysis showed that the offsprings of the five irradiated Chamaecrista spp cultivars were genetically different from the control (GuoZhong et al., 2007).

Mullainathan et al., (2014) reported ten primers were used to screen the polymorphism among the treated populations line tall, tall with chlorophyll deficient, leaf, flower, GMS and DNA damage in maturity mutants were analysed with control. Out of ten primers, four primers (PGF12, PGF03, PGF04 and OP107) were successfully amplified in all the samples.