CHAPTER -2

REVIEW OF LITERATURE
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Review of Literature

A brief review of important literature of various aspects particularly related to induced mutagenesis of chickpea (*Cicer arietinum* L.) and other economical important crops has been presented in this chapter.

2.1. Origin, biosystematics and cytogenetics

The domesticated chickpea (*Cicer arietinum* L.) is one of the seven Neolithic founder crops (other crops being einkorn wheat, emmer wheat, barley, lentil, pea and bitter vetch) and the wild progenitors of all of them are found together in the core area of the Fertile Crescent – a place near the upper reaches of the Tigris and Euphrates rivers in present-day southeastern Turkey/northern Syria (Lev-Yadun et al., 2000). It is also believed that cultivated chickpea have originated at least 7000 years ago in south eastern Turkey and adjoining areas of Iraq, Iran and former Soviet Union (Ramanujam, 1976) with *Cicer reticulatum* L. being the wild progenitor (Ladizinsky and Alder, 1976). Some believe that *C. reticulatum* is sub-species of *C. arietinum* L. (Singh and Singh, 1997). Earlier botanists had postulated several different origins. De Candolle (1883) traced the origin of chickpea to an area south of Caucasus and in the north of Persia. Vavilov (1926) recognized the Mediterranean, Central Asia and Indian regions as the probable centers of origin of chickpea. There is linguistic evidence that the large seeded chickpea reached India via Afghan capital, Kabul, about two centuries ago and acquired a name in Hindi as ‘Kabuli Chana’ (van der Maesen, 1972 & 1987). The small seeded chickpea is called ‘desi’ (local) and these denominations are commonly used to distinguish the two main groups of the cultivars. The earliest record of chickpea in India is from Atranji Khera in Uttar Pradesh and this dates back to 2000 B.C. (Chowdhury et al., 1970). It was introduced in Peninsular India probably between 500 and 300 B.C. (Vishnu-Mitre, 1974).
The genus *Cicer* belongs to the family Fabaceae and the tribe *Cicereae* (Iruela et al., 2002). However, some argue that it belongs to tribe *Vicieae* (Singh et al., 1997). The genus consists of 44 species of which 35 are perennial and nine are annual (van der Maesen et al., 2007; Toker, 2009), including the cultivated one.

Chickpea (*C. arietinum* L.) is a diploid and self-pollinated annual pulse crop known to have somatic chromosome number to be 2n=16 (Singh and Singh, 1997; Bharadwaj et al., 2010) with genome size 1C=740 Mbp (million base pair). There are reports of 2n=14 chromosome number, but presumably such plants of *Cicer* are rare and may not be able to maintain themselves in nature (Singh et al., 1997).

### 2.2. Induced mutagenesis

The concept of utilizing induced mutations in breeding new forms was advocated by Hugo de Vries (1901). For the first time a conclusive proof that ionizing radiations can induce mutations was reported by Muller (1927) when he succeeded in inducing certain variations in *Drosophila*. One year later Stadler (1928), while working on the effect of X-rays in maize, reported that it was possible to obtain very high mutation rates through irradiation. Ganger and Blakeslee (1927) did the same in *Datura stromonium* and Goodspeed (1929) in *Nicotiana*. Gustafsson (1947) reported effective and pioneering mutation work on the production of useful mutations in crop plants. Following Gustafsson's work, review and research analysis by several workers (Gaul, 1965; Brock, 1965; Kharakwal, 1996; Khan and Rehman, 1999; Chhun et al., 2003; Ilbas et al., 2005; Talame et al., 2008; Toker, 2009; Goyal and Khan, 2010a; Nakagawa et al., 2011) added an exhaustive information that has been generated on the role and application of induced mutations in several crop plants and were taken up by many breeders all around the world.

As the dawn of the “Atomic Age” following World War-II nuclear bombing, with devastating effects on the populations of two cities of Japan (Hiroshima and Nagasaki), great efforts were made to use atomic energy for peaceful purposes and by 1950, a number of projects on mutation induction research were initiated to use
radiations (physical mutagens) in agriculture research in various countries, using mainly radioactive isotopes of $^{32}$P and $^{35}$S, X-rays, alpha particles, beta particles, fast and thermal (slow) neutrons, ultra violet rays and gamma rays to induce mutations. A great number of experimental results showed that radiation effects could be modified and as a consequence major scientific efforts were devoted for a number of years to search for radiation treatment conditions or additional treatments (before or after the irradiation) that could eventually alter the “random” mutation induction into something more targeted, more specifically desirable and more economically useful (Micke, 1995).

Gamma rays are electromagnetic radiations with the shorter wavelength (shorter than X-rays), i.e. they represent electromagnetic radiations with the highest energy level. Gamma rays are generated from radioactive decay of some elements like $^{14}$C (Carbon-14), $^{60}$Co (Cobalt-60), radium etc. Of these, $^{60}$Co is commonly used for the production of gamma rays and are commonly used in mutation breeding. The great penetrating power of gamma rays makes them dangerous as they can cause considerable damage when they pass through the tissue (van Harten, 1998). During the process of irradiation and ultimately penetrating matter, these high energy rays liberate electrons in collision with atoms of different molecules, leaving positively charged free radicals or ions (van Harten, 1998). These ions, in turn, collide with other molecules, causing the release of further electrons. The net result is that a “core” of ions is formed along the track of each high-energy ray as it passes through matter or living tissues.

In the beginning, mutation breeding was based primarily upon X-rays, gamma rays, thermal neutrons and radio isotopes of certain heavy elements, however, the discovery of chemical mutagens is an important event in the history of plant breeding. It was 1930’s two chemicals, iodide and copper sulphate, that were known to act as a weak mutagens on *Drosophila* and during the World War–II the mutagenic activity of urethane was demonstrated (Donini and Sonnino, 1998). However, the first elaborate report was presented by Auerbach and Robeson (1942) who showed that mustard gas
could induce mutations as well as chromosomal breaks in *Drosophila*. Now it is well known fact that, in addition to several ionizing radiations, a number of chemical mutagens induce mutations in plants, when applied singly or in combination with other chemicals, and successively or simultaneously with physical mutagens (Konzak *et al.*, 1965; Ahloowalia and Maluszynski, 2001; Saleem *et al.*, 2005; Encheva, 2009).

Though there are several unanswered questions regarding the classification and mechanism of action of mutagens, yet more comprehensive account of them was given earlier by Sharma (1985) and later by van Harten (1998), Micke (1999) & Kodym and Afza (2003). The nature, essential properties and mode of action of physical and chemical mutagens has also been reviewed by Gottschalk (1978 a,b), Gottschalk and Wolff (1983a), Kaul (1989), Crueger (1993), Khan (1997), Kaul and Nirmala (1999), Siddiqui (1999), Ahloowalia *et al.* (2004), Al-Qurainy and Khan (2009), Goyal and Khan (2010a) & Nakagawa *et al.* (2011) which has fairly widened and enriched our knowledge on fundamental aspects of the mutational process and the possible mechanism of action of various physical and chemical mutagens.

Experiments on higher plants have shown that chemical mutagens, apart from easy handling and better efficiency, have much greater advantage and specificity than ionizing radiations due to a milder effect on the genetic material of a cell as against the physical mutagens which break the chromosome (Auerbach, 1965; Handro, 1981; Salnikova, 1995). Rapoport (1966) discovered overwhelming majority of strong chemical mutagens which are being used widely in genetic and breeding research. The chemical mutagens can be divided into three groups, viz. A) alkylating agents like ethyl methanesulphonate (EMS), methylmethane sulphonate (MMS), ethylethane sulphonate (EES), ethylene imines, diethylsulphate (dES), nitroso compounds; B) base analogues like 5-bromo uracil, 5-bromo deoxyuridine; and C) others like azides (sodium azide), antibiotics, acridines, nitrous acid and hydroxylamine. The speed of hydrolysis of the chemical mutagens is usually measured by the half life of the chemicals. Half life is the time required for disappearance of the half of the initial amount of active reaction agent. The preferred mutagens belong to the class of
alkylating agents. Alkylating agents (AA) are potent mutagens and can be classified broadly into monofunctional and bi- or polyfunctional ones, depending upon the number of alkyl groups present in the compound (Natarajan, 2005). Alkylation refers to the substitution of an alkyl group (e.g. $C_2H_5$ of EMS) for hydrogen in the nitrogenous bases (Sharma and Chopra, 1994). As per the works of Haughn and Somerville (1987), Ashburner (1990), Siddiqui (1999) & Natarajan (2005) the alkylation of DNA leads to the following effects:

(i) **Alkylation of the phosphate groups of DNA:** Alkylation leads to the formation of phosphate triesters which are unstable and release the alkyl group. However, if enough alkyl groups remain unreleased, then the attached alkyl groups interfere with DNA duplication. Sometimes the phosphate triester is hydrolyzed between the sugar and the phosphate and results in the breakage of the DNA backbone.

(ii) **Alkylation of bases:** The seventh position in the guanine is a preferred site for alkylation but it has been established that the major mutagenic effects arise from $O^6$ alkylation of guanine. $O^6$ alkyl-guanine can pair with thymine and leads to base pair transition.

(iii) **Depurination:** The alkylated guanine can separate from the deoxyribose leaving it depurinated. The gap can be filled up by any base during DNA replication leading to transversion or transition type of mutation.

The mutagenic action of ethylmethane sulphonate (EMS) on various morpho-physiological and biochemical parameters was studied earlier in *Drosophila* (Fahmy and Fahmy, 1957), bacteriophage (Loveless, 1959), *Escherichia coli* (Strauss and Rosemarie, 1964), *Arabidopsis* (Greene et al., 2003), *Lycoopersicon esculentum* (Saba and Mirza, 2002), soybean (Karthika and Subbalakshmi, 2006), *Jatropha curcas* (Dhakshanamoorthy et al., 2010), *Vigna* spp. (Kozgar et al., 2011), barley and wheat (Gustafsson, 1960; Ehrenberg, 1960; Swaminathan et al., 1962) and other cultivated crops of human importance. EMS is now being widely accepted as a powerful
mutagen and is used commonly in the induction of mutations in various crop plants because of its ability to induce a high frequency and wide spectrum of mutations (Swaminathan et al., 1962; Hussein et al., 1974; Khan et al., 1998; van Harten, 1998; Barro et al., 2001; Perry et al., 2003; Hohmann et al., 2005).

Since chemical mutagens have been proved to be more potent and efficient in inducing mutations than physical ones (Sharma, 1965; Blixt and Mossberg, 1967; Kharkwal, 1998 a,b), henceforth, they have become the method of choice for genetic studies and remain popular even with the advent of new technologies (Jain, 2002; Greene et al., 2003; Perry et al., 2003).

With the discoveries of mutagenic effects of radiations and chemical mutagens, combined treatments of them were used by the breeders in order to increase the mutation frequencies. Mehandjiev (2005) reported that the combined treatments of physical and chemical mutagens induced a wider mutation spectrum, which is of great significance to the experimental mutagenesis. He further suggested that the combined treatments enhance quantitative and qualitative changes in the spectrum of mutations, thus increasing or decreasing the frequency of the particular mutation types which do not occur in the spectra of the individual treatments. van Harten (1998) suggested that combined treatments would be even more attractive if synergistic effects would occur, either in that way that mutation frequencies should reach levels beyond the sum of both individual treatments, or if “unique” mutations should arise in this way. Various reports have been put forward which confirm the usefulness of combination treatments of physical and chemical mutagens for the improvement of crop varieties due to the results of superior mutation frequencies as compared to those of single treatments like in Glycine max (Patil et al., 2004; Khan and Tyagi, 2010a), Lens culinaris (Reddy and Annadurai, 1992), Lycopersicon esculentum (Zeerak, 1992a), Papaver somniferum (Chauhan and Patra, 1993), Vigna unguiculata (Girija and Dhanavel, 2009), Vigna mungo (Goyal and Khan, 2010b), Vigna radiata (Grover and Tejpaul, 1982; Singh, 2007a), Vicia faba (Bhat et al., 2007) and Brassica napus (Siddiqui et al., 2009). There are also many reports of the formation of different types of mutants with
different attributes produced through combination treatments of different types. Some of the noteworthy ones are an early maturing mutant variety “Boriana” of soybean, produced in M₂ by the combined treatments of gamma rays and EMS, which has a vegetation period 30 days shorter than the parental variety (Gecheva, 1983), mutant variety of sea buckthorn (*Hippophea rhamnoides*) with high yield and an increased content of the medicinal oil when combination treatment of 150 Gy of gamma rays and 0.01% nitrosomethyl urea were given (Privalov, 1986), mutant variety “Biser” of soybean - a highly productive and resistant to lodging - was produced by combined treating seeds of the “Beeson” variety with gamma rays and EMS (Mehandjiev, 2005) and semi dwarf mutants of oats (*Avena sativa*) were obtained when seeds were treated with 0.1% and 0.2% EMS for 2 hrs, followed by treatment with SA for 1 hr (Konzak, 1993), androgenic double-haploid mutants in barley after treatment with SA (sodium azide) to anther and microspore culture (Castillo *et al.*, 2001) and rice mutants with different genetic lesions after treated with different chemical and physical mutagens (Wu *et al.*, 2005).

2.2.1. **Dose effect and genotypic sensitivity**

Plant materials differ in sensitivity to mutagenic treatments. According to Acquaah (2007), it is difficult to find the precise dose but careful experimentation can identify an optimum dose rate. Overdose kills too many cells and often produces crippled plants, whereas underdose tends to produce few mutants. Many workers feel that a dose close to lethal dose-50 (LD₅₀) should be optimum. It is that dose of the mutagen which would kill 50% of the treated individuals. Solanki and Waldia (1997) are of the opinion that an optimum dose is the one which produces maximum frequency of mutations and causes minimum killings. van Harten (1998) also reported that it is better to perform a ‘seedling growth test’ with a range of doses to determine the optimal treatment conditions for a specific cultivar. Since, the genetic architecture of an organism is an important factor in determining the genotypic difference towards mutagens, henceforth, the LD₅₀ of a particular mutagen for a particular genotype varies greatly (Khan, 1990). Inter-varietal differences with regard to LD₅₀ in pulses were
reported by several workers like Khan (1988) in *Vigna mungo* & Kharakwal (1981 a,b) in *Cicer arietinum*. Khan *et al.* (1998) studied the mutagenic effect of maleic hydrazide (MH) in two varieties of *Vigna radiata* and found the var. PS-16 to be more sensitive than the var. K-851. Dose linked effectiveness of the mutagens like EMS and gamma rays, in terms of germination, reduction in pollen fertility, chlorophyll mutations and seedling height, were noted in peas (Salim *et al.*, 1974); *Pennisetum glaucum* (Singh *et al.*, 1978), *Vigna radiata* (Singh and Chaturvedi, 1980; Khan and Wani, 2004), *Lablab purpureus* (Kamau *et al.*, 2011). Khamankar (1984), while working on tomato plant, showed that the rate of mutation was different with different physical and chemical mutagens at certain loci as some of the gene loci were affected by one mutagen but not by the other. This type of differential sensitivity of genes to different mutagens is of considerable interest and pointed out that the mutation process with the chemical mutagens may be different from radiations. The effective dose of any mutagen in an individual crop is also varying. It has been found that polyploid species are slightly resistant to the action of mutagens than their diploid ones (Reddy *et al.*, 1991). Although, it is difficult to pinpoint the precise and exact cause for the differential sensitivity of genotypes to different mutagenic doses but several possible explanations have been put forward by several workers. Akbar *et al.* (1976) concluded from their studies in rice that the differences in radiosensitivity among rice varieties may be due to the difference in their recovery process involving enzyme activity.

With a view to enhance the mutation rate and also to alter the spectrum of mutations, many variations in treatment methodology have been tried (Chopra and Pai, 1979). Treatments of different mutagens have been given to dry as well as soaked seeds, bulbs and corms of different species, seedling at different developmental stages, at variable temperature and ionic concentrations of chemical mutagens. In general, the dose of mutagen comprises several parameters of which the most important are properties of mutagenic agent, duration of treatment, biological system in question and the environmental conditions.
2.2.2. Bio-physiological damages

The variations in terms of bio-physiological damages, gene mutations and chromosome mutations induced by mutagens in any mutation breeding programme have been used as criteria in determining the mechanism of action of the mutagen in question and also the sensitivity of the biological material towards the mutagenic treatments. Among the variations caused, gene and chromosomal mutations may be transferred from M₁ to the subsequent generations, whereas, biological and physiological damages are generally restricted to the M₁ generation.

It is possible to identify plants which suffered maximum damage due to mutagenic treatment using different types of parameters and in different experimental layout, either individually or in combination, of M₁ generation plants. Different parameters like seed germination, seedling height, plant survival at maturity, pollen and seed fertility, cytological abnormalities, aberrations on leaf surface, estimation of chlorophyll and biochemical contents, the activities of certain enzyme assays etc. have been studied by different workers in analyzing the biological and physiological changes in M₁ generation due to mutagenic treatments, both in laboratory and in field conditions. The effects of single and combination treatments of physical and chemical mutagens on different biological and physiological parameters in M₁ have been reported in Oryza sativa (Fujimoto and Yamagata, 1982; Sarawgi and Soni, 1994; Cheema and Atta, 2003), Vigna radiata (Khan, 1990; Sharma et al., 1995; Khan et al., 1994; Khan et al., 1998; Rehman et al., 2000; Khan and Wani, 2005a), Vigna mungo (Gautam et al., 1992), Triticale (Edwin and Reddy, 1993a), Brassica juncea (Singh et al., 1993), Triticum spp. (Xiuzher, 1994), Eleusine coracana (Kumar et al., 1996), Plantago ovata (Sareen and Kaul, 1999), Lens culinaris (Reddy et al., 1992; Verma et al., 1999), Capsicum annum (Siddiqui and Azad, 1998; Dhamayanthi and Reddy, 2000), Gossypium hirsutum (Muthusamy and Jayabal, 2002), Stipa capillata (Zaka, et al., 2002), Cicer arietinum (Barshile et al., 2006; Hameed et al., 2008), Solanum melongena (Alka et al., 2007), Euryale ferox (Verma et al., 2010), Lathyrus sativus
(Kumar and Dubey, 1998b), *Nicotiana tabacum* (Amarnath and Prasad, 1998) and many such examples are being reported for other crops of economic importance.

Brunner (1995) in *Vicia faba* reported that M1 parameters as seedling height, survival and fertility decreases with increasing doses of gamma and fast neutron radiations while chlorophyll and morphological mutant frequencies in segregating M2 population increase up to a maximum and decrease thereafter due to M1 injury.

Different workers have proposed different parameters as an important indicator to determine the mutagenic actions for specific plants by studying the biological and physiological damages. Konzak *et al.* (1972), Joshua and Bhatia (1983) & Kumar and Mani (1997) proposed that seedling height is a quick and simple method used as an index in determining the biological effects of various mutagens in M1 generation. Kodym and Afza (2003) observed that germination is not a good indicator for an effective mutagen dose. Blixt (1972) in *Pisum* & Solanki and Sharma (1999) in *Lens culinaris* found that seedling damage (leaf aberrations) to be the most effective index among all M1 parameters, whereas, Gautam *et al.* (1992) in *Vigna mungo* reported that there is a direct relationship of pollen and ovule sterility with higher doses of gamma rays and EMS. Mutagenic efficiency based on injury and lethality was found higher in combined treatments of gamma rays and NMU (N-nitroso-N-methylurea) than their respective individual treatments (Dixit and Dubey, 1986a). Combined treatments also showed greater reduction in seedling survival than the individual treatments. Lal *et al.* (2009) studied mutagenic effect of gamma rays and sodium azide and their different combinations in blackgram in M1 and observed that an increase in azide concentrations resulted in decrease in M1 germination. The plant survival was also affected with different doses of gamma rays and SA and was decreased with increasing doses. The combination treatments of gamma rays and sodium azide had more depressive effect on seedling growth. Singh and Chaturvedi (1980) reported mutagen induced damage, such as plant injury and lethality, in M1 generation arises due to physiological and chromosomal mutations. Verma *et al.* (2010) suggested that the alteration in chlorophyll contents in M1 generation of makhana (*Euryale ferox*) due
to gamma irradiation is a vital index in determining the mutagenic action. Gamma irradiation induces various physiological and biochemical alterations in the plants, like carbohydrate metabolism (Joshi et al., 1990; El-Fiki et al., 2003), photosynthetic activities (Kim et al., 2005; Seung et al., 2007; Moussa and Jaleel, 2011) and interference in the nucleic acid metabolism (Stoeva et al., 2011; Kovalchuk et al., 2001). The effect of the mutagens on the protein contents and the nitrate reductase activity (NRA) has also been reported by Barshile et al. (2009) in Cicer arietinum and Kozgar et al. (2011) in Vigna species.

2.2.3. Cytological aberrations

Induction of mutation in plant species is often associated with cytological abnormalities (Reddy, 1990). Cytogenetic information and the degree of cytological aberrations, either in mitosis or meiosis, is regarded as one of the dependable criteria used by plant mutation breeders for estimating the effect of mutagens on the crop plants and to manipulate chromosome segments or whole individual chromosome or sets of chromosomes to solve particular problem. Swaminathan et al. (1962) reported that the chromosomal mutations leading to the formation of non-functional gametes are the most common effect of mutagen induced sterility with reduced reproductive capacity. They further reported that the spectrum of meiotic chromosomal abnormalities is broad during diakinesis-metaphase and includes high proportion of univalents, moderate frequencies of multivalents, stickiness of chromosomes and non-orientation of bivalents. Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities (La Fleur and Jalal, 1972; Smith and Murphy, 1986; Consolaro et al., 1996; Kumar and Rai, 2007 & Kumar and Gupta, 2007).

In higher plants cytological abnormalities due to single and combination treatments of mutagens have been extensively reported by several workers (Swaminathan et al. (1962) & Kumar and Singh (2003) in Hordeum vulgare; Kalloo (1972) in Pisum sativum; Chowdhury and Nirmla (1976), Reddy (1990) & Edwin and

Different types of meiotic abnormalities like chromatin bridges, laggards, fragments, cytomixis, inversion, micronuclei and unequal separation of chromosomes were reported in pearl millet following treatments with gamma rays and EMS (Laxmi *et al.*, 1975). They further reported that gamma rays were more effective than EMS or combination treatments in inducing chromosomal abnormalities. Chromosomal abnormalities and disturbed meiotic behaviour at different stages like sticky chromosomes, fragments and ring chromosomes at metaphase and the laggards and bridges at anaphase were reported by Grover and Tejpal (1982) in *Vigna radiata* using gamma rays, MH and their combinations. Misorientations at metaphase, bridges at anaphase, fragmentation and multinucleolate conditions were observed by Shah *et al.* (1992) in *Vigna mungo* using gamma rays. The chromosomal aberrations were found to be significantly correlated with dose and the combined treatments enhanced chromosomal aberrations. Kumar and Gupta (1978) observed an induced asynaptic mutant of blackgram which had shown a large number of univalents and an irregular anaphase division.

A relative account of cytological effects of diethylsulphate on meiotic features and pollen fertility in *Vicia faba* was provided by Fatma and Khan (2009). There is a close relationship between pollen sterility and meiotic abnormalities as has been

2.2.4. Chlorophyll mutations

There are two important goals of mutation research viz., the enhancement of mutation frequency and alteration of mutation spectrum in a predictable manner. In the past, different approaches have been tried to achieve these goals (Gustafsson, 1963; Nilan, 1967; Swaminathan and Sharma, 1968; Chakarbarti, 1975; Maluszynski, 2001; Mehandjiev et al., 2001). The scoring of chlorophyll mutation frequency in M$_2$ generation is one of the most reliable measures for evaluating the mutagenic induced genetic alterations of the mutagen treatments used on the plant ideotype (Chaturvedi and Singh, 1990). Chlorophyll deficient chimeras in M$_1$ generation and their segregation in M$_2$ generation are often observed in a mutagenized population. Several authors have reported the occurrence of different types of chlorophyll mutations such as albina, xantha, chlorina, viridis, virescent, tigrina etc., in M$_2$ generation following treatments with various mutagenic agents in different crop species. Some of the noteworthy examples are of Dahiya (1973) & Wani et al. (2011b) in mungbean; Venkateswarlu et al. (1978) in pigeonpea; Venkateswarlu et al. (1988) in Catharanthus roseus; Prasad and Das (1980), Bawankar and Patil (2001) & Waghmare (2001) in grasspea; Dixit and Dubey (1986b), Reddy et al. (1993) & Paul and Singh (2002) in lentil; Arora and Kaul (1989) in Pisum sativum; Anwar and Reddy (1981), Reddi and Suneetha (1992) & Yamaguchi et al. (2006) in rice; Mohanasundaram et al. (2001), John (1999) & Girija and Dhanavel (2009) in cowpea; Singh et al. (1999) in urdbean; Prakash and Shambulingappa (1999) & Devi et al. (2002) in rice bean; Szarejko and Maluszynski (1999) in barley; Deepa and Devi (2000) in winged bean; Kumar et al., (2003) in limabean; Toker and Cagirgan (2004a), Khan et al. (2005a), Barshile et al. (2006) in chickpea & Khan and Tyagi (2009a) in soybean. The frequencies of chlorophyll mutations in different crops with different mutagens have been found to be markedly different. Chemical mutagens induce higher frequency of
chlorophyll mutations than radiations (Blixt, 1972; Filippetti et al., 1977; Sharma and Sharma, 1981; Tripathi and Dubey, 1992; Vandana et al., 1994; Kharkwal, 1998b; Singh et al., 2000a; Waghmare and Mehra, 2001; Karthika and Subbalakshmi, 2006; Lal et al., 2009; Arulbalachandran and Mullainathan, 2009a). Among the chemical mutagens, EMS was reported to induce higher frequency of chlorophyll mutations. The superiority of EMS in inducing chlorophyll mutations at a higher frequency was advocated by Swaminathan et al. (1962), Muntzing and Bose (1969), Hussein et al. (1974), Reddi and Suneetha (1992), Shah et al. (2006), Wani et al. (2011b). The frequency of induced chlorophyll mutations have also been reported to be of different in different mutagen treatments. Ionizing radiations generally produce a higher proportion of albina mutations than chemical mutagens (Swaminathan et al., 1962; Ando, 1970; Gupta and Yashvir, 1975; Subramanian, 1980; Cheema and Atta, 2003; Karthika and Subbalakshmi, 2006). However, Hemavathy and Ravindran (2005) found that the occurrence of albina is less compared to the chlorina and xantha in gamma rays treated population of Vigna mungo.

The use of combination treatments of physical and chemical mutagens alter the mutation frequency and spectrum (Arnason et al., 1963; Favert, 1963) and has gained a great momentum, in its usage, in the recent past in mutation breeding programmes. Chemical mutagens when combined with radiation are not only mutagenic themselves but also affect mutation in specific ways (Reddy and Smith, 1984). Singh et al. (1999) in Vigna mungo & Khan and Tyagi (2009a) in Glycine max has shown that the combined treatments are more effective in inducing chlorophyll mutations compared to individual treatments of gamma rays and EMS. In barley combination treatments of gamma rays and EMS showed synergistic effect on chlorophyll mutation frequency (Sharma, 1969a; Khalatkar and Bhatia, 1975). Similar observations of synergistic effects were made on rice treated with thermal neutrons and dES (Rao and Ayenger, 1964) and on black gram in the combination treatments of gamma rays and EMS (Gautam et al., 1992). In some cases, the antagonistic effects have also been reported in the combined treatments of gamma rays and EMS (Arora and Kaul, 1989).
However, in general, most of the mutagens given in combination exhibit synergism in legumes and cereals.

There is a strong indication that total mutation frequencies and spectrum are associated with the dose of mutagen (Hussein et al., 1974; Sarma et al., 1979; Reddy and Gupta, 1989; Reddy and Revathi, 1991; Venkatachalam and Jayabal, 1993; Singh et al., 1999; Amarnath and Prasad, 2000; Das and Kundagarami, 2000). However, this claim was repudiated by some workers (Pipie, 1972; Khan, 1990; Yamaguchi et al., 2009) who found no relationship between the dose of mutagen and the mutation spectrum. According to Vo Hung (1974), Khan and Siddiqui (1993), Kaul and Bhan (1977), Khan (1986), Reddi and Suneetha (1992), Raveendran and Jayabal (1997), Mitra and Bhowmik (1999) & Ganapathy et al. (2008), the highest dose is not always the most effective treatment.

2.2.5. Mutagenic effectiveness and efficiency

Mutagenic effectiveness and efficiency are two different properties which are important in mutation breeding programmes. Knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations (Smith, 1972; Kumar and Mani, 1997). Mutagenic effectiveness is a measure of the mutations induced per unit dose of a mutagen (time x concentration/dose), while mutagenic efficiency gives an idea of genetic damage (mutation) in relation to the total biological damage caused in M\textsubscript{1} generation (Konzak et al., 1965; Gautam et al., 1992; Khan, 1997; Khan and Wani, 2006b; Kamau et al., 2011 & Singh, 2011). Although, both are two different properties but the usefulness of any mutagen in plant breeding programme depends on both of them. It is not necessary that an effective mutagen shall be an efficient one also (Koli and Ramkrishna, 2002; Gaikwad and Kothekar, 2004; Khan et al., 2005b). Various factors like biological, environmental and chemical ones modify the effectiveness and efficiency of different mutagens and the mutation rate (Blixt, 1970; Fujimoto and Yamagata, 1982; Ogunbodede and Brunner, 1991; Kodym and Afza, 2003).
Mutagenic effectiveness and efficiency were also found to depend upon mutagen type and the genotype. There have been a number of reports revealing that the effectiveness and efficiency of mutagens vary to a greater extent in various crop plants as in clusterbean (Velu et al., 2007), cowpea (Dhanavel et al., 2008; Girija and Dhanavel, 2009), garden pea (Sharma et al., 2010), grasspea (Nerker, 1977; Kumar and Dubey, 1998a; Waghmare and Mehra, 2001), lentil (Dixit and Dubey, 1986a; Sharma, 1990; Gaikward and Kothekar, 2004; Solanki, 2005), limabeans (Kumar et al., 2003), mungbean (Mehraj-ud-din et al., 1999; Singh, 2007; Goyal et al., 2009; Wani et al., 2011b), soybean (Kavithamani et al., 2008; Pavadai et al., 2009; Khan and Tyagi, 2010a), rice (Rao and Rao, 1983; Reddi and Suneetha, 1992), wheat (Chowdhury, 1978), barley (Jagtap and Das, 1976), sorghum (Sree Ramulu, 1972; Reddy and Smith, 1984), fenugreek (Koli and Ramkrishna, 2002) and brinjal (Zeerak, 1992b). It has been noticed that among the monofunctional mutagens, while methylating agents are more toxic and thus have to be used only at lower concentrations (IAEA, 1970; Fujimoto and Yamagata, 1982; Khan and Siddiqui, 1992), ethylating agents, being less toxic, can be applied at relatively higher concentrations to yield more mutations. Khan and Wani (2005b) found that the order of mutagens based on effectiveness was MMS > SA > EMS whereas on the basis of their efficiency, the sequence was EMS > MMS > SA. It was also observed that moderate concentrations of the mutagens were most effective and efficient in inducing mutations. Kaul and Bhan (1977) reported that EMS is more effective and efficient mutagen than dES and gamma rays in rice. According to Mahapatra (1983), sodium azide was more effective and efficient than gamma rays, EMS and NMU. Makeen and Babu (2010) study the mutagenic effectiveness and efficiency of gamma rays, SA and their combination treatments in urdbean and observed that the effectiveness of gamma rays was higher than SA and combination treatments. HZ was found to be the most effective mutagen followed by SA and EMS in mugbean (Wani et al., 2011b). According to Kaul (1989), the most desirable mutagen is the one that is least damaging and highly useful mutation yielder.
2.2.6. Morphological mutations

Alterations in the morphological pattern of any type through mutagens is regarded as morphological mutations. Morphological mutations affecting important plant attributes in many cases prove to be promising from breeder’s point of view. Morphological mutants play a vital role to modify the characteristics of cultivars for the construction of ideotype and ultimately leads to development of new variety of crops. For the development of improved varieties such mutants were found to be more productive, when used in cross breeding (Pawar et al., 2010). Several morphological mutants based on different plant forms, leaf, maturity, pod and seed characters were reported with the application of single and combination treatments of physical and chemical mutagens in pulses (Blixt, 1972; Appa Rao and Reddy, 1975; Filippetti and De Pace, 1986; Jha, 1988; Vanniarajan et al., 1993; Venkatachalam and Jayabal, 1995; Ramesh and Dhananjay, 1996; Ravikesavan et al., 2001; Henry, 2002; Khan et al., 2005c; Solanki, 2005; Singh, 2007b; Selvam et al., 2010; Khan and Tyagi, 2010b; Wani et al., 2011a), cereals (Okuno and Kawai, 1978; Reddy and Gupta, 1988; Reddy, 1992a; Singh et al., 1998; Viswanatahan and Reddy, 1998; Kumar and Mani, 1977; Ali and Siddiqi, 1999; Singh et al., 1998) and other plants of economic importance (Chaghtai and Hasen, 1980; Zeerak, 1990; Datta and Laxmi, 1992; Venkatachalam and Jayabal, 1994; Marry and Jayabal, 1995; Kumar et al., 1996; Amarnath and Prasad, 2000; Datta and Sengupta, 2002; Cagirgan, 2006). In chickpea, like other pulse crops, morphological mutations have been isolated for flower colour and structure, growth habit, size and colour of the seeds (Khosh-Khui and Niknejad, 1971; Muehlbauer and Singh, 1987; Davis et al., 1990; Davis, 1991; Ahmed and Godward, 1993; Ghate, 1993; Knight, 1993; Pundir and Reddy, 1998; Gaur and Gour, 2001; Toker and Cagirgan, 2004a; Toker, 2009; Khan et al., 2004a & 2011). The presence of more than one mutation in a single plant was termed as ‘multiple mutation’ by Sharma (1969b). According to him, agents with higher mutagenic efficiency induce more multiple mutations, and such mutations may accumulate

Tyagi and Gupta (1991) reported that each gene which is of agronomic interest can mutate and hence a wide spectrum of viable mutants, morphological in nature, can be expected in mutation experiments. Based on segregation pattern of morphological mutants, Reddy and Gupta (1988) & Thakur and Sethi (1993) observed that most of the true breeding mutants were conditioned by single recessive genes. However, Konzak *et al.* (1969) argued that the different morphological mutants which bred true in future generations like tall, dwarf, semi-dwarf, bushy, prostate and bold seeded mutant types were found to be under the influence of polygenes.

Frequency of morphological mutations has been found to increase with increase in the dose of mutagen (Thakur and Sethi, 1995). Datta and Sengupta (2002) reported that spectrum of viable mutations was wider at lower doses of mutagens. Vaniranjan *et al.* (1993) observed the higher frequency of viable mutations at medium doses of gamma rays and EMS treatments. Thus, the spectrum and frequency of morphological mutations vary with mutagen and duration of treatment (Kumar and Mani, 1997) and the genetic differences of the experimental organism which have a key role in the recoverable frequency and spectrum of morphological mutations (Kharkwal, 1999; Sharma, 2001; Khan *et al.*, 2004a).

### 2.3. Quantitative traits

Most of the attributes of interest to a plant breeder are quantitative traits which are mostly controlled by polygenic interactions and in such situations, the efficiency of selecting a desirable mutant is generally lower than for specific trait controlled by a single gene. Emphasizing the significance of micro-mutations in plant breeding, Gaul (1965) stated that “there appears to be no doubt that micro-mutations may affect virtually all morphological and physiological characters as do large mutations and they might have higher mutation rate than the macromutations”. However, much difference of opinion exists among the breeders on the relative incidence of induced polygenic
variations (through induced mutagenesis) in negative or positive direction and shift of the mean in $M_2$ and later generations (Brock, 1965; Gaul and Aastveit, 1966; Goud, 1967; Faulkner, 1978; Rao et al., 1988; Siddiqui and Singh, 2010). Since mutagen derived variability for quantitative traits in crop plants is heritable and the response of the selection seems good (Frey, 1969), henceforth, many workers hold the view that induced mutations can be used to generate useful variation in the quantitatively inherited traits where appropriate selection is applied for improvement (Scossiroli, 1964; Lawrence, 1965; Brock, 1970; Chakrabarti, 1975; Khan, 1984; Kaul and Kumar, 1983; Tickoo and Chandra, 1999; Khan et al., 2004b; MacKay, 2010) and for the expression of mutated gene homozygosity is required because induced mutations occur more or less randomly in the genome and cannot be directed, only one of the two or more alleles of a locus is affected, inheritance is almost ever recessive (Micke, 1999).

For the last four decades the practical value of induced mutagenesis, in creating successful genetic variability for several desired traits in plant improvement programmes, has been well established and has been demonstrated by many workers in different crop categories viz., pulses like pigeonpea (Rao, 1984; Srivastava and Singh, 1993), cowpea (Murugan and Subramanian, 1993; Gunasekaran et al., 1998; Pandey, 2002), urdbean (Singh et al., 2001; Selvam et al., 2010), lentil (Solanki and Sharma, 2001, 2002; Khan et al., 2006a), faba bean (Filippetti and De Pace, 1986; Verma and Rao, 1994; Joshi and Verma, 2004), mungbean (Khan, 1984; Singh and Yadav, 1991; Mathew et al., 2005; Khan and Goyal, 2009), chickpea (Harer et al., 1999; Kharkwal, 2001; Khan and Wani, 2005b; Kozgar and Khan, 2009), cereals like wheat (Scossiroli, 1964; Konzak, 1973; Siddiqui, 1983; Khan, 1988; Nalini et al., 1993; Kalia et al., 2000; Jamil and Khan, 2002; Sakin and Yildirim, 2004), rice (Awan et al., 1980; Shanthi and Singh, 2001; Ishiy et al., 2006), barley (Gustafsson, 1963; Bhargava and Khalatkar, 1986; Nalini et al., 1993), triticale (Reddy, 1988, 1989; Viswanathan et al., 1994), other ornamental and medicinal plants (Amarnath and Prasad, 2000; Cagirgan, 2006; Datta and Laxmi, 1992; Datta and Sengupta, 2002; Kumar et al., 1996; Venkatachalam and Jayabalan, 1994). In all the cases, most of the
plant attributes of interest to plant breeders were quantitative traits which are governed by the principles of quantitative genetics.

A common practice in induced mutagenesis is to advance only normal looking $M_2$ plants to $M_3$ generation and apply the first dose of selection in $M_2$ is being adopted commonly by most of the breeders, after the elaborative and successful work of Brock (1965). This methodology has been advocated by Gupta and Swaminathan (1967), Tickoo and Jain (1979), Sharma (1986) & Wani and Khan (2006) with a conclusionary remarks of their work that the promising progenies can be identified with high degree of confidence in $M_2$ on the basis of mean and variance. However, Jana and Roy (1973) selected $M_2$ families on the basis of significantly changed mean only. Since most of the desired combinations of favourable alleles are likely to be lost in advanced generations due to intensive or even no selection for other traits thus the selection for quantitative traits, such as yield and yield attributing characters, should preferably be carried out in early generation. Sneep (1977), Saini and Gautam (1990), Sharma (1997), Micke (1999) and many other plant breeders also advocates for the early generation selection for quantitative traits. The efficiency of early generation ($M_2$) selection in mutation breeding experiments has been reported in the crops like lentil (Solanki and Sharma, 2002), mungbean (Tickoo and Chandra, 1999), sesame (Sheeba et al., 2003), soybean (Pavadai et al., 2010; Nakagawa, 2009 & Nakagawa et al., 2011). Since the quantitative traits selected in early generations indicate the degree of stability to the environmental fluctuations, henceforth, the potential transmissibility of these traits from parent to offsprings and from generation to generation are to be evaluated. For this, the estimates of heritability of various quantitative traits are essential (Mather and Jinks, 1971; Kaul and Garg, 1979; Scossiroli et al., 1966; Ignacimuthu and Babu, 1993; Brunner, 1995; Mohanty, 2001; Chaudhary et al., 2004; Khan et al., 2006a & Arulbalachandran et al., 2010). Trivedi et al. (2006) clearly brought out that in the treated population the estimates of heritability were larger and varied with trait to trait. Johnson et al. (1955) suggested that heritability in combination with genetic advance was more helpful in predicting the effect of

Both physical and chemical mutagens were used, alone or in combinations, in pulses for generating variability in quantitative traits like plant height, pods per plant, pod length, flowering and maturity period, seed weight, biological yield, number of fertile branches, number of seeds per pod, yield and harvest index (Kundu and Singh, 1981, 1982; Reddy et al., 1992; Srivastava and Singh, 1993; Kumar et al., 1995; Waghmare and Mehra, 2000; Rehman et al., 2001; Yaqoob and Rashid, 2001; Wani and Khan 2004; Wani et al., 2011c). The important primary yield components in pulses are considered pods bearing branches, pods per plant, seeds per pod and seed weight and several studies (Bahl, 1988; Kumar and Arora, 1991; Rao, 1996; Khan and Siddiqui, 1997; Guler et al., 2001; Kharkwal, 2003; Singh and Singh, 2003; Raut et al., 2004; Khan and Wani, 2005b; Mubeen et al., 2007; Makeen et al., 2009; Giri et al., 2010) have shown a close association between these components to the total plant yield in induced mutant lines. In chickpea, different workers have reported increased variability for various agronomic characters in mutagen treated population as observed by significant changes in the mean values and coefficient of variability as compared to control (Nerker and Mote, 1978; Haq and Shakoor, 1980; Kharkwal, 1981a; Kumar et al., 1981; Kozgar and Khan, 2009 & Barshile et al., 2009).

### 2.4. Mineral elements

Increased food insecurity and malnutrition conditions, especially in the developing countries like India, have forced the plant breeders to chalkout the policies of increasing the mineral elements in the crop in addition to the other quantitative traits which contribute to yield. Bouis (1996) is of the opinion that mineral and vitamin...
deficiencies affect a greater number of people in the world and if farmers could be induced to grow commonly eaten food staple crops, like chickpea, that fortify their seeds with essential vitamins and minerals. And, because of high consumption as dietary food, any increase in mineral concentration might well have a significant effect on human nutrition and health. Induced mutagenesis can be potent methodology for balanced increase in mineral elements (Wang et al., 2003a) in addition to yield and its attributing characters. The impact of mutagens on the trace elements like that of iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) and their genetic variation has not been extensively studied. Mutants with altered seed mineral profiles have been identified in pea (Wang et al., 2003a). The Consultative Group on International Agriculture Research Micronutrient Project (CGIARMP) indicated that it is possible to combine the high micronutrient trait, as Fe, Zn, Mn and Cu contents, with high yield, unlike protein content and yield that are mostly negatively correlated through breeding strategies (Gregoria, 2002). The report also showed that it will be possible to improve the content of several limiting micronutrients together, thus pushing population towards nutritional balance.

2.5. Seed protein and its profiling

With an aim to improve the seed protein content coupled with high grain yield of cereals and legumes the genetic fortification through induced mutagenesis has been done in the past and in fact at the end of late 1960's a voluminous international research programme was started by the I.A.E.A. in Vienna with this aim viz., to improve the seed protein quantity and quality of cereals and legumes by means of mutations (Gottschalk, 1986). Contrary reports have been put forward by several workers on the extent of success of induced mutations for high grain yield coupled with high protein contents of the mutants. Some research works by Hartwig (1979), Abo-Hegazi (1980), Gottschalk and Muller (1982), Matta and Gatehouse (1982), Gottschalk and Wolff (1983a), Gottschalk (1986) & Rehman et al. (2001) are of the view that high protein content is difficult to combine with high yield as these two traits reveal almost negative correlation. However, high yielding mutants coupled with high
protein contents were reported by Borah and Goswami (1995) in rice, Olejniczak (1986) in maize, Kalia et al. (2000) in wheat, Blixt (1979) in operate legumes, Ignacimuthu and Babu (1989a) in urdbean, Naik et al. (2002) in mungbean, Hassan et al. (2001) in chickpea & Hiremath et al. (2010) in groundnut. Gottschalk (1990) explained that there is no doubt that these traits are controlled by genes and mutations in these genes can alter the protein make up of the genotypes. It is, however, very difficult to discern their action reliably because protein content is known to be influenced by various endogenous and exogenous factors, like stem height, leaf area, time of maturation, seeds size, seed number, temperature, water stress, nitrogen feeding levels and other environmental factors (Gottschalk and Wolff, 1983b). The protein production in plants is highly influenced by the interaction of gene(s) and environmental factor(s) (Sengupta et al., 1986; Gottschalk, 1990; Singh et al., 1990).

The main difficulty in assessing the high seed protein mutant concurrent to high yield is that no handy screen methods are available by which mutagenically treated plants can be analyzed biochemically especially with regard to the composition of their seed proteins. Gottschalk (1986) suggested that it is possible to analyze the seed proteins of mutants quantitatively and qualitatively, which had been selected with regard to other useful traits but not with regard to improved seed proteins. And in this way, it is possible to obtain genotypes with increased protein production per plant, but they do not represent “protein mutants”, for example, the bold seeded mutants of Vigna mungo obtained by Singh (1996) with gamma rays treatment showed a slight increase in protein content over the control.

For the last couple of years the genetic diversity studies through protein profiling has been interestingly increased and the change in protein profiling among the isolated mutants is of paramount interest. The profiling of proteins in the isolated mutants via the electrophoresis involves the separation of different protein polypeptides on the basis of their molecular weights and the net charge they carry (Gepts et al., 1986; Anitha et al., 2008). The proteins can be reliably fractionated by SDS-PAGE (Laemmli, 1970). The protein profile could be used as molecular markers

In addition to the estimation of genetic variability in characters, such as yield and yield contributing traits, there is imperative need to undertake studies on correlation coefficients in the mutation breeding programmes. Bahl (1988) studied the change in correlations between various character pairs after mutagen treatments. Kumar and Arora (1991), Rao (1996), Guler et al. (2001), Kharkwal, (2003) & Raut et al. (2004) studied relationships among various plant characteristics and yield in chickpea.

The survey of important literature on induced mutations in pulse crops in general and in chickpea in particular reveals that:

- the information about the bio-physiological damages especially in terms of enzyme assays and chlorophyll contents, physiological studies of morphological mutants and the impact of mutagens on protein content, protein profiles and mineral composition of chickpea has not been extensively studied.
- the information on the effectiveness and efficiency of single and combination treatments of gamma rays and EMS and their possible role in generating polygenic variability in chickpea is scanty.
- in breeding programmes, a suitable character combination that affects plant yield to the maximum extent is important in formulating an effective selection programme. Correlation between yield and yield components are known in chickpea, however, correlations of yield to protein and mineral elements (iron, manganese, zinc and copper) are not well known.
In view of the above facts, it was felt desirable to use gamma rays, EMS and their combination treatments for generating polygenic variability for quantitative traits in chickpea, a self-pollinated crop, for getting maximum economical use of the plant in the era of food insecurity and malnutrition threats which grooms over developing countries like India for the past several years.