Scope and Practical Applications of Induced Mutagenesis for Genetic Improvement of Crop Plants

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Abstract

Mutation breeding is an important tool of enhancing genetic variability in crop plants so as to isolate the promising mutants of qualitative and quantitative traits for crop improvement. For last 7-8 decades the release of more than 3200 mutant varieties globally and a total of 343 mutant varieties in India is a testament of the fact that induced mutagenesis has played important role in global food security and sustainable development. Apart from improvement in economically important traits particularly yield, disease resistance, adaptability etc. through induction of micro and macro-mutations, the other parameters of the mutagenesis study included biological damage, effectiveness and efficiency of the mutagens, spectrum and frequency of chlorophyll mutations. M1 parameters like inhibition of seed germination, chromosomal aberrations and seedling injury collectively called as biological damage give a preliminary idea of sensitivity of crop plants to different mutagens as well as effectiveness of mutagenic treatments for identifying a suitable dose/concentration of the mutagen for a specific crop plant. On the other hand chlorophyll and morphological mutation frequency in M2 and subsequent generations give an idea of efficiency of mutagenesis in isolating promising mutant lines in the segregating generations. Induction of genetic variability is prerequisite for any crop improvement programme in mutation breeding. Various physical and chemical agents have been employed in different crop plants for inducing mutations and enhancing the genetic base. Due to small mutations in genes governing quantitative traits,
a large amount of variability is induced in the segregating generations of mutagen treated populations. However, the success of mutagenesis program largely depends on the effectiveness of selection employed in segregating generations for isolation of promising lines. In general mutant crop varieties have been developed for higher yield, better adaptability, enhanced resistances to biotic and abiotic factors, and improved nutritional and other quality traits. Several mutant varieties are more economical to grow and contribute to more environment friendly agriculture.

**Key words:** Mutagenesis, genetic variability, mutant varieties, crop improvement.

**Introduction**

Mutagenesis, a key area of genetical reasearch occupies prime position in biological researches from viruses to the plants, animals and humans in every country not only because of the understanding of the mechanism of mutation and the factors (internal or external) that has helped to elucidate the basic aspects of life phenomenon but also because it has profitably been utilized in raising a large number of economically superior and desirable genotypes of crop plants. In conventional breeding methods, the store of natural variability present either in the base population initially or introduced through hybridization, is subjected to recombination and selection so as to increase the frequency of favourable combinations of genes in the selected line. Mutation breeding helps in inducing greater magnitude of variability in various plant traits in a comparatively shorter time. Only through a careful screening and selection programme the magnitude of genetic variability induced by physical and chemical mutagens could be exploited for obtaining the desirable lines. Mutations provide an opportunity to create hitherto unknown alleles so that the plant breeder does not remain handicapped because of limited allelic variation at one or more gene loci of interest. Gottschalk (1986) stated that mutation breeding is a well functioning branch of plant breeding that can supplement the conventional methods in a favourable manner. Although the induction of mutations has been accepted as a useful tool in the plant breeding programme, the success in plant improvement programmes, however, depends basically on controlling and directing the induced mutation process for the production of desired mutations. One of the chief advantages of mutation breeding is its ability to improve a single feature in a variety without significantly altering the otherwise desirable make up of agronomic characters. Another advantage of mutation breeding is the creation of genetic variability which
enhances the scope for selection. Development of genotypes showing improvement over the existing varieties for higher yield and other desirable characteristics is the ultimate aim of mutation breeding experiments. The polygenic traits such as grain yield, early maturity, quality characters, grain quality, abiotic stress and biotic resistance have been improved by mutagenesis (Kharkwal, 1996). These findings supplement that mutagenesis is a potential tool to be employed in the crop improvement. For past 4-5 decades mutation breeding has been at the centre of many national and international breeding programmes and significant achievements have been made in sexually and asexually propagated crop plants. These aspects are being discussed in detail in the proceeding section.

**Mutagenesis as a means of crop improvement**

Mutation means a sudden heritable change in the genetic material at the gene or chromosomal level (Chahal and Gosal, 2002). The term mutation was introduced by Hugo de Vries (1901) in *Oenothera lamarkiana*. Occurrence of spontaneous mutations were proposed by Morgan (1911) in Fruitfly (Drosophila). Mutagenic action of X-rays was discovered by Muller in 1927 on Drosophila and of gamma rays and X-rays in 1928 by Stadler in Barley (*Hordeum vulgare*) and Maize (*Zea mays*). Altenberg (1928) observed that the frequency of Translocations was increased by radiation. Success with X-rays was achieved by Stadler (1928) in Barley and by Goodspeed (1928) in *Datura* and *Nicotiana*. The principles of induced mutations in seed propagated crops were established in the forties, mainly by Gustafsson in 1941. Since then, mutagenesis as been successfully used for the improvement of crops through the induction of mutations at loci that control economically important traits and/or by eliminating undesirable genes from elite breeding lines. It has been demonstrated that genetic variability for several desired characters can be induced successfully through mutation and its practical value in plant improvement programs has been well established. The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype. During the past seventy years, worldwide more than 3200 mutant varieties have been released through induced mutations (Maluszynski et al., 2000; Chikelu Mba 2013). In several mutation derived varieties, the changed traits have resulted in increasing the yield and quality of the crop, improving the agronomic inputs and consumer acceptance (Ahlowalia et al., 2004). Almost about 77%, of the released mutant varieties belong to seed propagated crops probably indicating
the ease of application of mutagenic treatments to seeds for the induction of mutations as against stem cuttings, tubers and other vegetative propagules. Among various types of crop plants, almost half (48%) of all mutant crop varieties recorded in the Mutant Variety Database (MVD) are cereals, rice crop being at the top of the list with the highest number of mutants released and alone accounts for 53% of the mutant cereals under cultivation followed by barley which makes up 20% of all cereal mutant varieties globally (Chikelu Mba, 2013). Among different countries, China, India and Japan are the top three countries with the most officially released mutant crop varieties (Cickelu and Mba, 2013).

Induced mutagenesis has been successfully utilized in several crop plants viz., rice (Chakrabarti, 1995), common beans (Nichterlein, 1999), Artemisia (Rekha and Kak, 1997; Rekha and Langer, 2007), Chickpea (Wani and Anis, 2008, 2011) suggesting the potential of this technique for crop improvement. Mutagenesis has been successfully employed in rapeseed and mustard by the plant breeders to alter the genetic architecture of plant and isolate the mutants with desired economic characters such as plant height, number of pods per plant, number of grain per pod, 1000- grain weight, grain yield, oil content and disease resistance (Robbelen, 1990; Mahla et al., 1990; Rehman, 1996; Shah et al., 1998, 1999; Javed et al., 2000). Mutation breeding has been used to produce many cultivars with improved economic value and study of genetic and plant developmental phenomena (Van et al., 1990; Bertagne et al., 1996). The Joint Division of the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA) in Vienna, Austria which maintains a Mutant Varieties Database (MVD) for these crop varieties introduced some newly developed mutant rice varieties with modified starch characteristics and early maturity in China and Bangladesh respectively (Chikelu Mba, 2013). The conversion of non-edible linseed (Linum usitatissimum) oil into an edible form (Linola) is one of the major success stories of mutagenesis programmes. Linola has been developed by the Australian Commonwealth Scientific and Industrial Research Organization (Askew, 1993; Larkin, 1998). With mutations to the fatty acid synthesis pathway, the alpha-linolenic acid (ALA) content was reduced to 2% (as against 50% in the wild type). Linola is now being produced in Australia, Canada, the United Kingdom and the United States.

Kharkwal and Shu (2009) who reviewed the contributions of induced crop mutants to global food security concluded that there was considerable evidence that mutant crop varieties would continue to contribute significantly to addressing the food and nutritional securities of many
countries especially in view of the potentials for harnessing novel traits in enhancing the adaptabilities of crops to climate change and variations.

The success of mutation breeding in India is impressive when we look at the number of mutant varieties released so far and contributions made towards food security and sustainable development in the agriculture sector (Chopra, 2005, Kharkwal and Shu 2009). The Indian Agricultural Research Institute (IARI) in New Delhi; Bhabha Atomic Research Center (BARC) in Mumbai, Tamil Nadu Agricultural University (TNAU) in Coimbatore, and the National Botanical Research Institute (NBRI) in Lucknow, are some of the major research centers actively engaged in mutation breeding for several crops and have contributed substantially to the development and release of a large number of mutant varieties. Kharkwal et al., (2004) listed a total of 309 mutant cultivars of crops, belonging to 56 plant species that were approved and/or released in India by the end of the twentieth century. An updated list of 343 mutant cultivars released in India, however, has been given by Kharkwal and Shu (2009). According to these authors, the largest number of mutant cultivars have been produced in ornamentals (119) followed closely by legumes (85) and cereals (74).

Various mutagenic agents have been used to induce favourable mutations at high frequency that include ionizing radiation and chemical mutagens (Ahlowalia and Maluszynsky, 2001). Physical mutagens like X-rays, gamma rays, fast neutrons, thermal neutrons, ultraviolet and beta radiations have been frequently used for induced mutagenesis (Yaqoob and Rashid, 2001). Apart from physical mutagens, several chemical mutagens were also frequently used for induced mutagenesis in crops including Ethyl Methane Sulphonate (EMS), Ethylene imine (EI), Methyl Nitroso Urea (MNU), N-nitroso-N-methyl urea (NMU), Ethyl Nitroso Urea (ENU) and sodium azide (SA) (Sharma and Chopra, 2000). The ethylated agents such as EMS have been found to be more effective and efficient than physical mutagens in crops like Lentil (Gaikwad and Kothekar, 2004), Cowpea (John, 1999), pea (Waghmare and Mehra, 2001) and Chickpea (Kharkwal, 1998). Seed mutagenesis through EMS treatment has been used for induction of male sterility in wheat (Maan and Williams, 1984), herbicide resistance in soybean (Sebastian et al., 1989), early flowering in Spring rape (Thurling and Depittayanan, 1992), increased pollen viability and fruit rot resistance in bell pepper (Ashok et al., 1995) as well as quantitative variations in different yield traits in Avena sativa L. (Krishna and Vasudevan, 1984). EMS has been successfully used to develop fenugreek mutants with the ability to produce early maturing mutants with a
determinate growth habit, high seed yield, seed quality and adaptation to a short growing season (Basu et al., 2008). Sodium azide is known to affect the seed germination, shoot length, and root length and also induces high frequency of chlorophyll deficient mutations.

**Dose effect / L.D 50**

The amount of mutagens (physical or chemical) to be used for mutation induction varies from species to species. Criteria such as LD 50 (50% viability) or GR 50 (50% growth reduction) are used to choose the dose range. LD 50 or GR 50 is the dose of mutagen that is lethal to 50 % of treated individuals. The dose required for high mutation efficiency of a physical or chemical mutagen depends on properties of mutagenic agents and of biological system in question. In general, the dose effect of physical and chemical mutagenic treatments comprises several parameters, of which the most important are dose rate, concentration, duration of treatments, temperature and pH during treatments.

In chick pea Singh (1988) reported LD 50 value for gamma rays at 460 Gy (var. G130) and 483 Gy (var. H208) and for EMS at 0.25 % (var G130) and 0.2% (var. H208). In both the varieties 0.4 % EMS treatment was most lethal. Kharkwal (1981) reported higher lethality in 0.2 % EMS in comparison with 400 and 500 Gy gamma rays. Higher LD 50 values for the gamma rays in chick pea in comparison to other pulse crops such as 300Gy in Black gram (Khan, 1988), 200 Gy in Lentil (Singh, 1983) and 100Gy in pea (Singh, 1988) indicate its greater resistance to the mutagen. Further, variations have been observed for LD-50 values in different chick pea varieties, which are attributed to their differential radio-sensitivity. A decline in the survival of a mutated population has been associated with the increase in the dose of mutagen (Farooq and Nizam, 1979; Singh, 1988), which has resulted from cytological damage and /or physiological disturbances as also reported earlier by Sato and Gaul (1967). Umavathi and Mullainatha (2015) recorded the LD50 values in chickpea based on the growth reduction of seedlings after treatments with mutagen. The effective doses/concentrations which caused 50% growth reduction were observed in 40kR in gamma rays and 30mM in EMS. Kangarasu et al (2014) reported 20 and 30 Gy of gamma rays and between 75 and 125 mM L.D 50 in cassava. These and other studies on LD50 in various crop plants strongly suggest that different genotypes respond differentially to different mutagens and/or mutagenic treatments. It thus becomes
imperative to work out appropriate dose/concentration of physical or chemical mutagen for successful mutagenesis programme.

**Mutagenic sensitivity and biological damage**

The effects of physical and chemical mutagens and their combination treatments on different biological parameters such as germination response, seedling survival, seedling injury, chromosomal aberrations, sterility and induction of micro and macro mutations in M₂ have been studied by many workers (Khan et al., 1994; Vanniarajan et al., 1994; Sharma et al., 1995; Khan et al., 1999; Sareen and Kaul, 1999; Verma et al., 1999; Mitra and Bhowmick, 1999; Khan and Wani, 2005). Such parameters are considered as the main indices for the overall response of a mutagenic agent. Mutagenic sensitivity is known to be influenced by an array of factors, such as type of mutagen and dose, moisture content of seed, treatment conditions, stage of development, ploidy level and genotype of the material. Varied mutagenic sensitivity in different genotypes was first reported by Gregory (1955) in groundnut and by Lamprechet (1956) in pea. Similar varietal differences were recorded in production of viable and chlorophyll mutations in *Nigella sativa* (Mitra and Bhowmick, 1999) and in *Vigna mungo* (Rehman, 2000) following gamma rays and EMS treatments. Sharma and Sharma (1981) observed differential mutagenic response of gamma rays and NMU in microsperma and macrosperma Lentils. They reported better viability of chlorophyll mutations like xantha and chlorina in the microsperma than in the macrosperma varieties. Venkatachalam and Jayabalans (1995) while using EMS, SA and gamma rays found distinct differences with respect to mutagenic sensitivity in ground nut (*Arachis hypogea*). Distinct varietal differences to SA in *Vigna radiata* was observed by Khan et al. (2004). In chickpea, Kharkwal (1998) reported that varieties of desi type were more resistant towards mutagenic treatments than kabuli type. Akbar et al. (1976) concluded that differences in radiosensitivity may be due to differences in their recovery process including enzyme activity. Mutagenic response to cytological aberrations has been reported by many workers (Rao and Laxmi, 1980; Suganthi and Reddy, 1992; Rehman, 2000). Mitra and Bhowmick (1996) observed no varietal differences with regard to mitotic index as well as meiotic abnormalities in *Nigella sativa*. Both cultivars of *Nigella sativa* were found equally radiosensitive. Ahmad and Godward (1981) reported radio sensitivity in nine cultivars of chick pea. Out of these nine, two cultivars CSIMF and F10 were identified as the most radio-resistant and radiosensitive,
respectively. Similarly Kharkwal (1998) reported mutagenic sensitivity in four varieties of chick pea on the basis of total germination rate, seedling damage, pollen sterility and plant survival. It has been concluded that the varieties with large assortment of recessive alleles governing traits show greater sensitivity and frequency of M₂ mutants than the varieties having more dominant alleles governing a trait (Gelin et al., 1958; Blixt, 1970). A few members of alkane sulphonate series have been found to be exceptionally mutagenic in a variety of organisms. While studying the mutagenic action of EMS in barley and wheat (Swaminathan et al., 1962; Freese, 1963; Gaul, 1964), it has been observed that EMS preferentially reacts with guanine and cytosine and that it was capable of producing more number of various morphological mutants as compared to gamma rays. Gupta and Yashvir (1975) reported a radio-protective effect of EMS in Abelmoschus esculentum by recording a higher germination percentage in the combined treatments of gamma rays and EMS than in corresponding EMS treatments. Similar radio-protective effect has been reported in barley (Khalatkar and Bhatia, 1975) where it has been observed that the seedling injury, chromosomal aberrations, pollen and seed sterility were less in combined treatments than in separate treatments of gamma rays and EMS. Gamma rays were reported to inhibit the uptake of EMS due to the generalized action of radiation on metabolic processes in the cells. Singh and Chaturvedi (1980) reported mutagen induced damage such as plant injury and lethality in M₁ generation arising due to physiological, chromosomal and factor mutations. A direct relationship of pollen and ovule sterility with higher doses of gamma rays and EMS was also observed. Increase in pollen sterility and decrease in germination with increasing doses of gamma rays and EMS has also been reported in Vigna mungo (Gautam et al., 1992) and Capsicum annuum (Rao and Laxmi, 1980). Mutagenic efficiency based on injury and lethality has been found higher in combined treatments of gamma rays and NMU than their respective individual treatments (Dixit and Dubey, 1986). On the other hand in some cases combined treatments showed greater reduction in seedling survival than the individual treatments. Bhatnagar (1984) reported the adverse effect of combined treatments on germination and survival of plants in chick pea. The pollen sterility increased in combined treatments indicating the additive or synergistic effect. Lal et al., (2009) while studying the mutagenic sensitivity of gamma rays, sodium azide and their different combinations in M₁ generation of black gram concluded that the combination treatments of gamma rays and SA had more depressive effect on germination and seedling growth. A dose related reduction in seed
germination and pollen fertility by both gamma rays and EMS have been shown by various workers (Nerker, 1970; Rao and Laxmi, 1980; Khanna and Maherchandani, 1981; Gautam et al., 1992). Similar findings were also reported in *Pearl millet* (Singh et al., 1978), *Arachis hypogea* (Venkatachalam and Jayabal, 1995), *Nigella sativa* (Mitra and Bhowmick, 1999) *Vigna radiata* (Singh and Chaturvedi, 1980; Khan et al., 2004) and *Lens culinaris* (Khan, 2002).

Several workers have attempted to explain the causes responsible for inhibition of seed germination. Reduction in seed germination in mutagenic treatments may be due to delay or inhibition in physiological and biological processes necessary for seed germination which includes enzyme activity (Chrispeeds and Varner, 1967), hormonal imbalance (Ananthaswamy et al., 1971) and inhibition of mitotic process (Sato and Gaul, 1967). Yusuf and Nair (1974) inferred that gamma irradiation interfered with the synthesis of enzymes and at the same time accelerated the degradation of existing enzymes involved in the formation of auxins and thus reduces the germination of seeds. Reduced seed germination due to mutagenic treatments may also be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009). Turkan et al. (2006) attributed reduction in germination and root or shoot length to the cell cycle arrest caused by higher doses of sodium azide. Mehetre et al., (1994) in soybean have attributed reduction in germination percentage to the seed injury caused by higher exposures of gamma rays. Kumar and Yadav (2010) have attributed delay and reduced seed germination to the effect of mutagen on meristematic tissues and chromosomal damages. Kleinhofs et al., (1978) reported that SA may hamper ATP biosynthesis resulting in decreased availability of ATP molecule which may slow the germination rate and reduce the germination percentage. Chowdhury and Tah (2011) in *Dianthus caryophyllus* suggested that the reduction in germination was due to disturbed base pair relationship and disturbance in the formation of enzymes caused by colchicine, EMS and sodium azide.

Seedling length is widely used as an index in determining the biological effects of various physical and chemical mutagens in M₁ generation (Konzak et al., 1972). Various explanations have been provided to explain the phenomenon of reduced seedling growth. Riley (1954) suggested that it could be due to chromosomal abnormality, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanisms and chromosomal damage-cum-mitotic inhibition. Gray and Scholes (1951) and Lea (1955) reported that reduction in seedling growth is
due to an uneven damage of the meristematic cells as a consequence of genetic injury. The badly damaged cells would produce only a few cell progeny and growth will recur from those cells which are least damaged genetically. Sparrow and Sparrow (1965) suggested that the growth inhibition arises from the interference with the cell elongation. Inhibition of impaired meiosis could also be the reason for reduced growth. Reduced seedling growth has also been attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances (Gunckel and Sparrow, 1954; Gordon, 1959; Singh, 1974; Usuf and Nair, 1974). Srivastava et al., (2011) in wheat suggested that the reduction in seedling survival is due to the hinderance caused by the sodium azide on different metabolic pathway of the cells. Similar findings have also been reported by Rachovska and Dimova (2000) in wheat, Khan et al., (2004) in mungbean, Ilbas et al., (2005) in barley, Adamu and Aliyu (2007) in tomato and Mostafa (2011) in sunflower.

The adverse effects of various physical and chemical mutagens on plant survival have been reported by many workers and a dose dependant reduction has also been observed. The reduction in seedling survival has been attributed to cytogenetic damage and physiological disturbances (Sato and Gaul, 1967). The greater sensitivity at higher mutagenic level has been attributed to various factors such as changes in the metabolic activity of the cells, inhibitory effects of mutagens and to the disturbance of balance between promoter and inhibitors of growth regulators (Krishna et al., 1984).

The high percentage of pollen sterility induced by all types of mutagens and in all crop plants studied so far may be due to cumulative effects of various aberrant meiotic stages as well as physiological and genetic damages that are induced probably by the breakage of chromosome through formation of an anti metabolic agent in the cell or may be due to irregular disjunction of chromosomes at anaphase (Mathusamy and Jayabalan, 2002; Khan and Wani, 2005; Mensah et al, 2007, Kumar and Rai 2006, Lal et al 2009, Kumar et al 2009). The structure and physiology of the pollen grains is under genetic control and irregular or abnormal meiosis may cause significant changes in the pollen properties leading to high pollen sterility. Contrary to this, a high pollen sterility coupled with low frequency of meiotic abnormalities may be attributed to small undetectable deletions or gene mutations (Sato and Gaul 1967).
Induction of cytological abnormalities

Cytological analysis with respect to mitotic and meiotic behaviour is considered one of the most dependable indices to estimate the potency of mutagen. It also provides a considerable clue to assess sensitivity of plants for different mutagens. Physical and chemical mutagens are known to produce chromosomal aberrations leading to abnormal chromosome behaviour during meiosis and consequently giving varying degree of sterility. According to (Kumar and Rai, 2007), the induction of cytological disturbances in the meiotic cells is of great value, as it results in genetic damage that is handed over to the next generation. Different types of chromosomal abnormalities have been reported by different workers in different plant materials after treatment with physical and chemical mutagens (Ahmad and Godward, 1981; Ahmad, 1993; Anis and Wani, 1997; Kumar and Dubey, 1998c; Verma et al., 1999; Dhamayanthi and Reddy, 2000; Khan et al., 2009; Kumar and Verma, 2011).

Gamma rays, MH and their combination treatments have been shown to induce disturbed mitotic and meiotic behaviour in Vigna radiata (Grover and Tejpaul 1982). The sticky chromosomes, fragments and ring chromosomes at metaphase and the laggards and bridges at anaphase were noticed by these workers. The chromosomal aberrations were found to be significantly co-related with dose, whereas, combined treatment enhanced chromosomal aberrations compared to separate treatments. Grover and Virk (1986) reported induced chromosomal aberrations in mungbean after treatments with gamma rays, N’methyl-N-nitro-N-nitroso-guanidine (MNNG), EMS and Hydroxyl amine (HA).The maximum frequency of chromosomal aberrations was noticed with gamma rays followed by MNNG, EMS and HA. The quadrivalents, trivalents and univalents were encountered at Metaphase-I, whereas, irregular distribution of chromosomes accompanied by laggards and chromatin bridges were observed at anaphase-I. Mitotic abnormalities like miss-orientation at metaphase, bridges at anaphase, fragmentation and multinucleate condition were also observed by Shah et al. (1992) in gamma rays treated Vigna mungo. Vandana and Dubey (1996) reported the meiotic anomalies induced by EMS and DES in Vicia faba. These anomalies were found to increase with the increase in the concentrations of mutagens applied. Overall frequency of meiotic anomalies induced by various concentrations of DES was higher than those of EMS. A relative account of cytological and developmental effects of gamma rays, EMS and MMS on meiotic features and pollen fertility in Vicia faba L. was
provided by Bhat et al. (2005). The various kinds of chromosomal abnormalities and reduction in pollen fertility were found to be dose dependent. The induction of meiotic abnormalities was observed to be higher under MMS treatments, followed by gamma rays and EMS, suggesting that MMS could be more effective in inducing chromosomal abnormalities than gamma rays and EMS. Khan and Tyagi (2009) reported bridges and laggards in soybean when treated with EMS, gamma rays and their combination. In EMS treated *Cicer arietinum* L. Sharma and Kumar (2004) observed different types of meiotic abnormalities such as stickiness, univalents, multivalent, unorientation of chromosomes, precocious separation of chromosomes at metaphase and bridges, laggards and unequal separation of chromosomes at anaphase. In general, the meiotic abnormalities increased along with the increase in concentration of EMS and varietal sensitivity to mutagenic response was also noticed. Goyal and Khan (2009) treated two varieties of Urdbean (*Vigna mungo* L.) Hepper with different concentrations of EMS and HZ. Chromosomal aberrations like univalents, multivalent, laggards, bridges, micronuclei, stickiness, cytomixis and precocious movement were noticed in mutagen treated populations. Chromosomal aberrations were found to be correlated with the concentration of chemical mutagens. The maximum frequency of abnormalities was induced by EMS in both the varieties of Urdbean. Kumar and Srivastava (2010) studied the mutagenic potential of gamma rays and laser rays on seeds of Safflower (*Carthamus tinctorius* L.). Results have shown that a wide spectrum of chromosomal aberrations was encountered in mutagen treated populations but the most frequent anomaly was the stickiness of chromosomes. Gamma rays were found more effective than laser rays in inducing chromosomal aberrations.

Mutagens have been shown to induce stickiness of chromosomes which is seen as intense chromatin clustering during the prophase and metaphase stages. The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense with the formation of pycnotic nuclei that may involve the entire genome, culminating in chromatin degeneration. Chromosome stickiness may be caused by genetic or environmental factors. Genetically controlled stickiness has been described in many cultivated plants such as maize (Caetano-Periera *et al.*, 1995), *Pearl millet* (Rao *et al.*, 1990) and wheat (Zanella *et al.*, 1991). Several agents have been reported to cause chromosome stickiness, including x-rays (Stephenson, 1956), gamma rays (Rao and Rao, 1977; Al-Achkar *et al.*, 1989), temperature (Eriksson, 1968), herbicides (Badr and Ibrahim, 1987) and some chemicals present in soil
(Caetano-Pereira et al., 1995). However, the primary cause and biochemical basis of chromosome stickiness are still unknown. Gaulden (1987) postulated that sticky chromosomes may result from the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutations in the structural genes coding for them (hereditary stickiness) or by the action of mutagens on the proteins (induced stickiness).

The occurrence of univalents at metaphase and lagging chromosomes at anaphase are two commonly observed abnormalities in mutagen treated populations. The induction of univalents at metaphase has been attributed to mutagen induced structural change in chromosomes and mutations that might be responsible for the failure of pairing among homologous chromosomes, whereas, the lagging chromosomes might be due to delayed terminalization, stickiness of chromosomal ends, abnormal spindle formation or because of failure of chromosome movement (Tarar and Danyansagar, 1980; Permjit and Grover, 1985; Jayabalan and Rao, 1987; Bhat et al., 2006).

In angiosperms, cytoplasmic connection between PMC’s at various stages is a widely observed phenomenon and has been reported by many workers (Heslop-Harrison, 1966; Risueno et al., 1969 and Whelan, 1974). It has been postulated that these connections must form an important avenue of exchange between PMC’s leading to the transfer of nuclear material through them from one meiocyte to another, the process being called as cytomixis. Although, cytoplasmic connections are very common in angiosperms, the movement of nuclear material through them is rare. In general, cytomixis has been detected at a higher frequency in genetically imbalanced species such as hybrids, as well as in apomictic, haploid and polyploidy species (Yen et al., 1993). Among the factors proposed to cause cytomixis are the influence of genes, fixation effects, pathological conditions, herbicides and temperature (Caetano-Pereira and Pagliarini, 1997). Cytomixis may have serious genetic consequences by causing deviation in chromosome number and may represent an additional mechanism for the origin of aneuploidy and polyploidy (Sarvella, 1958).

From the above discussion it seems clear that the primary effect of all types of mutagens is reaction with DNA leading to various types of structural and numerical aberrations besides point mutations. A mutagen like gamma rays is weaker than EMS or other chemical mutagen in one
crop species whereas, the other crop species is more sensitive to physical rather than chemical mutagen. The effectiveness of any mutagen in inducing higher proportion of mutations thus depends upon concentration / dose of the mutagen, treatment duration, genetic background of the crop species and the plant part treated.

**Chlorophyll mutations**

The scoring of chlorophyll mutations in M₂ generation is considered as a dependable measure of genetic effect of mutagenic treatments (Nilan and Konzak, 1961; Gautam et al., 1998). Although, the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability (Devi and Mullainathan, 2011). According to Miller (1968) in spite of impaired seed production, the chlorophyll mutants are potentially useful in understanding of different physiological functions, various biochemical reactions and pathological invasion. The induction of chlorophyll mutations by physical and chemical mutagens has been reported in chickpea (Wani, 2004); blackgram (Gautam et al., 1992); Nigella sativa (Mitra and Bhowmik, 1999); rice (Ando and Montalvan, 2001); Limabean (Kumar et al., 2003); chili (Devi and Mullainathan, 2011); blackgram (Lal et al. 2009) and horsegram (Shirshat et al. 2010). Several chlorophyll mutants like chlorina, viridis, chlorotica, albina and xantha have been observed in these studies following treatments with physical or chemical mutagens and their combinations. A dose dependant increase in frequency of chlorophyll mutations has also been reported.

Hemavathy and Ravindran (2006) reported that in urdbean occurrence of albina was less than the other types, when treated with different doses of gamma rays. Maximum frequency of chlorina and xantha was recorded at higher doses of gamma rays. Giri and Apparao (2011) observed different chlorophyll mutants like chlorina, xantha, albina and striata in pigeonpea (*Cajanus cajan* L.) after treatments with EMS. The frequency of chlorophyll mutations increased at lower concentrations and decreased at higher concentrations of EMS. Higher frequency of chlorophyll mutations were recorded in 20Mm concentration, while lower frequency was observed in 40 mM concentration of EMS. Khan and Tyagi (2010) reported four types of chlorophyll mutants viz., albina, xantha, chlorina and viridis in gamma rays and gamma rays + EMS treated population of soybean. Gamma rays were found to be more effective in inducing chlorophyll mutations. Khan
et al., 2005 subjected seeds of two chick pea (*Cicer arietinum* L.) varieties; Avrodhi and BG-256 to EMS, SA and HZ with varying concentrations. Different types of chlorophyll mutants obtained included albina, chlorina, tigrina, viridis and xantha. It was observed that lower and moderate concentrations of EMS gave higher frequency of chlorophyll mutations whereas no such trend was noticed with SA and HZ.

In rice Bhan and Kaul (1976) noted an enhanced chlorophyll mutation frequency with increasing dose of gamma rays, EMS and dES alone and in combination. Albina type chlorophyll mutants constituted a major class in chlorophyll mutants in M₂ in both physical and chemical treatments. EMS was responsible for inducing significantly higher proportion of albina type than did gamma rays. Ando and Montalvan (2001) reported induction of different types of chlorophyll mutations viz., Albina, Viridis, Xantha, Tigrina and Striata in rice after treatments with gamma rays and SA. They reported the highest percentage of chlorophyll mutations in azide treatments, followed by the combined treatment and gamma rays. Among chlorophyll mutations the albina type was predominant followed by Viridis in the treated populations. Kumar et al. (2003) exposed seeds of Lima bean (*Phaseolus lunatus* L.) to gamma rays and Ethyl methane sulfonate (EMS). Results revealed that EMS was more pronounced in inducing chlorophyll mutations than gamma rays in M₂ generation and the frequency of Viridis type was more as compared to Albina. Further it was observed that the initial dose had given more percentage of chlorophyll mutants which then decreased with increasing dose / concentration of mutagens.

Chlorophyll development seems to be controlled by many genes located on several chromosomes (Goud, 1967) which could be adjacent to centromere and proximal segments of chromosomes (Swaminathan, 1964 and 1965). The origin of chlorophyll deficiencies is mainly due to mutations in genes, which are responsible for synthesis of photosynthetic pigments. The chlorophyll mutants are usually lethal but semi lethal and viable mutants are also known (Kothekar et al., 1994).

**Mutations affecting morphology**

Morphological mutations affecting different plant parts can be of enormous practical utility and many of them have been released directly as crop varieties (Shah et al., 2010). A wide range of morphological mutations induced by physical and chemical mutagens have been
reported in different crop plants such as blackgram (Raisinghani and Mahna, 1994), sesame (Mary and Jayabalan, 1995), barley (Ramesh et al., 2001), cowpea (Kumar et al., 2009), chickpea (Wani, 2011; Shah et al., 2011; Khan and Goyal, 2011). Rao and Jana (1976) subjected the seeds of Black gram (*Phaseolus mungo*) to X-rays and EMS treatments with the objective for obtaining some promising mutants. The induced leaf mutants scored comprised of crinkled leaf, waxy leaf, narrow leaf and unifoliate mutants. Crinkled leaf and waxy leaf mutants had normal fertility and vitality whereas the narrow leaf mutant was partially sterile and the unifoliate. An extreme dwarf mutant was also isolated which was completely sterile. Chandra and Tewari (1978) in bean (*Phaseolus aureus*) observed that increasing doses of gamma rays and neutrons caused a gradual reduction in germination of seeds and pollen and ovule fertility. Irradiation caused the appearance of leaf abnormalities including unifoliate, bifoliate, trifoliate, tetrafoliate and pentafoaliate characters. Under the influence of neutrons both tetra and pentafoaliate leaves were observed on the same plant apparently associated with enhanced luxuriance of plants which resulted in enhanced pod formation. Moh (1972) induced variations in seed coat colour of some black bean (*Phaseolus vulgaris*) varieties of Latin America. The seeds were treated with EMS and gamma rays and a special screening technique was employed for isolation of the potential mutants at a very early stage of seedling development, in which seed coat colour mutants were correlated with green hypocotyl colour. Mutagenesis resulted in inducing some seed coat colour mutants varying from white, yellow to various degrees of brown and their seed coat colour was associated with a change in hypocotyl colour from red to green. All these mutants were bearing white flowers instead of red in the parents, but their morphology, growth habit and disease resistance were similar to that of the parents. Mouli and Patil (1976) subjected peanuts (*Arachis hypogea*) to gamma irradiation. They isolated a suppressed branched mutant with larger leaves, altered flowering pattern, reduced shelling, smaller kernels and branch length as compared to normal in the autumn and spring growing seasons respectively. An extremely poor pod shelling was observed in autumn grown plants as compared to spring grown ones. Silva and Barbosa (1996) treated seeds of *Phaseolus vulgaris* L. with varying concentrations of sodium azide. Many morphological deviates were found in M$_2$, including reduction in plant height, reduced leaf size and thicker leaves. Anomalies prevailing in all treatments were three cotyledonary leaves, small and elongated leaflets usually with lighter colour, leaflets with folded margins and darker colour, and plants with shorter internodes and excess of branches. Sangsiri et
al., (2005) reported a number of mutant characters in gamma rays treated mungbean varieties. Mutant characters were grouped as chlorophyll, leaf, flower and pod mutants. Gamma ray induced morphological mutations have also been reported by Tah (2006) in Mungbean. Kumar et al., (2003) reported several viable mutants induced by gamma rays in Lima bean (*Phaseolus lunatus* L.) which included earliness, erect plants, profuse flowering and high yielding mutants. Wani (2011) reported a series of morphological mutants in chickpea isolated in separate and combined treatments of gamma rays and EMS. The various types of mutants reported included plant height, leaf, pod and seed mutants. Combination treatments in general were found more effective and efficient in inducing various types of morphological mutants.

Differences in the frequency of induced morphological mutations have been reported earlier by many workers (Tripathi and Dubey, 1992; Vandana and Dubey, 1994). Solanki and Sharma (1999) and Kharkwal (2001) reported that the chemical mutagens, particularly alkylating agents are more effective than ionizing radiations in inducing morphological mutations. The more frequent induction of certain mutation types by a particular mutagen has been attributed to the fact that the genes controlling these characters may be more responsive to chemicals or ionizing radiations. This could be due to differential mode of action of the mutagens on different base sequences in various genes. Nilan (1967) concluded that different mutagens and mutagen treatment change the relative proportion of different mutation types. It is possible that chemical mutagens may prove to be a better alternative for inducing morphological mutations, as they induce mutations at a much higher rate and cause less chromosomal disturbances than radiations (Sharma, 2001). Molina-cano *et al.* (2003) reported Mildew resistant mutants of *Hordeum vulgare* induced by sodium azide mutagen. Seeds of *Spathoglottis plicata* Blume, a terrestrial orchid were treated with sodium azide which induced strikingly attractive flower colour modification thereby improved its floricultural significance (Roy and Biswas, 2005). Increase in stearic acid content was induced in Sunflower up to 35% when treated with sodium azide mutagen (Scoric *et al.*, 2008). In mutagen treated populations, one would expect isolation of morphological mutatnts in *M*₂ and subsequent generations than in the *M*₁ generation. The absence of morphological mutation types in *M*₁ and their appearance in *M*₂ generation might be attributed to one of these two assumptions: (i) the induction of mutants, each of which was controlled by one or few number of recessive genes, in the *M*₁ and their segregation in a homozygous state in the *M*₂. (ii) The induction of mutants, in *M*₁, each of which was governed
by a number of genes, every gene had a small effect and the accumulation of such genes in one plant as a result of segregation in the second generation (Nofal et al., 2011).

**Mutagenic effectiveness and efficiency**

The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also on its efficiency. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen, while as 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, chromosome breakage, sterility, lethality, etc. (Konzak et al., 1965; Gaul et al., 1972). Thus, two agents may be equal in mutagenic effectiveness because, at a given dose, they induce a mutation with the same frequency. However, when they diverge in their ability to produce undesirable changes such as sterility and lethality then they may be said to differ in mutagenic efficiency. Studies on effectiveness and efficiency of the physical and chemical mutagens have been carried out in various crops by several workers (Khan, 1999; Solanki, 2005; Rekha and Langer, 2007; Basu et al., 2008; Shah et al., 2008; Wani 2009; Girija and Dhanavel, 2009; Shirsat et al., 2010).

The ethylated agents like Ethyl methane sulfonate (EMS) have been found more effective and efficient than physical mutagens in crops like chick pea (Kharkwal, 1998), Cowpea (Jhon, 1999), Lathyrus sativus (Waghmare and Mehra, 2001) and lentil (Gaikwad and Kothekar, 2004). Thilagavathi and Mullainathan (2009) reported that EMS was more effective and efficient for viable mutants than gamma rays in Black gram. Deepalakashmi and Kumar (2003) studied the efficiency and effectiveness of physical and chemical mutagens in Urdbean and reported that gamma rays were found to be more effective than EMS in causing lethality and sterility as well as in producing chlorophyll and viable mutants. Dhanavel et al. (2008) reported decrease in mutagenic effectiveness with an increase in concentration of EMS, DES and SA in cowpea. Shirsat et al. (2010) reported sodium azide more effective and efficient than EMS and NEU in horsegram.. Khan et al., (2005), after EMS, SA and HZ treatments in chickpea (Cicer arietinum L.) observed that the mutagenic effectiveness followed a dose dependent decreasing trend, the order of mutagenic effectiveness was HZ > SA > EMS. On the other and the order of efficiency varied depending upon the criteria used viz., seedling injury (MI/I), pollen sterility (MI/S) and
meiotic abnormalities (Mf/Me). In pea (*Pisum sativum* L.) results recorded for mutagenic effectiveness and efficiency (Dhulgande *et al.*, 2011) revealed that the EMS concentration exhibited the highest values of effectiveness followed by gamma rays treatment. Based upon different criteria used, the mutagenic efficiency varied for EMS and gamma rays treated populations in the two varieties under consideration. In general almost all studies on mutagenic effectiveness and efficiency have revealed lower or intermediate doses as most effective in inducing mutations. The decrease in effectiveness at higher dose / treatments has been attributed to the failure in proportional increase of mutation frequency induced at higher treatments (Singh and Chaturvedi 1980, Wani 2000). Mutagenic efficiency calculated on the basis of lethality, sterility, injury and meiotic aberrations with respect to induced morphological mutations in M$_2$ population basis has shown variation depending upon the criterion selected for its estimation. In general, the lower or intermediate dose treatments have proved to be most efficient on the basis of all the criteria used. Higher efficiency at lower and intermediate doses of mutagens has been explained due to the fact that the biological damage (lethality, injury and sterility) increased with the dose at a rate greater than the frequency of mutations (Konzak *et al.*, 1965).

**Induced variability in quantitative traits**

Breeding is the most commonly used method for crop improvement and genetic variability is the basis of any breeding program. Genetic variability is also important to adapt a population to the inevitable changes in the environment and to promote the survival of the species. Mutagenesis has proved to be a handy tool to enhance the natural mutational rate, thereby enlarging the genetic variability and increasing the scope for obtaining desired selections (Lagoda, 2007). The role of mutation breeding in increasing the genetic variability and its practical value for plant improvement programmes has been proved beyond doubt in several crop plants (Tickoo and Chandra, 1999; Waghmare and Mehra, 2000; Solanki and Sharma, 2002; Ahloowalia and Maluszynski, 2001; Ahloowalia *et al.*, 2004; Das and Misra, 2005; Khan and Qureshi, 2006; Wani and Anis, 2008; Khan and Goyal 2009). The inheritance of quantitative traits is controlled by the interaction of many genes or polygenes, with each gene contributing having an additive effect on the total phenotypic variability. In crop improvement programmes, it is the quantitative variation for yield and its component traits that is important to a plant breeder. Since most of the economically important characters are influenced by environment, estimates of
genetic parameters like phenotypic and genotypic coefficient of variation (PCV & GCV), heritability ($h^2$) and genetic advance (GA) in segregating generations of mutagen treated populations gives an indication about the scope of improvement in the trait concerned, through selection (Sheeba et al, 2003). It has been a consistent observation in mutation breeding experiments that mean values for various quantitative traits shift in both positive and negative direction, the positive shift being more pronounced at the lower or intermediate dose treatments, whereas negative shift being observed at higher dose treatments. Reduction of mean values for various quantitative traits in mutagenic populations has been attributed to induction of more mutations in negative direction, whereas, increase in mean could be due to induction of more positive mutations in the polygenes governing the character (Singh and Rao, 2008).

By using physical and chemical mutagens, many workers have found mean values for various quantitative traits decreasing significantly in M$_2$ generation (Brock, 1965; Scossiroli, 1966; Tickoo and Chandra, 1999; Muduli and Misra, 2008; Larik et al., 2009). They attributed the decline to either physiological damage caused chiefly by chemical mutagens or chromosomal aberrations caused mainly by irradiations. These disturbances get eliminated progressively in the subsequent generations. Another group of workers believe that mean remains unchanged although there is an increase in variance due to mutagenic treatments indicating bidirectional mutations (Upadhyaya and Singh, 1979). These workers believe that the mean performance of a population having equal proportion of favorable and unfavorable genes would remain unchanged since mutations in plus and minus direction will be equally likely. Shift in mean values in both positive and negative direction after mutagenic treatments has been reported by many workers (Sinha and Joshi, 1986; Singh et al., 2000; Waghmare and Mehra, 2000; Khan and Qureshi, 2006; Mensah and Obadoni, 2007). Since quantitative traits have a complex genetic constitution involving large number of genes interacting with one another, consequently variations in both directions is expected. The success of mutation breeding, however, largely depends on the effective selection process employed by the researcher. For isolation of promising mutant lines for yield and yield contributing traits in the segregating generations, the point mutations in genes controlling these traits are ideal. Thus several workers believe that selection of normal looking plants from non segregating M$_2$ families is effective in isolating promising lines for crop improvement (Sarkar and Sharma, 1988; Vanniarajan et al., 1996; Solanki and Sharma, 2001) while others believe that selection in M$_3$ is more effective than M$_2$ (Sharma, 1986). Sharma
(1986) concluded that efficiency of mutation breeding for polygenic traits could be increased by selecting M₁ plants with maximum damage, normal looking plants from the macromutational M₂ families, as well as non segregating families with high variance and desired shift in mean followed by confirmation of these selections in M₃. The estimates of genetic parameters like GCV, heritability and genetic advance are essential, since they indicate the degree of stability to the environmental fluctuations and the potential transmissibility of a character from parent to offspring and from generation to generation. The increase in variability associated with an increase in heritability and genetic advance of quantitative traits in the segregating generations is an indication that the induced variability is genetic in nature and effective selections could be made in these generations. While increase in heritability estimates, from M₂ to M₃ generation has been attributed due to increased homozygosity of genes controlling polygenic traits (Sharma and Sharma, 1982; Coimbra et al., 2004), the increase in heritability associated with increase in genetic advance particularly for yield and its contributing traits suggest the effectiveness of selection in early segregating generations. According to Johanson et al. (1955) heritability estimates along with genetic advance is usually more helpful than heritability alone in predicting the resultant effects of selection. Genetic advance is indicative of the expected genetic progress for a particular trait under selection (Kaul and Garg, 1982) and consequently carries much significance in self pollinated crops. The higher values of heritability and genetic advance suggest that mutations have mostly occurred at the loci having additive effects (Lawrence, 1965) and such traits are likely to respond effectively to phenotypic selection (Johnson, 1955; Sheeba et al. 2003).

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Mutant Varieties Database of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Available online:


