II. Review of Literature

2.1. Induced mutations

Induced mutations have been used mainly to improve particular characters in well-adapted local varieties or to generate variation difficult to be found in germplasm collections. Mutant traits commonly exploited are: earliness, semi-dwarfness, disease resistance, higher yield and better quality, and these have lead to new cultivars (Maluszynski et al., 1995). The vast majority of induced mutations are recessive and as a consequence are normally detected and selected in the second or third (M2 and M3) generation after mutagenic treatment.

About 70% of mutant cultivars were released as direct mutants, i.e., without any further breeding; the remaining 30% were developed through cross-breeding programmes and mutants or mutant varieties served as a source of desirable alleles (Maluszynski et al., 2000). Mutation techniques have contributed significantly to plant improvement worldwide, and have made an outstanding impact on the productivity and economic value of some crops (Maluszynski and Szarejko, 2005).

Mutation breeding

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. Genetic variation among genotypes and relationships between major yield contributing traits/characters is of vital importance to breeding programmes that aim to produce important cultivars in crops like green gram. Mutation induction with radiation was most frequently used methods to develop direct mutant varieties, as improvement by acclimatization, selection and hybridization have proven to be time consuming, laborious with limited genetic variation (Yahoob and Rashid, 2001).

2.2. Choices of mutagens

Elliot (1958) described that physical mutagens namely X-rays, gamma rays, fast neutrons, thermal neutrons, ultraviolet and beta radiations have been frequently used for induced mutagenesis. Except ultraviolet rays, all radiation types were found to ionise atoms in a tissue by detaching electrons from the atoms (Anonymous, 1977).

Szarejko and Forster (2007) recorded that chemical mutagens are available for mutagenic treatment of crop plants. Nevertheless, several chemical mutagens have been applied of which EMS, NMU, ENU and sodium azide are the preferred agents in plant mutation induction. Alkylating agents are the most important chemical mutagens used in mutation breeding. They
added ethyl or methyl groups to bases in the nucleotide structure, which lead to activating a silent gene or altering a particular gene action (Snustad and Simmons, 2006).

**Physical mutagens**

Radiation has been successfully used for the development of new flower colour/shape mutants in *Dendranthema*. Therefore, induced mutagenesis through irradiation or chemical treatment has become a very important method for plant breeding, including flower breeding. IAEA (2004) analysed that 2335 varieties were released through mutagenesis in the world, in which ornamental crops and decorative crops are 552 varieties. While, India has a particularly impressive share as it has commercially released 46 mutant cultivars in *Chrysanthemum* alone in year 2004 (Chopra, 2005).

Irradiation-induced mutation breeding is effective in improving sweet potato characters such as yield, starch and soluble sugar content, carotenoid content of storage roots and disease resistance (Wang et al., 2007). Irradiation has also been successfully used for mutation breeding in various crops and ornamental plants and has proven an adopt means of encouraging the expression of recessive genes and producing new genetic variations (Song and Kang, 2003).

Batan (2006) pointed out that gamma ray irradiation at low dose levels or (micro mutation) is less influencing changes in quantitative characters of
plants and chromosomes compared with the macro mutation using gamma ray irradiation at high doses. Mutation induction can be done on the plants by mutagenic treatment of certain materials of plants such as seeds, stem cuttings, pollen, root rhizome, tissue culture and others.

**Chemical mutagens**

Chemical mutagenesis is a simple approach to create mutation in plants for their improvement of potential agronomic traits. Mutations are the tools and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu et al., 2007). Mutation methodology had been used to produce many cultivars with improved economic values by (Bertagne-Sagnard et al., 1996).

Singh et al. (2001) analysed the quantitative characters in the M2 generation such as number of pods per plant, number of seeds per plant, hundred seed weight and yield per plant were higher in EMS treatment in green gram than the control. Similar results were reported by Dhole et al., (2003) in plant height, number of branches per plant, number of pods per plant, 100 seed weight (g) and seed yield were increased with effect of EMS in soybean on M2 generation. Among different concentrations was showed high quantitative mean performance in M2 generation. This was confirmed with earlier reports on legumes with effect of different mutagen. Improved
quantitative traits namely, plant height, number of branches per plant, hundred seed weight and plant yield with effect of EMS in M$_2$ generation of chick pea by Wani and Anis (2001).

### 2.3. Seed germination and seedling survival

Falque (1994) described that the decrease in chilli germination with increasing dosage could be attributed to the occurrence of seeds without completely developed embryos. The main cause of germination reduction in plants has been attributed to the occurrence of seeds without developed embryos. The fact that chilli seeds which were treated with low gamma rays grew better than those exposed to higher doses and this was so because irradiation may cause a block in cellular DNA, hence causing plant growth to stop or slow (Mokobia and Anomohanran, 2004). The survival rate of the control plants was certainly higher because their seeds were not irradiated with gamma rays. At a certain level of radiation, the plant can grow at early stage of growth and they cannot survive at certain duration probably due to DNA breakage and inability to repair them. Plants exposed to 600 Gy for example started to grow well at the early stage but they could not survive till 34 days after planting.

Bewley and Black (1985) elucidated that the seeds were good explants to create mutations in a genome of a cell. After treatment of chemical mutagens, seeds show the effects of mutagen as modified morphological traits
from disturbed physiological processes. Germination is the process by which a seed initiates growth after a period of quiescence. Seed germination was scored based on the appearance of the cotyledons, plumule and radicle. Evidence of these structures signified the last stage of seed germination.

Yuan (1992) reported that chemical mutagens affect the germination process in seeds. The percent of germination in seeds depend on the nature of the mutagen and its treatment dose. Many of these mutagens have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals. Van der Veen (1966) studied that the chemical mutagen generally produced induced mutations. That lead to base pair substitution resulting in amino acid changes, which change the function of proteins, but do not abolish their functions as deletions or frame shift mutations mostly do. These chemomutagens also induced a broad variation of morphological and yield structure changes when compared to normal plants.

Chrispeeds and Varner (1967) had explained the seed germination in mutagenic treatments due to delay or inhibition of physiological and biological processes necessary for seed germination which include enzyme activity, hormonal imbalance and inhibition of mitotic process. The inhibitory effect of sodium azide on germination could be anions which were strong inhibitors of cytochrome oxidase, which in turn inhibited oxidative phosphorylation (Kleinholds et al., 1978). In addition, it was a potent inhibitor of the proton pump that altered the membrane potential of mitochondria. These effects
together may hamper ATP biosynthesis resulting in decreased availability of
ATP which may slow the germination rate and reduce the germination
percentage (Zhang et al., 2000).

The mutagenic agents (gamma rays, fast neutrons and EMS) affected
seedling height, reducing it with increasing dosage. Based on the mutagen
damage on seedling height, the LD_{50} and LD_{30} values for 15 sunflower
genotypes were estimated for the three mutagens. Retardation of growth due to
the mutagenic treatments has been used to determine the dose rate for
mutation induction. It is the most functional parameter to be used in radio-
biological investigations because it was generally considered to be a result of
primary injury due to nuclear DNA damage. Sensitivity in seedlings height
had been demonstrated in earlier dose response studies of bean Cheah and Lim
(1982), soybean (Koo et al., 1972) and other crops.

Raut et al. (1982) described that the germination percentage and
survival percentage in variety PKV-1 and JS-335 studied during M_{2} and M_{3}
generations exhibited similar kind of results as observed in M_{1} generation
soybean plants. But the magnitude of reduction was very less, particularly in
M_{2} and M_{3} generation.

2.4. Estimation of LD_{50} value

Optimal dose can be defined as the dosage leading to adequate genetic
variation accompanied by the lowest plant lethality (Snustad and Simmons,
2006). Mutagen dose, treatment period and their interaction can be considered as the main factors also influenced by pre-treatment, temperature, pH, and post-treatment (Hu and Rutger, 1992). Lethal dose 50 (LD$_{50}$) is generally used as a criterion to define the optimum mutagenic dose. Bacelis (2001) investigated the effects of different concentrations of EMS, ENU and NMU on variability of two flax varieties and reported 0.025% ENU, 0.012% NMU and 0.3% EMS as their optimal doses. Patil et al. (2011) also introduced 0.1 to 0.2% EMS concentrations as optimum dosages to induce maximum variations in soybean populations. Fowler and Stefansson (1972) evaluated EMS for mutagenesis in rapeseed (Brassica napus L.) and observed that increasing EMS concentration from 0 to 1.0% adversely affected germination percentage, plant vigour and seed yield. Germination test is an indication of the potential of a seed lot to emerge under field conditions.

2.5. FIELD OBSERVATION

The predominance of the albino type over viridis, in the spectrum that resulted from the treatment with physical and chemical mutagens, is not consistently shown in the literature. Ando (1970), found a larger albino rate using ethylene oxide (EO), Ethyleneimine (EI) and EMS, as well as gamma-rays. Similar results were reached by Miah and Awan (1971) with neutrons and gamma-rays and by Rao (1977) with EMS and gamma-rays. On the other hand, Kaul and Bhan (1977) found a higher albino rate in one of the two varieties studied; nevertheless, in a third variety, a larger proportion of viridis
was found. Finally, Rao and Rao (1983) produced higher proportions of albinos by applying MMS, NMU and HA.

**Viable mutations**

The mutants found mainly of leaf chlorophyll mutation such as albino, light-green leaf, variegated leaf, waxy leaf, white streak leaf and xantha leaf. Leaf mutations were lanceolate leaflet, narrow rugose leaflet, multiple leaflet, round-cuneat leaflet, unifoliate leaf and wrinkled leaf. Flower mutation gave looks like cock’s comb with pollen sterility. Similar mutants were reported by Lamseejan *et al.* (1983). A lobed pod mutation with fewer seeds per pod was also found. This trait may associate with partial sterility, causing constriction at the point where there was undeveloped seed. These mutants were not found in the control populations.

The morphological mutants common in the M₂ generation are tall, erectoid, unifoliate, dwarf, bushy, trailing, clustered pod sterility etc. The inheritance of these morphological mutants in the M₃ generations indicated that these are monogenic recessive in nature (Saini and Mahna, 1989). Tall mutant with high leaf areas and yields were selected in the M₂ and M₃ generations and supposed to have a substantial effect on the seed yield with greater leaf area (Chow *et al.*, 1987). Some of the other qualitative traits included leaf mutants (dark green, waxy, multiple and lobed), pod mutants (large and top podding) and semi-dwarf plants (Srinives and Hual-alai, 1999).
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Talukdar et al. (2001) described that visible morphological dwarf mutants can be generated by inducing polyploidy with chemical mutagens like colchicine. Trait dwarfism can be obtained in the $M_2$ generation and generally exhibit a slower growth rate and reduced plant height from early flower seedling stage until maturity. Internodes of a dwarf mutant can be winged and the main axis can be perpendicular to the soil. The orientation of early-formed branches may also be found very close and inclined to the main axis compared to the control. Dwarf mutants can be late in initiation and days to 50% flowering, however it can mature earlier compared to the control. The total number of branches, flowering nodes, pods per plant and seed yield per plant decreased significantly, but the number of seeds per pod, pod size, hundred seed weight and harvest index decreased marginally.

Quantitative characters in $M_1$, $M_2$, $M_3$ and $M_4$ generations

Plant yield was found to be associated with the increase in the number of fertile branches, pods and hundred seed weight. A high degree of correlation between number of pods per plant and number of fertile branches was reported earlier by Khan and Siddiqui (1997) in mungbean and Kharkwal (2003) in chickpea.

Yield per plant was found to have significant positive correlation with number of pods per plant and fertile branches per plant. These findings are in conformity with those reported by Vijayalakshmi et al. (2000) in chickpea.
100-seed weight was not correlated with yield per plant in the present study. However, Amaranath et al. (1990) and Singh and Singh (1999) reported positive correlation of 100-seed weight with total plant yield in soybean. Seed protein content of the mutants did not alter significantly in any of the EMS treatments studied. However, the coefficient of variation was higher in mutagenic treatments as compared to control, indicating that improvement in seed protein content is possible. Seed protein did not show any association with yield per plant, indicating the independent genetic control of protein content and total plant yield. Sandhu et al. (1979) suggested that the lack of association between grain yield and grain protein can be easily combined in a single genotype.

The selection of high-yielding potential mutant lines was made under low water supply conditions. Selection of individual plants was done every generation from M₂ to M₅ under field conditions, taking into account the following visual criteria: healthy plants, determinate growth habit, greater number of fruits per plant or bigger fruit compared to the parental variety. Yield per plant, fruit number per plant, average fruit weight, equatorial and polar fruit diameters were recorded in individually selected plants. The best genotypes were chosen by means of multivariate and genetical distances (Sigarroa and Cornide, 2002), according to the highest-yielding genotypes.

Singh and Kole (2005) assessed using morphological traits in M₇ generation of soybean mutant lines by gamma irradiation. It can be inferred to
the results that irradiation induce significant genetic variability in the majority of studied traits such as number of nodule per plant and harvest index. Arulbalachandran et al. (2010) reported that increase in studied traits in mutant lines may be attributed to chromosomal damages. Also separation of mutant lines from parent cultivar showed gamma rays effect in creating genetic diversity that resulted into separate mutant lines from parent cultivar. According to the means of studied traits in each group mutant lines groups (second, third and fourth groups) in comparison with parent group (first group) had highest value. Since these traits are most important agronomical traits such as yield and yield components traits therefore relevant mutant lines can be selected from segregated group.

**Chlorophyll mutants**

Frequency and types of the chlorophyll mutations albino, albina-terminalis, xantha, aurea, chlorina, and chlorotica varied in different mutagens. Germinated albino mutants were completely devoid of chlorophyll and could survive only a few days. Albina-terminalis had normal green leaves up to the fifth node and were then totally white to the apex. Aurea (golden yellow-coloured seedlings) and xantha (pale yellow-coloured seedlings) mutants could not survive more than a few days due to a block in chlorophyll synthesis (Blixt, 1961). Several types of chlorophyll deficiencies were noted as light coloration of leaves (chlorotica) and yellowing of the leaf (chlorina), which persisted throughout the growing period and weak plants were produced due
to low photosynthesis efficiency (Ladygin et al., 2004). Frequency of chlorophyll mutants increased as irradiation and chemical mutagen doses increased. Frequency of the xantha-type mutation was higher than chlorina and albino mutants. Frequency of albino-terminalis, aurea, and chlorotica mutants were very low.

**Effectiveness**

Mutagenic effectiveness reflected rate of mutation in relation to mutagen dose, whereas mutagenic efficiency was the mutation rate in relation to lethality or biological injury. The usefulness of a mutagen depended both on its effectiveness and efficiency, efficient mutagenesis being production of maximum desirable changes accompanied by the least possible undesirable changes. Mutagenic effectiveness was a measure of frequency of mutations induced by unit dose of mutagen, whereas mutagenic efficiency was indicated the proportion of mutations as against undesirable biological effects such as gross chromosomal aberrations, lethality and sterility (Konzak et al., 1965).

Dhanavel et al. (2008) revealed that the degree of effectiveness and efficiency varied between different mutagens and also between the two varieties. Lower or intermediate dose treatments proved to be more effective and efficient the decrease in effectiveness at higher dose treatments may be attributed to the failure in proportional increase of mutation frequency with the increase in dose/conc. of the mutagens. The higher efficiency at lower and intermediate doses of mutagens as observed in the present study might be due
to the fact that the biological damage increased with an increase in dose at a rate greater than the frequency of mutations (Konzak, 1965). Greater effectiveness and efficiency of lower or intermediate treatments of chemical mutagens alone or in combination with gamma rays has also been reported (Khan et al., 2005).

According to Konzak et al. (1965), the reason for greater efficiency of lower doses/treatments is due to the fact that the biological damage such as injury, lethality and sterility increases with the increase in mutagenic treatments at a faster rate than the mutations. In other words lower or intermediate dose/concentration cause relatively less damage enabling the organism to express the induced mutations successfully. It could be well stated here that physical mutagens have been exploited to a greater extent for inducing mutations in crop plants and majority of the varieties released through induced mutations belong to physical mutagens. However, some genotypes in crop plants respond more to chemical mutagens than physical ones and in such genotypes appropriate dose/conc. followed by efficiency handling of the mutagenized population could yield better results in terms of economic traits like yield, adaptability, protein content etc.

Efficiency

Mutagenic efficiency is the production of desirable changes which are free from associations with undesirable genetic alterations. This is generally
measured by the proportion of the mutation frequency in relation to damages associated to mutagenic treatments such as: height reduction, chromosomes breakages, sterility, lethality, etc. (Konzak et al., 1965 and Gaul et al., 1972). The possibility of inducing new variability by mutagenic agents is, therefore, of great interest to genetic improvement. This interest becomes even greater when associated with the possibility of assessing qualitatively and visibly the increases in mutation frequency through chlorophyll mutations.

Gustafsson (1963) believed that ionizing radiations produce high frequency of albino mutations while the chemical mutagens produce other types of chlorophyll mutations in cereal crops. But, in chickpea, the frequency of four types of chlorophyll mutations was much higher in the EMS treatments in four genotypes than gamma irradiation. Some other studies have also reported that EMS induces a wide spectrum and high frequency of chlorophyll mutations as in mungbean (Khan et al., 2005) and barley (Gaul, 1964). The comparative superiority of chemical mutagens over gamma rays producing a higher frequency and spectrum of chlorophyll mutations suggest that the chemical mutagens are more efficient in inducing mutations of genes needed for chlorophyll development. Swaminathan et al. (1962) proposed that such high frequency is due to the preferential action of EMS on chlorophyll development genes located near centromere. The results also indicate that in spite of poor efficiency of gamma rays in producing certain type of chlorophyll mutations, the chemical mutagen was more specific in inducing
certain type of chlorophyll mutations. Higher frequency and a wider spectrum of chlorophyll mutants in chemical mutagen have been reported by Bhattacharya (2003).

Kharkwal (1998) reported dose dependent decrease in the frequency of chlorophyll mutations with gamma irradiation. For chemical mutagen higher frequency of chlorophyll mutations with low doses of mutagens was observed in Pb2000, Pb-1 and CH40/91 by Yadav (1987). It seems that the strong mutagens reach their saturation point even at lower doses in the highly mutable genotypes and further increase in dose does not add to the mutation frequency. With increase in dose beyond a point, the strong mutagens become more toxic than the higher doses of relatively weaker mutagens (Gustafsson, 1947).

2.6. Biochemical analysis

Dale et al. (1997) reported that there was a significant, though relatively small interaction between potato cultivars and irradiation level. It showed slightly higher reduction in chlorophyll synthesis with increased levels of gamma irradiation. Moreover it has also been reported in Citrus that non-irradiated plantlets demonstrated the highest amount of chlorophyll content as compared to irradiated (10-50 Gy) plantlets (Kiong Ling et al., 2008).
Arulbalachandran (2006) observed significant increase in protein content in black gram using gamma rays, EMS, DES and colchicine. Similarly, Mercykutty et al. (1990) reported a positive shift of mean values in total seed protein content of autotetraploid components to their respective diploid in pea with effect of colchicine.

Kiong Ling et al. (2008) reported that the total soluble protein content in irradiated and non-irradiated plantlets and observed that plantlets irradiated at high doses (30, 40 and 50 Gy) displayed a higher total soluble protein content and relatively low doses (10 and 20 Gy) caused a reduction of total soluble protein content.

2.7. Genetic variability

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.
Ranjan Jalgaonkar et al. (1990) observed that phenotypic coefficient of variation (PCV) was higher in magnitude than the genotypic coefficient of variation (GCV) for all the characters. The close resemblance between the corresponding estimates of both PCV and GCV in almost all the characters suggested that the environment had little effect in those characters expression. The GCV provides a measure to compare genetic variability present in various quantitative characters. The highest value of it was recorded for total branches per plant, moderate for days to branching and seeds per inflorescence and lowest for total flowers per plant. Flower diameter (cm) showed no GCV because genotypic variance was negative. The higher value clearly indicated high degree of genotypic variability in these quantitative traits in *Dianthus caryophyllus*. PCV which measured total relative variation was highest for total branches per plant, seeds per inflorescence, leaf area, days to branching and seed germination, lowest for flower diameter (cm), total number of flowers per plant and plant height (cm) at 50% flowering phase. Similar result was reported for plant height (cm) by Pathania et al. (1988). High values of GCV suggest better improvement scope for these traits by selection. However, the estimation of heritable variation with the help of genetic coefficient of variation alone may be misleading. Burton (1952) suggested that the genetic coefficient of variation together with heritability estimates gave the better picture of the extent of heritable variation.

Heritability (H²)

Singh (1991) recorded that high heritability for plant height at 50% flowering phase and total leaves per plant, moderate heritability for days to branching, total branches per plant, seeds per inflorescence and total flowers per plant, lowest days to seed germination and leaf area. Flower diameter showed negative value due to its genotypic variance. High heritability combined with high genetic gain as per cent of mean was observed for total leaves per plant and total number of branches per plant. This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance, hence amenable for simple selection. High heritability with moderate genetic gain as per cent of mean was recorded for plant height at 50% flowering, days to branching and seeds per inflorescence indicating that these characters were governed by additive gene interaction. High heritability coupled with low genetic gain as
per cent of mean was recorded for total flowers per plant indicating non-additive gene action for these traits.

Mensah and Obadoni (2007) determined that high heritability, coupled with high expected gains were observed for number of pods per plant and number of seeds per plant indicating that additive gene effects played an important role in the expression of such traits. Thus, these traits could be effective in the selection of high yielding cultivars/genotypes. The characters in which heritability has already been reported among legumes include plant height, pods per plant, 100-seed weight and seed yield.

Patel and Shah (1982) reported that cluster per plant and days to flowering showed high heritability associated with moderate heritability was noted for number of pods per plant, seeds per plant, 100-seed weight, yield per plant and seed protein content in urdbbean. Rao et al. (2003) recorded high heritability for leaf area, nodule number, nodule weight, plant dry weight in Phaseolus with the effect of EMS.

**Genetic advance (GA)**

Basak and Ganguli (1996) indicated by high genetic advance for flag leaf angle in rice. Kole and Hasib (2003) reported low genetic advance in days to first flower, number of panicle, length of panicle in aromatic rice. In M₃ generation showed a considerable increase in GCV, heritability and expected
genetic advance in the treated population of mungbean as reported by Wani and Khan (2006).

**Genetic advance as per cent of mean (GA %)**

Genetic advance as per cent of mean under selection in the M₂ populations varied with treatments and characters studied. It also increased in the treatments and it was relatively higher for different quantitative characters studied. The genetic advance as per cent of mean was highest for grain yield per plant followed by pods per plant, 100-seed weight and leaves per plant. While, low genetic advance was observed for seeds per pod, number of branches per plant, days to maturity and plant height in lentil (Dixit and Dubey, 1985). Similar differential estimates of genetic advance in different mutagenic treatment populations for different traits have also been reported by Kalia et al. (2001).

Deepalakshmi and Ananadakumar (2004) observed high heritability and genetic advance as per cent 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/conc. of EMS.

**2.8. Cytological analysis:** The effect of chemical mutagens to study the induction of chromosomal aberrations in various crop plants such as cereals (Smith, 1946), Chickpea (Shamsuzzaman et al., 1994). Natarajan (1983) stated
that genotoxic agents induce chromosomal alterations, such as aberrations, micronuclei and sister chromatid exchange as well as mutations both \textit{in vivo} and \textit{in vitro}. Ionising radiation and typical radiometric agents such as bieomycin are efficient inducers of chromosomal aberrations.

Bhaskaran and Swaminathan (1962) put forward an investigation of the effect of X-ray and fast neutrons on the mitotic aberrations of diploid, tetraploid and hexaploid wheat and diploid and tetraploid barley. There is an increase in chromosome aberrations along with the increase in doses; the frequency of aberration was highest in the hexaploid wheat and least in the diploid wheat.

Kleinhofs \textit{et al.} (1978) described that the chromosomal damages may be the prominent causes of reduced seed germination and decreased yield as compared to control. The reduction in germination percentage might have been due to the effect of mutagen on meristematic tissues of the seed. The mutagenic treatments also delayed the germination process and the initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth, ATP and DNA synthesis.

Different mutagen induced different types of structural changes, like fragments, bridges etc. (Bose and Banerjee, 1977). Mikaelson \textit{et al.} (1968) observed fragments anomalies with EMS induction. Jana \textit{et al.} (1974) described inhibited chromosomes with broken chromosome with Maleic
hydrazide. Bridges at anaphase and telophase stages observed on somatic cells
of wheat under ethylene glycol treatment were reported by Kalloo (1972),
Singh and Godward (1974). Anaphase bridges were observed in Vicia faba
with different types of chemicals by Gopalan and Ngagi (1984). Due to sticky
nature of chromatin bridges found at anaphase and telophase stages (Jain and
Sarbhoy, 1987) and that is also caused due to mutagenesis.

2.9. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
(SDS-PAGE)

Seed protein patterns obtained by electrophoresis have been
successfully used to resolve the taxonomic and evolutionary relationships
among crops and their wild relatives (Das and Mukarjee, 1995).

Ghafoor et al. (2005) stated that the seed protein obtained in several
sequences where pattern differences enabled discrimination between various
genotypes of black gram through SDS-PAGE analysis in gamma rays
treatments. Similarly, Anitha et al. (2008) generated a total of 22 bands and in
which polymorphisms were observed mostly in regions between 14-38 kDa in
black gram.

2.10. RAPD

Random Amplified Polymorphic DNA (RAPD) analysis has been used
for describing levels and patterns of diversity and genetic structure in a
number of plant species (Nebaure et al., 1999, Bartish et al., 2000). RAPD
markers have also been used to determine evolutionary relationships and
levels of genetic variation among wild and cultivated populations of a number of crop species, including *Capsicum* spp. (Rodríguez *et al.*, 1999).

According to Danylchenko and Sorochinsky (2005), RAPD molecular marker method is applicable for the detection of changes in the DNA structure after different genotoxical treatments (radiation). 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 in some cases can be explained as a result of different DNA structural changes (breaks, transpositions, deletions, etc). Thus, the estimate on the existence of mutation and structural alterations in plant DNA after impact of radiation on the bases of DNA patterns could be obtained after RAPD markers with the set of primers. A similar opinion was reported by Wachira *et al.* (1995).

Floria (2002) detected little natural genetic variability in tomato. However, using induced mutation it has been possible to increase the genetic variability and obtain new tomato genotypes. Based on the large differences between the selected mutants and the donor variety in their agronomic, morphological and biochemical characteristics, together with the RAPD analysis, it can be concluded that R16-300, R20-300 and R4-300 genotypes are beneficial mutants and that mutation induction can be used effectively to obtain drought tolerant tomato varieties.
The RAPD analysis of the M₃ generation plants exposed to gamma rays and EMS produce clear difference from the untreated control, thus indicating that mutagenic treatments produce polymorphic regions in the paprika mutant. This may be due to the reason that the variation might be stabilized upon consecutive generations. Erdem and Oldacay (2004) recommended that radiation is one of the best known physical mutagens. It dissociates the atoms of water molecule 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 in turn damages the DNA structure and biological activity.