"Studies on the Induction of Mutations in Fenugreek (*Trigonella foenum-graecum* L.)"

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Certificate

This is to certify that the dissertation entitled **"Studies on the Induction of Mutations in Fenugreek (*Trigonella foenum-graecum* L.)"** being submitted to the Department of Botany, University of Kashmir for the award of Degree of Master of Philosophy in Botany is a research work done by **Shagufta Bashir** under our supervision. To the best of our knowledge and belief, no part of this work has been submitted to this or any other University in India for award of M.Phil degree or any other degree.

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The family Fabaceae or Leguminosae (also known as pea and bean family) is the third largest flowering plant family following Orchidaceae and Asteraceae. The family includes 727 genera with 19,235 species (Lewis *et al*., 2005). Legumes grow in diverse agro- ecological conditions, and the habit varies from herbs to gaint trees. Taxonomists have divided Fabaceae into three major subfamilies based on morphological features, particularly flower traits such as Paplionoideae [476 genera and 14,000 species], Caesalpinoideae [162 genera and 3000 species], and Mimosoideae [77 genera and 3000 species], (Doyle and Lucknow, 2003).

The family Fabaceae includes many useful crops for food, forage, fiber, wood and ornamental purposes. A few legumes such as chickpea, soybean, fababean, fenugreek, lentil, pea etc are consumed as grain legumes. The grain legumes are plants used as food in the form of unripe pods, mature seeds or immature dry seeds, directly or indirectly (Rachie and Roberts, 1974). The grain legumes not only provide variety to human diet but also supply dietary proteins for vegetarian populations who do not consume animal and fish proteins in their diet. Considering the growing problem of malnutrition, use of legume species as high protein food is very important. Moreover, legumes are also capable of symbiotic nitrogen fixation enriching the soil condition suitable for both mix and alternate cropping (Bromfield, 2001).

The genus *Trigonella* is one of the largest genera of the tribe Trifoliatae in the family Fabaceae and sub family Paplionoideae (Balodi and Rao, 1991). The generic name, *Trigonella*, comes from Latin meaning “Little Triangle,” in reference to the triangular shape of the small yellowish-white flowers. The

species epithet *foenum-graecum* means 'Greek Hay' and according to Rosengarten (1969) the Romans, who got the plant from Greece where it was a very common crop in ancient times, gave it this name.

Fenugreek, an annual legume cash crop of India (Fazli and Hardman, 1968) is cosmopolitan in distribution. In several parts of Asia, the young plants are used as pot herbs and the seeds as a spice or as herbal medicine (Lust, 1986; Petropoulos, 2002). The species name '*foenum- graecum*' means "Greek Hay" indicating its use as a forage crop in the past (Petropoulos, 2002).

Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and Traditional Chinese Medicines (Tiran, 2003). According to Lust (1986) fenugreek is one of the oldest known medicinal plants in the recorded history. Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Basch *et al*., 2003).

Fenugreek is reported to have anti-diabetic, anti-fertility, anti-cancer, anti-microbial, anti-parasitic and hypocholesterolemic effects (Al-Habori and Raman, 2002). In India, Fenugreek is used as a lactation stimulant (Tiran, 2003). Fenugreek seed in powdered or germinated form exhibits anti-diabetic properties (Hannan *et al*., 2003; Thakaran, 2003; Broca *et al*., 2004) hypocholesterolemic effects (Venkatesan, 2003; Suboh *et al*., 2004), anti-cancer effects (Devasena and Menon, 2003) and effect on thyroxine- induced hyperglycemia (Tahiliani and Kar, 2003).

Fenugreek has been reported to generate a high yield of good quality forage and hay or silage (Mir *et al*., 1998). As forage, it is bloat free, unlike alfalfa and contains animal growth promoting substances (diosgenin) not present in any other forage legumes (Mir *et al*., 1997). These monohydroxyl sapogenins (i.e., diosgenin and yamogenin) are used as precursors in steroid hormone semi synthesis (Anis and Aminuddin, 1985; Bruneton, 1995). Other pharmacological properties of Fenugreek include anti-inflammatory, antiulcer, sexual stimulant

(Mishkinsky *et al*., 1997) as well as diuretic (Tanira *et al*., 1989) and antioxidant (Ravikumar and Anuradha, 1999) etc.

The biological and pharmacological properties of fenugreek are attributed to the variety of its constituents, namely; steroids, N –compounds, polyphenolic substances, volatile constituents, amino acids etc. (Mehrafarin *et al*., 2010). Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fibre (galactomannans), 20-30% proteins high in lysine and Tryptophan, 5-10% fixed oils (lipids), Pyridine alkaloids, mainly trigonelline (0.2 - 0.38%), choline (0.5%), free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 - 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin), cholesterol and sitosterol, vitamin A, B_1, C and nicotinic acid (Budavari, 1996; Newall *et al*., 1996; Mehrafarin *et al*., 2010).

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.

The application of mutagenesis in agriculture for improving the crop plants presented a new departure from the conventional breeding methods. 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.

The induction of mutation 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 (Kharakwal, 1996). These findings supplement that mutagenesis is a potential tool to be employed in the crop improvement.

In fenugreek, several workers have tried for artificial induction of mutations through the use of mutagens (Laxmi and Datta, 1986; Abbasi and Anis, 2002; Basu *et al*., 2008). Despite the release of different cultivars, fenugreek production has not increased to any noticeable extent over the last 1-2 decades. The present work is therefore, designed to evaluate the morphological and cytological effects of some mutagens (physical and chemical) in fenugreek with the main objective of inducing changes in the genotype to enhance genetic variability in this plant so as to broaden its genetic base for selection of

desirable genotypes for commercial cultivation. The main objectives of the present study were:

1. To study the effect of different mutagenic treatments on various biological parameters.
2. To investigate the chromosome behaviour of treated populations with respect to controls
3. To quantify the magnitude of the genetic variability induced in various quantitative traits.
4. To isolate promising mutants based on changes in phenotypic traits.

2.1 Distribution

Fenugreek is an important cultivated crop in parts of Europe, Northern Africa, West and South America and Australia (Fazli and Hardman, 1968; Edison, 1995; Jongbloed, 2004; Acharya *et al*., 2006). Major fenugreek producing countries are Russia, India, Pakistan, Germany, Argentina, Egypt, Canada, Iran, Canada, USA and China (Basu, 2008). India is the largest producer of fenugreek in the world where Rajasthan, Gujarat, Uttaranchal, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states (Debranjan and Tara, 2010). Rajasthan produces the lion's share of India's production, accounting for over 80% of the nation's total fenugreek output.

2.2 Origin of Fenugreek

Fenugreek (*Trigonella foenum-graecum* L.) locally known as methi in India is an annual crop of the Legume family. The carbonized fenugreek seed was recovered by Saraswat (1984) from a Rohira village in the Sangrur district of Punjab, India indicating its use in trade by people of the Harappan civilization as far back as 2000–1700 B.C. Fenugreek is one of the oldest known medicinal plants recognized in recorded history (Lust, 1986).

There are varied opinions about the probable ancestry of *Trigonella foenum-graecum.* Vavilov (1951) suggested that fenugreek is native to the Mediterranean region of the “Old World”, while, de Candolle (1964) and Fazli and Hardman (1968) proposed an Asian origin for the crop. Dangi *et al*. (2004) have suggested that *T. aerulea* and *T. foenum-graecum* originated in Turkey. Many authors believe that the species of *T. foenum-graecum* evolved from *T. gladiata*, which had possibly given rise to some new extinct forms of *T. foenum-graecum* (Petropoulos, 2002).

There are divergent opinions about the exact number of species of fenugreek Linnaeus suggested existence of as many as 260 species of fenugreek

(Petropoulos, 2002) while as 128 species of fenugreek were reported by Vasil'chenko (1953), 70 by Hector (1936) and Hutchinson (1964) and 97 by Fazli (1967). A total of 18 different species of fenugreek (*Trigonella*) are currently recognized in the primary literature. Some of the common species are: *T. anguina, T. arabica, T. caerulea, T. corniculata, T. cariensis, T. rigida, T. suavissima, T. torulosa, T. spinos, T. polycerata, T. radiata, T. platycarpos, T. hamosa, T. cretica, T. occulta, T. arcuata* and *T. striata* (Tutin and Heywood, 1964; Fazli and Hardman, 1968; Petropoulos, 2002).

2.3 Ploidy level

According to Darlington and Wylie (1945) the haploid chromosome number (n) of *Trigonella* can be 8, 9, 11 or 14. Most species including *Trigonella foenum-graecum* L. are diploids; with 2n =16. However, *T. hamosa* from Egypt was found to have 16 and 44 chromosomes; *T. polycerata* from the Mediterannean region of South West Asia has 28, 30 and 32 chromosomes and *T. ornithopodioides* is reported to have 18 chromosomes. These data suggest that some *Trigonella* species have undergone several rounds of chromosome doubling and rediploidization through gene and chromosome elimination (Petropoulos, 2002).

Singh and Singh (1976) isolated five double trisomics from *Trigonella* along with primary trisomics from the progeny of autotetraploids which had a chromosomal constitution of 2n + 1 + 1 = 18. Roy and Singh (1968) also produced tetraploid Fenugreek by treating shoot apices with colchicine. Joshi and Raghuvanshi (1968) have demonstrated that chromosome number in fenugreek can increase through the presence of B–chromosome. Ahmad *et al.* (1999) has reported B- chromosomes in many fenugreek accessions.

2.4 Mutagenesis as a means of crop improvement

Mutation means a sudden heritable change in the genetic material at the gene or chromosome level (Chahal and Gosal, 2002). Mutations are the tools used to

study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliya, 2007). Mutagenesis alters the genetic makeup of plants by interference and modification of genes (Koornneef, 2002). Mutants with new alleles and genes are created which enhances genetic variation (Koornneef, 2002). Production of heritable changes is an important aspect of many breeding programmes and breeders use mutations to produce these changes (Neuffer *et al*., 1997).

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.*

Mutation breeding is an important method 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. The principles of induced mutations in seed propagated crops were established in the forties, mainly by Gustafsson in 1941. During the past seventy years, worldwide more than 2250 varieties have been released through induced mutations (Maluszynski *et al*., 2000). 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). Induced mutagenesis has been successfully utilized in several crop plants viz., rice (Chakrabarti, 1995), common beans (Nichterlein, 1999), artemesia (Rekha and Kak, 1997; Rekha and Langer, 2007; Chickpea (Wani

and Anis, 2008) suggesting the potential of this technique for crop improvement.

Many agronomically important mutations affecting plant and grain characters have been identified, including alteration of grain color, stem rust resistance and earliness in wheat (Chopra, 2005). The success of mutation breeding in ornamentals and horticultural crops in India is impressive with 46 mutant varieties commercially released (Chopra, 2005). Mutation breeding has been successfully used for turning the non–edible oil from Linseed (*Linum usitatissimum*), into an edible seed oil (Linola).

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 (Rehman *et al*., 1987; Robbelen, 1990; Mahla *et al*., 1990; Rehman, 1996; Shah *et al*., 1990, 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). 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. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001).

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 (Kharakwal, 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).Gamma rays generally influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Gunckel and Sparrow, 1961). There are some reports which showed that higher exposures of gamma rays were usually inhibitory (Radhadevi and Nayar, 1996; Kumari and Singh, 1996), whereas, lower exposures were sometimes stimulatory (Sparrow, 1966; Taylor, 1968; Raghava and Raghava, 1989; Thapa, 1999).

Sjodin (1962) reported that the material and energy necessary for initial growth are already available in the seed, and so the young embryo has no need to form new substances, but only to activate those already stored in the cotyledons. The role of low doses of gamma radiations may be to increase the enzymatic activation and awakening the young embryo, which result in an increasing rate

of cell division, which effects not only germination, but also vegetative growth and flowering. Exposing the dry seeds to low γ-irradiation doses resulted in increasing yield of some plants such as Sunflower (Abo-Hegazi *et al*., 1988) and *Ammi visnage* (El-Shafie *et al*., 1993).

Gustafsson (1947) observed the occurrence of induced, useful mutations in Barley for such characters as height of straw, earliness and lateness, strength of straw, chemical properties, brewing characteristics, protein content, 1000 grain weight and littering capacity. These observations led him to suggest that the radiations could be used as a new tool in plant breeding.

Induced mutation through gamma rays have played a significant role in the alteration of plant architecture and selection of mutants with enhanced yield potential in rapeseed and mustard (Rahman, 1996; Shah *et al.*, 1999). Uma and Salimath (2001) in cowpeas, Ashraf *et al.* (2003) in basmati rice and Khatri *et al.* (2005) in *Brassica juncea* reported early flowering due to gamma irradiation. Kaul and Singh (1972) subjected dormant seeds of *Datura metel* to different doses of gamma rays with an aim to investigate the effect of gamma rays on its seeds, on the growth and metabolic activities of the plant and frequency and spectrum of viable mutations. Irradiation with different doses resulted in the production of chromosomal aberrations including deletions, duplications and translocations both at mitosis as well as meiosis. With increasing doses of radiations increase in seed lethality, seedling injury and production of chimeral effects such as morphological freaks were observed. In M_1 Treated population an increase in the concentration of total alkaloids was noted which was mere at lower doses of irradiation.

Developing rice embryos at various stages of their development were irradiated by Bhaduri and Brahmachari (1976) *in vitro* and *vivo*. A number of mutations were obtained after the irradiation of developed embryos as compared to the irradiation of undeveloped embryos. It was found that with the progress in the

development of embryos their resistance to X-rays gets increased. Irradiation treatments resulted in variations in both positive and negative directions.

Sodium azide is known to affect the seed germination, shoot length, and root length and also induces high frequency of chlorophyll deficient mutations. Reduction in seed germination in mutagenic treatments has been explained due to delay or inhibition in physiological and biological processes necessary for seed germination which include inhibition of mitotic process (Sato and Gaul, 1967), hormonal imbalance (Ananthaswamy *et al*., 1971) and enzyme activity (Chrispeeds and Varner, 1976),

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).

Nilan *et al.* (1973) used sodium azide as a mutagen in barley for the first time. They reported high frequency (17.3%) of chlorophyll mutations at pH 3, a low frequency at pH 7 and no effect at pH 11.

Rao and Rao (1983) produced higher proportions of albinos in rice by applying Methyl Methane Sulfonate (MMS), N-nitrous-N-methyl Urethane (NMU) and Hydroxyl amine (HA) as mutagens. Reddy and Vishwanathan (1999) induced rust resistance in hexaploid wheat variety “WH147” by using gamma rays and EMS.

Joshua *et al*. (1974a) observed that combined treatment of fast neutrons and diethyl sulphate on barley resulted in synergistic effects on both anaphase fragments and bridges. Storage of seeds after neutron irradiation had no significant effect on frequency of fragments and bridges. Joshua *et al*. (1974b)

further studied the effect of fast neutrons and gamma rays on seedling height and chromosome aberrations in barley. In response to the treatments a synergistic effect in seedling height was noted. Further the presence of chromosome fragments and anaphase bridges showed an additive effect with combined treatments.

Wanjari and Phadnis (1977) subjected the seeds of *Mamordica charantia* L. to different concentrations of colchicine to induce polyploidy. All treatment concentrations of colchicine proved to be lethal when sprouted seeds were treated. Subjecting pre-soaked seeds to 0.3% colchicine was found effective in inducing tetraploidy. Increased doses resulted in decreased germination, poor survival and poor plant growth at early stages. Induced tetraploids were devoid of vigorous growth and exhibited delayed flowering. However, their foliar size was bigger than that of diploids. Meiotic studies in tetraploids revealed multivalent formation at metaphase–I and irregularities such as laggards during anaphase.

Raut and Thombre (1977) were successful in inducing tetraploidy in *Impatiens balsamina* L. by treating its seeds with 0.05 and 0.1% colchicine. In colchicine treated population plants showing arrested growth, height retardation, abnormal plumules and leaf shape were observed. Autotetraploids possessed bigger flower size and longer blooming period.

2.5 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 460Gy (var. G130) and 483Gy (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. Kharakwal (1981) reported higher lethality in 0.2% EMS in comparison with 400 and 500Gy 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), 200Gy 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).

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; Wani, 2007). Dose linked effectiveness of EMS and gamma rays were noted in chickpea in terms of germination, reduction in pollen fertility, chlorophyll mutations and seedling height (Khanna, 1991; Parveen, 2006). Similar findings were also reported in *Pearl millet* (Singh *et al*., 1978), *Arachis hypogea* (Venkatachalam and Jayabalan, 1995), *Nigella sativa* (Mitra and Bownick, 1999) *Vigna radiata* (Singh and Chaturvedi, 1980; Khan *et al*., 2004) and *Lens culinaris* (Khan, 2002; Wani, 2003).

2.6 Mutagenic sensitivity

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. The effects of

physical and chemical mutagens can be compared by studying parameters like reduction in germination, reduction in plant height, delay in emergence of M_1 seedlings and induction of micro and macro mutations in M_2. Such parameters are considered as the main indices for the overall response of a mutagenic agent.

Same mutagen dose can cause different degrees of effect in different species. 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 Bownick, 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 Jayabalan (1995) while using EMS, SA and gamma rays found distinct differences in ground nut (*Arachis hypogea*). Distinct varietal differences to SA in *Vigna radiata* was observed by Khan *et al*. (2004). Geeta and Vaidyanthan (1977) observed different phenotypic response of two soybean cultivars to ethidium bromide and gamma rays. Differences to radio-sensitivity were also reported by Khan (1999) in black gram and Nerker (1976) in *Lathyrus sativus*. Akbar *et al*. (1976) concluded that differences in radiosensitivity may be due to differences in their recovery process including enzyme activity. In chickpea, Kharakwal (1998) and Parveen (2006) reported that varieties of desi type were more resistant towards mutagenic treatments than kabuli type.

Mutagenic response to cytological aberrations has been reported by many workers (Rao and Laxmi, 1980); Suganthi and Reddy, 1992; Rehman, 2000). Mitra and Bownick (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. Kharakwal (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 M_2 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. Freese (1963) and Heslot (1977) gave a detailed account of chemical mutagens like ethyl methane sulphonate (EMS) and diethyl sulphate (dES). The mutagenic action of EMS was studied earlier in *Drosophila* (Fahmy and Fahmy, 1957), bacteriophage (Loveless, 1959), barley and wheat (Gustafsson, 1960; Ehren berg, 1960; Swaminathan *et al*., 1962). Gaul (1964) in barley observed that EMS was capable of producing more number of various morphological mutants as compared to gamma rays. At moderate level, EMS is known to react preferentially with guanine and cytosine (Freese, 1963).

2.7 Biological damage

The effects of physical and chemical mutagens and their combination treatments on different biological parameters such as germination, survival, injury and sterility 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 Bownick, 1999; Khan and Wani, 2005). Reduction in seedling height following treatments with gamma rays and EMS was observed in barley (Sharma, 1970).

Gupta and Yashvir (1975) reported a radio-protective effect of EMS in *Abelmoschus esculentum*. The combined treatments of gamma rays and EMS showed higher germination percentage than in corresponding EMS treatments. Chaudhary (1983) reported a symmetric reduction in germination in different varieties of wheat with higher doses of gamma rays. Parveen (2006) reported the effect of seed treatment with different concentration of EMS on germination and growth of seedlings in chickpea. There was a proportionate decrease in germination percentage with the increasing concentrations of EMS.

The effect of gamma rays, EMS and their combination on M_1 parameters in barley was studied by Khalatkar and Bhatia (1975). They observed that the seedling injury, chromosomal aberrations, pollen and seed sterility were less in combined treatments than in separate treatments. 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 M_1 generation arising due to physiological, chromosomal and factor mutations. Khan and Siddiqui (1987) studied the effect of Methyl Methane Sulphonate (MMS) on pollen fertility in the Var. T-9 of urdbean. A direct relationship of pollen and ovule sterility with higher doses of gamma rays and EMS in *Vigna mungo* was reported by Gautam *et al*. (1992). Increase in pollen sterility and decrease in germination with increasing doses of gamma rays in *Capsicum annuum* was reported by Rao and Laxmi (1980).

The mutation rate of NMU was found to be 1.5-2.0 times higher than gamma rays on plant survival and sterility (Sharma and Sharma, 1981) in microsperma and macrosperma lentils. Rapoport (1966) has called the alkylating agents as super mutagens in view of their higher mutagenic effect. The EMS treatment was found to cause higher sterility than gamma rays in chick pea (Kharakwal, 1981). Mutagenic efficiency based on injury and lethality was found higher in combined treatments of gamma rays and NMU than their respective individual

treatments (Dixit and Dubey, 1986). Combined treatments also 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. Reduction in seed germination with increase in dose of gamma rays in chickpea was reported by Khanna and Maherchandani (1981) and Khanna (1991).

Lal *et al.* (2009) studied mutagenic sensitivity of gamma rays, sodium azide and their different combinations in M_1 generation of black gram and observed that an increase in azide concentrations resulted in decrease in M_1 germination. The plant survival was also affected with different doses of gamma rays and SA. The combination treatments of gamma rays and SA had more depressive effect on seedling growth.

2.8 Induction of cytological abnormalities

The study of chromosomal behaviour during mitosis and meiosis is considered to be a suitable method for evaluating the effect of mutagens and also helps in determining the radio-sensitivity of plants to both physical and chemical mutagens. Auerbach and Robson (1942) presented first elaborate report that Mustard gas could induce mutations as well as chromosomal aberrations in *Drosophila*.

Gamma rays, MH and their combination treatments have been shown to induce disturbed mitotic 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. The combined treatment enhanced chromosomal aberrations. Similarly, the meiotic process was also affected. The quadrivalents presumably due to translocations, were occasionally encountered on metaphase-I. Irregular

disjunction of chromosomes at anaphase-I, accompanied by laggards was also observed.

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.Var.G-65 was found to be more sensitive with treatments of 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 misorientation 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. However, EMS treatments induced higher proportion of anomalies in pairing whereas DES induced higher proportion of anomalies during anaphasic disjunction.

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 followed by gamma rays and EMS. Khan and Tyagi (2009) reported bridges and laggards in soybean when treated with EMS, gamma rays and their combination.

In maize, sticky chromosomes were first reported by Beadle (1932) and are seen as intense chromatin clustering in the pachytene stage. 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 (Erikisson, 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).

Katyayani *et al*. (1980) studied the mutagenic effects of Maleic Hydrazide (MH) and Ethyl methane sulfonate (EMS) on germinating seeds of *Trigonella foenum-graecum* L. Results showed that higher concentrations of EMS (0.05-0.1%) and MH (0.1%) exercised retarding effects on seedling growth while low concentrations of both the chemicals, particularly 0.001% EMS and up to 0.05% MH resulted in its promotion. Induction of binucleate, trinucleate and tetranucleate conditions and some chromosomal aberrations including bridges, fragments were noted in 0.01 and 0.001% concentrations of MH and EMS,

respectively. It was also observed that seed treatment with 0.001 and 0.01% MH and EMS resulted in induction of early flowering.

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). The first description was made by Gates (1908), who observed delicate threads of cytoplasm connecting adjacent pollen mother cells in *Oenothera*. Gates (1911) subsequently suggested that these connections must form an important avenue of exchange between PMC's and described the transfer of nuclear material through them from one meiocyte to another, calling the process 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).

Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities (Pagliarini and Pereira, 1992; Pagliarini *et al*., 1993; Consolaro *et al*., 1996; Khazanehdari and Jones, 1997). In most of the mungbean varieties, pollen fertility showed a close relationship with meiotic abnormalities (Khan, 1990). The least mutation frequency at higher doses may be attributed to chromosomal aberration or saturation in the mutational events which may result in the elimination of mutant cells during growth (Blixt and Gottschalk, 1975).

Sharma and Kumar (2004) treated seeds of two cultivars viz., CSG-89.62 and KPG-59 of *Cicer arietinum* L. with four different concentrations i.e., 0.1, 0.2, 0.3 and 0.4% of ethylmethane sulphonate. They 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 in both the cultivars. However, cultivar KPG-59 showed more chromosomal abnormalities as compared to cultivar CSG-89.62 at the same treatment.

Goyal and Khan (2009) treated seeds of two varieties, PU-19 and T-9 of Urdbean (*Vigna mungo* L.) Hepper) with four concentrations (0.1, 0.2, 0.3 and 0.4%) of EMS and (0.01, 0.02, 0.03 and 0.04%) of 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 were encountered in both the laser and gamma rays treatments but the most frequent anomaly dominated was the stickiness of chromosomes. The percentage of chromosomal aberrations observed in case of gamma rays treated set was higher than laser rays suggesting that gamma rays could be successfully employed for creating additional genetic variability in Safflower.

2.9 Chlorophyll mutations

Chlorophyll mutations are one of the important criteria to determine effectiveness of the mutagens. Chlorophyll mutations are easily detectable and

have been extensively used to find out sensitivity of crop plants to mutagens. According to Miller (1968) inspite of impaired seed production, the chlorophyll mutants are potentially useful in understanding of different physiological functions, various biochemical reactions and pathological invasion. Several chlorophyll mutants like chlorina, viridis, chlorotica, albina and xantha have been observed following treatments with physical or chemical mutagens and their combinations (Thakare, 1988; Vandana, 1991; Singh *et al.*, 1999; Arulbalachandran and Mulainathan, 2009; Khan and Tyagi, 2009).

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 40mM 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 order to study the frequency and spectrum of chlorophyll mutations in rice in relation to the genotype and nature of the mutagen Bhan and Kaul (1976) treated the seeds of three rice varieties with gamma rays and two alkylating

agents (EMS and dES) alone and in combination. They noted an enhanced chlorophyll mutation frequency with increasing dose but the dose showing 90 % seedling lethality showed a drop in mutation frequency. Albina type chlorophyll mutants constituted a major class in chlorophyll mutants in M_2 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) treated seeds of rice (*Oryza sativa* L.) cultivar IAC-1246 with gamma rays and sodium azide (SA). They observed induction of different types of chlorophyll mutations viz., Albina, Viridis, Xantha, Tigrina and Striata. They reported that the azide treatment recorded the highest percentage of chlorophyll mutations, 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 M_2 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.

2.10 Mutations affecting morphology

Plant morphology is considered to be an important tool for isolation of desirable mutants. Several induced morphological mutations have been reported in literature showing alterations in the morphology of various plant parts.

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*) var. S-8 and var. Pusa Baisakhi 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 pentafoliate characters. Under the influence of neutrons both tetra and pentafoliate leaves were observed on the same plant of cv. S-8 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. Though the varieties under improvement were disease resistant and good yielding yet were considered inferior because of their seed coat colour. The seeds were treated with EMS and gamma rays and a special screening technique was employed in which seed coat colour mutants were correlated with green hypocotyl colour, for isolation of the potential mutants at a very early stage of seedling development. Mutagenesis resulted in inducing some seed coat colour mutants who varied 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. Further studies revealed that these induced characters were recessive and their inheritance followed a simple Mendalian manner.

Kaul and Chaudhry (1972) conducted mutagenic studies in *Atropa belladonna* after exposing its seeds to different doses of gamma rays. Studies were aimed at assessing the variability in polygenic characters released in M_1 and M_2

generations of *belladonna*. A higher variability was noted in M_2 generations than in M_1. After observing a greater variability for tiller number and alkaloid content than that for plant height and leaf length, it was inferred that different characters may respond differently to different mutagenic treatments.

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.

Narsinghani and Kumar (1976) in a mutation breeding programme subjected the seeds of Cowpea (*Vigna sinensis* L.) to EMS and MMS treatments. In M_1 and M_2 generations, reduction in survival percentage, mean pod number, seed yield per plant and average pollen fertility was observed which was less in M_2. A few long podded mutants, chlorophyll mutations and leaflet modifications were also recorded. Meiotic studies revealed the presence of reciprocal translocations, inversions and other anomalies. In comparison to M_1 a decrease in total aberrations was recorded in M_2.

Silva and Barbosa (1996) treated seeds of *Phaseolus vulgaris* L. cv. Milionaro 1732 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) treated seeds of mungbean varieties KPS 2, VC 6468-11-1B with gamma rays. A number of mutant characters were recorded in M_2 generation. Mutant characters were grouped as chlorophyll, leaf, flower and pod mutants. Gamma ray induced morphological mutations have also been

reported by Morishita (2001) in Buckwheat and 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.

2.11 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, chromosomes breakage, sterility, lethality, etc. (Konzak *et al.*, 1965; Gaul *et al.*, 1972). Studies on effectiveness and efficiency of the physical and chemical mutagens have been carried out in various crops by several workers (Khan *et al*., 1988; Badami and Bhalla, 1992; Khan, 1999; Mehraj-ud-din *et al*., 1999; Koli and Ramakrishna, 2002).

The ethylated agents like Ethyl methane sulfonate (EMS) have been found more effective and efficient than physical mutagens in crops like chick pea (Kharakwal, 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 producing chlorophyll and viable mutants. Gamma rays were also found more efficient in causing lethality and sterility.

Dhanavel *et al.* (2008) reported decrease in mutagenic effectiveness with an increase in concentration of EMS, DES and SA in cowpea. It is obvious that the higher efficiency at lower and intermediate doses of mutagens may be due to the fact that the biological damage (Lethality and sterility) increased with the dose at a rate greater than the frequency of mutations (Konzak *et al.*, 1965).

Shirsat *et al*. (2010) treated two varieties of horsegram, viz., SINA (K-42) and KS-2 with three concentrations of EMS (0.05%, 0.1% and 0.125%), SA (0.001%, 0.002% and 0.003%) and NEU (0.001%, 0.003% and 0.005%). In M_2 generation SA was found more effective followed by Nitroso ethyl urea in both the varieties. In variety SINA the highest effectiveness was seen at 0.001 % SA treatment and the lowest value at 0.125 % EMS. In case of variety KS-2, the highest effectiveness value was recorded at 0.001% SA concentration and lowest value at 0.10 % EMS. SA was also found more efficient than EMS and NEU in both the varieties of horsegram.

Khan *et al.* (2005) exposed seeds of two chickpea (*Cicer arietinum* L.) varieties viz., Avrodhi and BG-256 to EMS, SA and HZ for 6 hours. They observed that the mutagenic effectiveness for EMS and SA followed a dose dependent decreasing trend in both varieties. In case of HZ, mutagenic effectiveness exhibited a dose dependent increase but decreased abruptly at the highest dose in both the varieties, the order of mutagenic effectiveness was HZ > SA > EMS. To determine the efficiency of the mutagens three criteria viz., seedling injury (Mf/I), pollen sterility (Mf/S) and meiotic abnormalities (Mf/Me) were taken into consideration. Based on seedling injury, the order of efficiency was HZ > EMS > SA whereas on the basis of sterility, it was EMS > HZ > SA.

Based on meiotic aberrations induced, EMS was found to be efficient followed by SA and HZ in var. Avrodhi and HZ proved to be the most efficient mutagen followed by EMS and SA in var. BG-256.

Dhulgande *et al*. (2011) treated seeds of two varieties of pea (*Pisum sativum* L.) namely DDR-53 and DMR-55 with varying doses/concentrations of gamma rays and EMS. Results recorded for mutagenic effectiveness and efficiency revealed that the EMS concentration induced the highest values of effectiveness followed by gamma rays treatment in both the varieties. It was further observed that the lower dose or concentration of the two mutagens proved to be most effective than the higher ones in all the mutagenic treatments. The effectiveness values decreased with increasing dose or concentration of the mutagens. For mutagenic efficiency, it was noted that the efficiency decreased for lethality , pollen sterility and mitotic aberrations from EMS concentration to gamma rays in variety DDR-53 while in variety DMR-55 the efficiency for lethality and pollen sterility increased from EMS to gamma rays but efficiency for mitotic aberrations decreased from EMS to gamma rays .

2.12 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. The role of mutation breeding in increasing the genetic variability for quantitative traits in various crop plants have been proved beyond doubt (Singh and Yadav, 1991; Vyas and Chauhan, 1994; Khan *et al.*, 1994, 1998, 1999; Khan and Siddiqui, 1995; Das and Chakraborty, 1998; Jabeen and Mirza, 2002; Kumar and Mishra, 2004; Khan and Wani, 2006; Singh *et al*., 2006; and Khan and Goyal, 2009).

Jabeen and Mirza (2002) treated the seeds of Capsicum annuum cv. Longhi with varying concentrations of EMS. Data was recorded on eight different

characters in M_1 generation including leaf area, number of leaves, number of branches, height of plants, days to flowering, days to fruiting, number of fruits per plant and chlorophyll content. Results have shown that the variance was increased for all the characters under study in the treated populations compared with control suggesting an increase in genetic variability. However, it was noted that increase in EMS concentration resulted in gradual decrease of seed germination. EMS has been widely used for Fenugreek improvement with respect to steroidal sapogenins and oil constituents (Petropoulos, 1973, 2002; Zhu *et al*., 2003).

Wani and Khan (2006) treated the seeds of mungbean var. PDM-11 with EMS (0.1% and 0.2%) and Hydrazine hydrates (HZ) (0.01% and 0.02%) to induce mutations. Results revealed that the variability in the treated population was higher than the control for all the quantitative traits, namely fertile branches per plant, pods per plant, 100 seed weight and seed yield per plant. However, EMS was found to be more effective. Similar increases in the number of pods of some other varieties of urdbean have been reported by Tickoo and Chandra (1999) using EMS, Nitroso methyl urea, Hydroxyl amine and gamma rays.

Mensah *et al*. (2007) exposed seeds of Sesame (*Sesame indicum* L.) to varying concentration of sodium azide and colchicine solutions ranging from 0-0.250%. They observed dose related effects of the mutagenic treatments on quantitative traits resulting in reductions in traits such as germination and survival percentages, plant height, number of fruit per plant, but increase in leaf area, maturity time and fruit size. Low doses of both mutagens (<0.125%) produced early maturing and robust / high yield variants.

Yakoob and Rashid (2001) reported that various quantitative traits can be improved in various genotypes through variable gamma ray doses. Kumar and Rai (2006) studied the effect of different doses of gamma rays (100Gy to 500Gy) in soybean. They have shown that with increasing doses of gamma rays the pollen germination percentage and fertility continuously decreased as

compared to control. However, the pollen tube lengths showed an improvement over control up to 100Gy followed by simultaneous decrease at higher doses. Among the morphological parameters, seed setting was found to be adversely affected along with the increasing doses of gamma rays. Number of seeds and number of pods/plant also registered a considerable decrease over the control along with the increasing doses of gamma rays except for 100Gy dose at which number of pods/plant showed a slight enhancement over control.

In some cases reduction in flower size was also noticed. Kon *et al*., 2007 exposed seeds of long bean (*Vigna sesquipedalis)* to different doses of gamma rays (300, 400, 500, 600 and 800Gy). The study revealed that germination percentage, plant height, survival percentage, root length, root and shoot dry weight decreased with increasing dose of gamma ray. The 800Gy gamma ray in particular had a pronounced effect on these morphological characteristics probably because of injury it might have caused to the seeds of Long bean. As a result, poor growth and development was noticed.

Das and Prasad (1978) studied the influence of differential and combined treatment of gamma rays (10-30 KR) and Methyl Ethane Sulfonate (0.2%) on some varieties of *Lathyrus sativus* L. It was observed that height of the plant and number of branches per plant showed dose dependent reduction in all the varieties in M_1 and both increase and decrease in M_2 as compared to control.

Wani and Anis (2001) treated seeds of two Chickpea varieties viz., Avrodhi-T3 and KPG-59 with gamma rays and EMS, separately as well as in combination. Data on germination percentage, percent survival and pollen sterility were recorded. Seed germination and plant survival decreased. Whereas, pollen sterility increased with an increase in dose/concentration of the mutagens. Combination treatments proved to be more effective than the individual treatments.

Trigonella foenum-graecum commonly known as fenugreek, an annual dicotyledonous herbaceous plant belongs to the family Leguminosae with branched stems, trifoliate ovate-orbicular leaves, roots bearing nodules, white flowers, paplionaceous corolla, stamens diadelphous [1+(9)], ovary superior, ovules many, pods bearing golden yellow seeds. Seeds vary from rectangular to round in outline with a deep groove between the radical and cotyledons. In general, two types of flowering shoots are observed. The common ones bear axillary flowers showing an indeterminate growth habit, whereas so called "blind shoots" have axillary and terminal flowers, becoming "tip bearers" for seed pods. Both cleistogamous (closed) and aneictogamous (open) flowers have been described (Petropoulos, 1973) but the vast majority of fenugreek flowers are closed or cleistogamous.

3.1. Materials

3.1.1. Variety used

Pure line seeds of local variety of *Trigonella foenum-graecum* L. were used in the present study. The certified, healthy and dry seeds (10% moisture content) of this variety were procured from Sher-i-Kashmir University of Agricultural Sciences and Technology (SKAUST-K), Srinagar . This variety

is well adapted to the agro climatic conditions of Kashmir including the experimental site [Kashmir University Botanical Garden- KUBG].

3.1.2. Mutagens used

The seeds of fenugreek were treated with different doses / treatments of physical and chemical mutagens . The physical mutagen used was gamma rays and chemical mutagens included Ethyl Methane Sulfonate (EMS) and Sodium azide (SA).

3.1.2.1. Gamma rays

Uniform healthy dry seeds (10% moisture content) of the fenugreek were exposed to different doses of gamma rays (100Gy, 200Gy, 300Gy and 400Gy) with a dose rate of 14.5 Kr/hr. from ^{60}Cobalt source at the Babha Atomic Research Centre (BARC) Zakura, Srinagar.

3.1.2.2. Ethyl methane sulphonate [EMS (CH_3 OSO_2 C_2H_5)]

Fenugreek seeds were treated with different concentrations of EMS (HI MEDIA) (0.1%, 0.2%, 0.3% and 0.4%).

3.1.2.3. Sodium azide [SA (NaN_3)]

Seeds of fenugreek were treated with varying concentrations of SA (LC-LOBA) (0.1%, 0.2%, 0.3% and 0.4%).

3.2. Preparation of mutagenic solution

One percent stock solution of EMS and SA was prepared and from this stock solution different concentrations of EMS and SA were prepared by using the formula $N_1V_1 = N_2V_2$, where,

N_1 = Strength of stock solution

Table 1: Details of Mutagenic Treatment given to fenugreek seeds

Mutagen used	Dose/conc.	Duration of presoaking (hrs.)	Duration of treatment (hrs.)
Control	DDW	12.0	–
Gamma rays (Gy)	100	–	–
	200	–	–
	300	–	–
	400	–	–
EMS (%)	0.1	12.0	6.0
	0.2	12.0	6.0
	0.3	12.0	6.0
	0.4	12.0	6.0
SA (%)	0.1	12.0	6.0
	0.2	12.0	6.0
	0.3	12.0	6.0
	0.4	12.0	6.0

N = 150, pH = 7

V_1 = Volume of stock solution

N_1 = Strength of desired solution

N_2 =Volume of desired solution

The specificity of action of chemical mutagen depends upon the particular conditions of treatment, the more important of which are temperature and hydrogen ion concentration. In the course of present study, EMS and SA solutions were prepared by dissolving appropriate quantity of this chemical in Sorensen's phosphate buffer having a pH of 7.0 and the final pH was adjusted to 7.0 by adding few drops of normal NaOH solution.

3.2.1. Method of treatment with chemical mutagen

Prior to the mutagenic treatment, the seeds were presoaked in distilled water for a period of 12hr. Seed presoaking allows the cells to reach a metabolic state when they are relatively more sensitive to mutagenic action. The control seeds were also immersed in distilled water for the same duration. Thus the control seeds although not treated with the chemical mutagens, were exposed to similar physiological conditions before sowing as that of treated seeds. The seeds were given intermittent shaking throughout the treatment period (6hr) to provide sufficient aeration. For uniform absorption, large quantities of mutagenic solution, approximately three times the volume of seeds (Konzak *et al*., 1965) were used. After the completion of treatment period, the seeds were thoroughly washed in running tap water for 20min to remove the excess chemicals from the seed surface before they were sown in the field.

3.2.2. Sample size

A set of 150 seeds were chosen for each dose/treatment including the control. Out of these 150 seeds, one hundred seeds for each treatment and control were sown in the field for morphological and cytological studies, whereas the remaining set of 50 seeds was allowed to germinate on moist cotton in Petriplates for measuring root-shoot length.

3.2.3. Sowing of seeds in the field

Nursery beds were prepared for sowing seeds and raising M_1 generation. In April, 2010, the treated as well as untreated (control) seeds were sown in three replicates in a Complete Randomized Block Design (CRBD) at the Kashmir University Botanical Garden, (KUBG), Srinagar. The distance between the seeds along a row was kept 5cm whereas row to row distance was maintained at 10cm in each experimental plot in a replication.

3.3. Mechanism of action of physical and chemical mutagens

a) Physical mutagens

Gamma rays are the most energetic form of electromagnetic radiation, possessing the energy level from 10 kilo electron volts (kev) to several hundred kev, and they are considered the most penetrating in comparison to other radiation such as alpha and beta rays (Kovacs and Keresztes, 2002). The common sources of gamma rays are ^{137}Cs (half-life 30 years, energy 0.66 Mev) and ^{60}Co (half-life 53 years, energies 1.33 Mev, 1.17 Mev). Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modification of the anti-oxidative system and accumulation of phenolic compounds (Kim *et al*., 2004, Wi *et al*., 2005).

The biological effects produced by a radiation depend upon the total amount of radiation energy absorbed or delivered in an organism. The dose of radiation is measured as the amount of energy absorbed per unit mass of the irradiated object and is commonly expressed as rad. One rad equals 100 erg/g or 10^{-2} j/kg of irradiated object. The unit of radiation dose is called Gray (Gy). Gy equals 100 rad or 1 J/kg of the irradiated object.

b) Chemical mutagen

Ethyl methane sulphonate, a mono functional alkylating agent is a colourless liquid soluble in water with a boiling point 85.86^{o}C/10mm Hg.

In the action of EMS on biological systems, triester is the main target. It has been established by many workers that primary reaction centres in the

DNA are the alkylation of its phosphate groups. The resulting triester is unstable and tends to lose the alkyl group. The unstable phosphate triester can hydrolyse between the phosphate and deoxyribose resulting in the backbone breakage.

EMS is known to induce alkylation of purine and pyrimidine bases. Alkylation is thought to occur most readily with guanine at position N_7 followed by Adenine at N_3 and rarely with N in cytosine. However, no reaction with thymine has been detected. Alkylation of the nitrogenous ring ultimately leads to the removal of the alkylated base i.e., depurination or depyrimidation and may lead to back bone breaks.

EMS is also known to induce transitions e.g., alkylation of guanine results in the formation of o^6-ethyl guanine , which can pair with thymine (T) but not with cytosine (C). Through subsequent DNA repair, the original G/C pair can then be replaced with A/T (Greene *et al*., 2003).

In majority of cases (99%), EMS induces C-to-T changes resulting in C/G to T/A substitutions (Kreig, 1963).

Sodium azide, an ionic compound and highly soluble in water is one of the most powerful mutagens in crop plants. Sodium azide has been reported to induce high frequency of point mutations (base substitutions) and few detectable chromosomal aberrations (Nilan *et al*., 1973). The mutagenicity is mediated through the production of an organic metabolite of azide compound (Owais and Klienhofs, 1988).This metabolite enters into the nucleus, interacts with DNA and creates point mutations in the genome. Azide ion (N_3^-) plays an important role in causing mutation by interacting with enzymes and DNA in the cell. These azide anions (N_3^-) are strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation process. In addition it is a potent inhibitor of the proton pump (Kleinhofs *et al*., 1978) and alters the mitochondrial membrane potential (Zhang, 2000). These effects, caused by NaN_3, together may hamper ATP biosynthesis resulting in decreased

availability of ATP molecule which may slow the germination rate and reduce the germination percentage.

3.4. Evaluation of M_1 generation

3.4.1. Seed germination

The data on seed germination was recorded right from the emergence of first shoot in each treatment including control. After recording the data, percentage of seed germination was calculated by using the formula

$$\text{Germination (\%)} = \frac{\text{No.of seeds germinated}}{\text{No.of seeds sown}} \times 100$$

3.4.2. Seedling height (cm)

Seedling height was estimated on 9^{th} day of germination by measuring root and shoot lengths of 15 randomly selected seedlings from each treatment as well as control. Seedling injury as measured by the reduction in root and shoot length and calculated in terms of percentage of root and shoot injury.

$$\text{Percent injury} = \frac{\text{Control-Treated}}{\text{Control}} \times 100$$

3.4.3. Plant survival

The surviving plants in different treatments were counted at the time of maturity and the survival percentage and percent lethality were calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100$$

$$\text{Lethality (\%)} = \frac{\text{Control-Treated}}{\text{Control}} \times 100$$

3.4.4. Cytological studies

Cytological studies were carried out on pollen mother cells by fixing young flower buds from each treatment as well as control. The purpose of fixation is to kill the tissue without causing any distortion of the components to be studied

and facilitate proper staining of the tissues. It should not only increase visibility of the chromosome structure but should also clarify the details of chromosome morphology such as chromatin or heterochromatin regions and primary and secondary constrictions.

Flower buds of appropriate size were collected randomly from 10-15 plants from each concentration including control and fixed in 1:3 aceto-alcohol solutions (1part glacial acetic acid and 3 parts absolute alcohol) for 24hrs. Thereafter, the buds were rinsed in alcohol and transferred in 70% alcohol for preservation. Anthers were squashed in 1% aceto-carmine stain for meiotic analysis (Swaminathan *et al*., 1954). Preliminary observations were taken from temporary slides. The slides were later made permanent by using n-butyl alcohol and glacial acetic acid schedule (Bhaduri and Ghosh, 1954). Analysis of various stages of meiosis was done from fresh and temporary preparations, chiefly at metaphase and anaphase stages. The photomicrographs were taken from temporary as well as permanent slides with the aid of photo micrographic unit (Leica DM LS2) at USIC, Kashmir University using 10x eyepiece × 100x 0bjective lense. Percent meiotic abnormalities were calculated by using the following formula:

$$\text{Percent meiotic abnormalities} = \frac{\text{Total number of meiotic abnormalities}}{\text{Total number of PMCs}} \times 100$$

3.4.5. Pollen fertility

The pollen fertility was estimated from fresh pollen samples. The pollen grains which took up the stain and have a regular outline were considered as fertile, while the hyaline (empty) ones without stain and having irregular shape were considered as sterile. The following formulae were used to calculate percent fertility and sterility.

$$\text{Pollen fertility \%} = \frac{\text{Number of fertile pollen}}{\text{Total number of pollen}} \times 100$$

$$\text{Pollen sterility \%} = \frac{\text{Control-treated}}{\text{Control}} \times 100$$

3.4.6. Quantitative characters of M_1 generation

The following morphological parameters were recorded in M_1 generation

i) Plant height (cm): The height of 15 randomly selected plants was measured from the point above the ground to the tip of the main axis of the plant.

ii) Number of pods per plant: Total number of pods per plant for a selected number of 15 plants from each concentration including control was recorded.

iii) Length of pods per plant (cm): The length of five pods per plant from 15 randomly selected plants in each treatment including control was recorded.

iv) Number of seeds per pod: Five pods per plant from 15 randomly selected plants in each treatment were used to calculate the mean seeds per pod.

v) Seed yield per plant (g): Randomly selected 15 plants per treatment were used for calculating the mean seed yield per plant.

3.5. Evaluation of M_2 generation

Seeds from each M_1 plant were harvested separately in treated as well as control populations. Thereafter the seeds in each treatment were bulked and a random sample of 300 seeds was selected from the bulk for raising M_2 generation. Each replicate containing 100 seeds, thus a total of 4800 seeds including control were used for raising M_2 population.

3.5.1. Observations recorded in M_2 generation

i) Chlorophyll and Morphological mutations

The treated as well as control populations were carefully screened for chlorophyll mutations from the emergence till the age of 4-5 weeks after germination, whereas morphological mutations were scored throughout the growth period of plants in the field. For identification and classification of

mutations Gustafsson method (1940) was followed with suitable modifications. Mutation frequency was calculated by the following formula:

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutated plants}}{\text{Total number of plants}} \times 100$$

ii) Mutagenic effectiveness and efficiency

Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen, whereas mutagenic efficiency is the ratio of frequency of mutations to various biological damages induced in M_1 generation like injury, lethality, sterility and meiotic aberrations. Mutagenic effectiveness and efficiency of gamma rays, EMS and SA were calculated by using the formulae as given by Konzak *et al.* (1965).

1) Mutagenic effectiveness

a) Physical mutagen: $\text{Effectiveness} = \frac{\text{Mutation frequency (Mf)}}{\text{Dose in Kiloroentgen (Kr)}} \times 100$

b) Chemical mutagen: $\text{Effectiveness} = \frac{\text{Mutation frequency (Mf)}}{\text{(Conc. of mutagen)} \times \text{(time of treatment)}} \times 100$

2) Mutagenic efficiency

$$\text{Efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{\% lethality (L)or \% injury (I)or \% sterility (S)or \% meiotic abnormalities(M)}}$$

3.5.2. Quantitative characters of M_2 generation

Observations were recorded on 15 normal looking plants from treated as well as control populations. The plants segregating for macromutations were not used for such analysis. The following five quantitative traits were thoroughly studied in M_2 generation.

1. **Plant height (cm):** The height of plant was measured at maturity in centimeters from the base up to the apex of plant.
2. **Number of pods per plant:** These were counted at maturity and recorded as the number of pods borne on the whole plant.

3. **Pod length (cm):** Mean length of five pods per plant was first calculated followed by the calculation of pooled mean from different mean values.
4. **Number of seeds per pod:** Mean seeds for five selected pods per plant were first calculated and then the pooled mean was calculated from different mean values.
5. **Seed yield per plant (g):** It was first recorded as the weight of total number of seeds harvested per plant followed by the calculation of pooled mean from different mean values.

3.6. Statistical analysis

Data collected for above mentioned morphological characters in M_1 and M_2 generations were subjected to statistical analysis to assess the extent of induced variations, as indicated below:

a) Mean ( ): The arithmetic mean was computed by taking the sum of a number of observations ($X_1 + X_2 + X_3 \ldots + X_n$) and dividing it by total number of observations (N) recorded, thus

$(X) = X_1 + X_2 + X_3 \ldots\ldots + X_{15}/N$

$(X) = \sum X/ N$

Where (X) = arithmetic mean

$\sum x$ = sum of all values of the variable, X i.e., $X_1, X_2, X_3 \ldots\ldots X_{15}$

N = number of observations

b) Standard deviation (S.D): standard deviation is the positive square root of the average of squares of deviations of all observations from their means. It is computed by applying following formula.

$S.D. = \sqrt{(X_1 - X)^2 + (X_2 - X)^2 + (X_3 - X)^2 \ldots\ldots\ldots + (X_{15} - X)^2 / N}$

Where (X) = mean of observations involved

X_1, X_2, X_3 = observations

N = number of observations

c) Coefficient of variability: It measures the relative magnitude of variation present in observations relative to magnitude of their arithmetic mean. It is defined as a ratio of standard deviation to arithmetic mean expressed as percentage, and is a unit less number. The following formula was applied to compute coefficient of variability (C.V.)

$$C.V. = \text{——————} \times 100$$

$$C.V. = \frac{\cdot}{(\ )} \times 100$$

Where S.D. = Standard deviation

(X) = arithmetic mean

d) Analysis of variance was performed to determine variations and differences between populations, using software SPSS-10. Treated and untreated population mean comparisons were made using the Tukeys HSD test at the p=0.05 level.

4.1. STUDIES IN M_1 GENERATION

The mutagenic effects of gamma rays, EMS and Sodium azide were studied on seed germination, seedling height, plant survival, pollen fertility, chromosomal aberrations and various quantitative characters in M_1 generation of fenugreek.

Data on seed germination, plant survival and pollen fertility are presented in Table 2.

4.1.1. Seed germination

Germination percentage was found to be significantly reduced in all the mutagenic treatments (Figs. 1-3). The maximum inhibition in germination was recorded at higher treatments of all mutagens. Seed germination was about 95% in control. In gamma rays it ranged from 72% (100Gy) to 42% (400Gy). In case of EMS and SA treatments it ranged from 75% (0.1%) to 49% (0.4%) and 78% (0.1%) to 61% (0.4%) respectively.

The pooled mean values showed that gamma rays were most effective in reducing the seed germination followed by EMS and SA (Table 2).

4.1.2. Plant survival

Plant survival was higher in control (93.68%) than in all the three mutagenic treatments (Table 2; Figs 1-3). Plant survival tended to decrease with the increase in the dose / concentration of mutagens except 300Gy in gamma rays where it was slightly higher (75%) as compared to 200Gy (74.07%). In case of gamma rays highest lethality was observed at 400Gy (33.92%). In both EMS and SA treatments maximum lethality was noticed at 0.4% concentration (28.11% and 30.01% respectively).The pooled mean values for survival

percentage depicted maximum survival in case of SA (80.88%) followed by EMS (79.29%) and gamma rays (74.27%) [Table2].

4.1.3. Pollen fertility

Data recorded showed higher percentage of pollen fertility in control (95.42%). The effect of physical and chemical mutagens on pollen fertility was uniform, in that, higher concentrations led to decrease in pollen fertility. In other words, there was increase in pollen sterility with an increase in dose /conc. of the mutagen. The maximum sterility was observed at 0.4% EMS (58.01%). In case of gamma rays and SA treatment maximum sterility was at 400Gy and 0.4% (56.06% and 42.40%) respectively. The pooled mean values have shown that EMS was most effective followed by gamma rays and sodium azide in reducing pollen fertility (Table 2; Figs 1-3).

4.1.4. Seedling height

Data recorded on seedling height measured in terms of root+shoot length is presented in Table 3. It is evident from the Fig. 4 that seedling height decreased with an increase in dose / concentration of mutagens. Control populations exhibited the highest seedling height of 7.78cm. Among the mutagenic treatments maximum injury was recorded in 400Gy treatment (74.29%) followed by 0.4% SA treatment (63.75%). The pooled mean values for seedling height and percent injury indicated that gamma rays were more effective followed by EMS and SA treatments.

Table 2: Effect of gamma rays, EMS and SA on seed germination, plant survival and pollen fertility in M_1 generation in *Trigonella foenum-graecum* L.

Treatments	Germination (%)	Plant survival (%)	Lethality (% L)	Pollen fertility (%)	Sterility (%S)
Control	95.00	93.68	–	95.42	–
Gamma rays					
100Gy	72.00	86.11	8.08	75.53	20.85
200Gy	54.00	74.07	20.93	67.04	29.74
300Gy	48.00	75.00	19.94	55.80	41.52
400Gy	42.00	61.90	33.92	43.94	53.95
Mean	**54.00**	**74.27**	**20.72**	**60.58**	**36.51**
EMS					
0.1%	75.00	88.00	6.06	78.67	17.55
0.2%	80.00	83.75	10.60	68.76	27.93
0.3%	73.00	78.08	16.65	52.30	45.19
0.4%	49.00	67.35	28.11	40.07	58.01
Mean	**69.25**	**79.29**	**15.35**	**59.95**	**37.17**
SA					
0.1%	78.00	91.02	2.84	81.68	14.40
0.2%	72.00	87.50	6.60	71.96	24.59
0.3%	73.00	79.45	15.19	65.35	31.51
0.4%	61.00	65.57	30.01	57.60	39.64
Mean	**71.00**	**80.88**	**13.66**	**69.14**	**27.53**

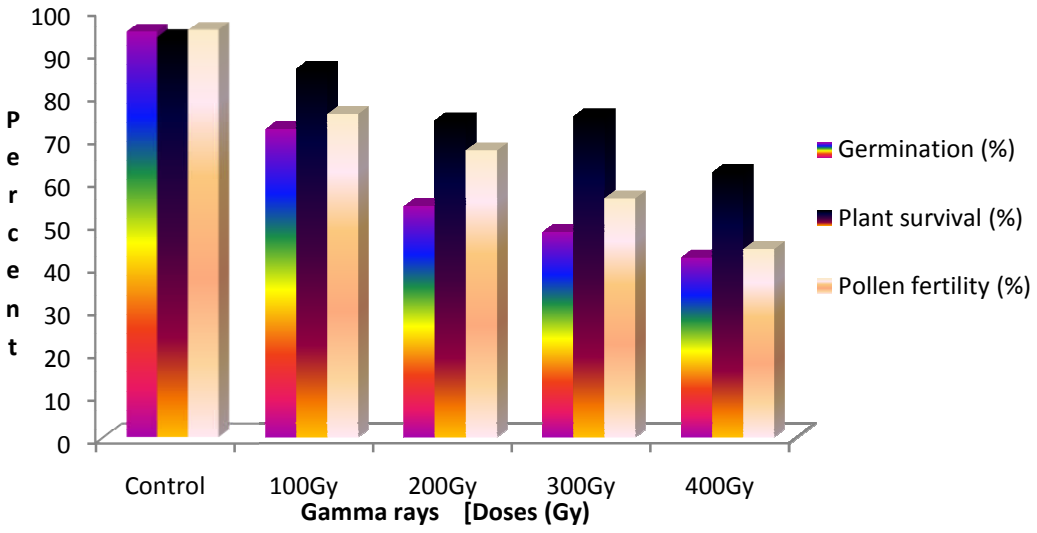


Fig. 1: Graphic representation of Gamma rays on seed germination, plant survival and pollen fertility in *Trigonella foenum-graecum* L.

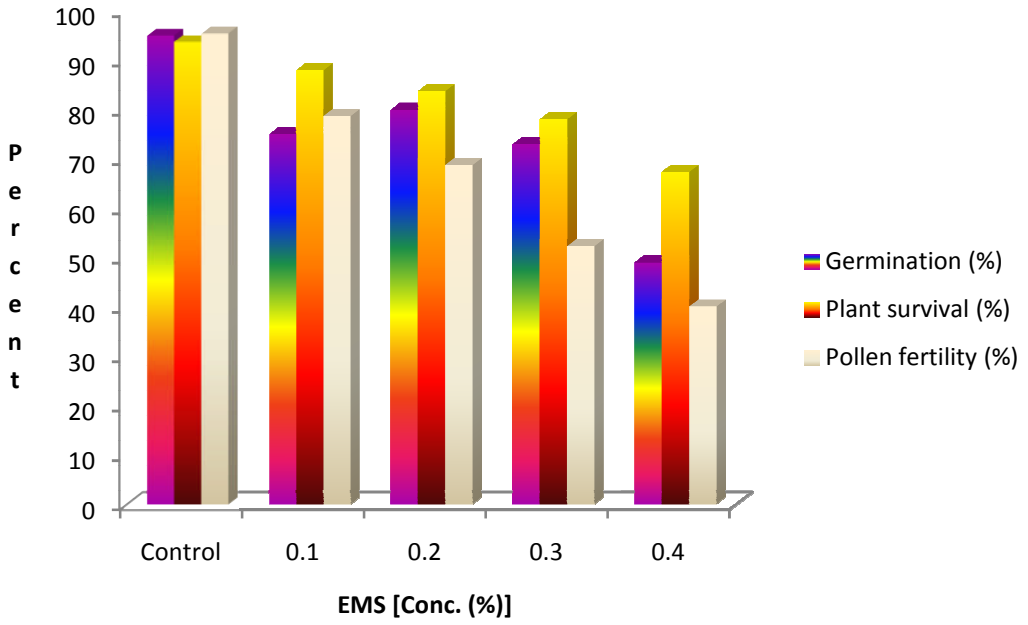


Fig. 2: Graphic representation of EMS on seed germination, plant survival and pollen fertility in *Trigonella foenum-graecum* L.

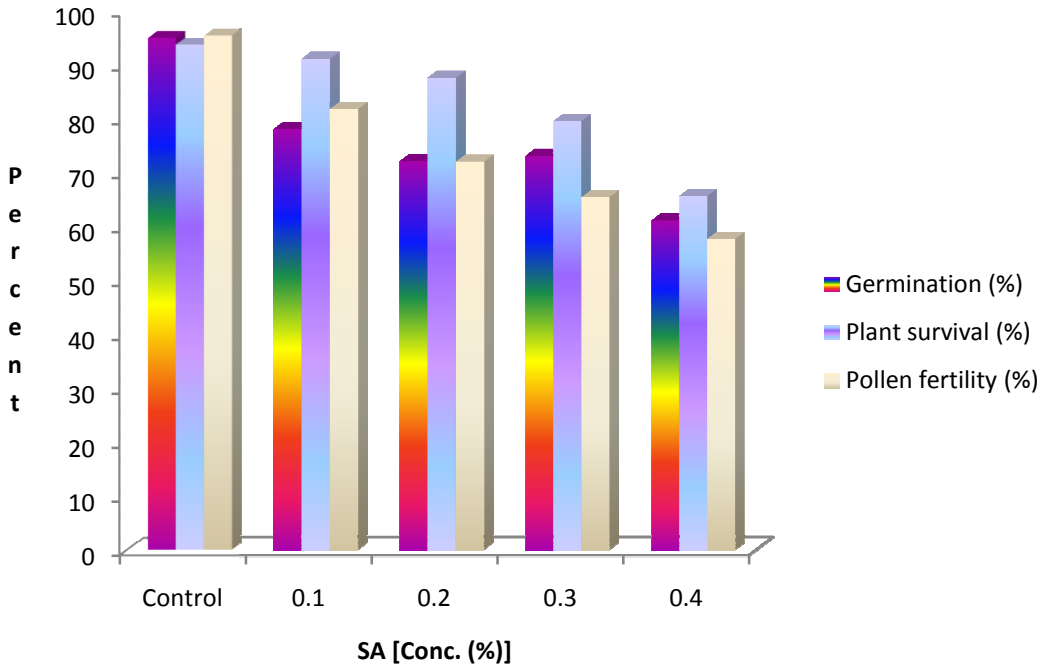


Fig. 3: Graphic representation of SA on seed germination, plant survival and pollen fertility in *Trigonella foenum-graecum* L.

4.1.5 Meiotic studies

Meiosis in the control (diploid) was regular and without any irregularities with 8 bivalents (2n=16) observed both at diakinesis and metaphase -1. Chromosome segregation was normal at anaphase and telophase stages resulting in normal tetrads. The data on chromosomal behaviour in control and treated populations is presented in the Table 4, Plate 1-4, Fig. 5). The treated populations exhibited various frequencies of meiotic abnormalities such as stickiness, univalents, precocious segregation, laggards, bridges, disturbed polarity and cytomixis. From the table it is clear that the frequency of chromosome abnormalities increased with increase in doses / concentrations of each mutagen.

Table 3: Effect of gamma rays, EMS and SA on seedling height [root+shoot length (cm)] in *Trigonella foenum-graecum* L.

Treatments	Root length (cm)	Shoot length (cm)	Total length (R+S)	Percent injury (I)
Control	3.36	4.42	7.78	–
Gamma rays				
100Gy	2.96	4.30	7.26	6.68
200Gy	2.40	3.46	5.86	26.37
300Gy	1.76	2.14	3.90	49.87
400Gy	0.98	1.02	2.00	74.29
Mean	**2.02**	**2.73**	**4.75**	**39.30**
EMS				
0.1%	2.92	4.02	6.94	10.80
0.2%	2.62	3.18	5.80	24.45
0.3%	2.24	2.34	4.58	41.13
0.4%	1.64	1.30	2.94	62.21
Mean	**2.35**	**2.71**	**5.06**	**34.65**
SA				
0.1%	3.14	4.26	7.40	4.88
0.2%	2.62	3.50	6.12	21.34
0.3%	2.02	2.32	4.34	44.21
0.4%	1.38	1.44	2.82	63.75
Mean	**2.29**	**2.88**	**5.17**	**33.54**

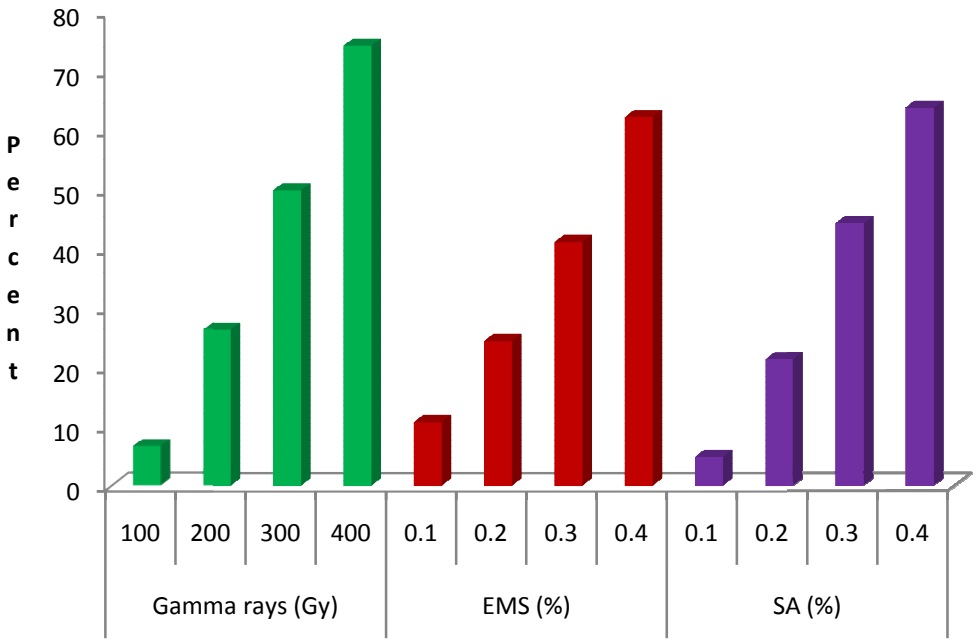


Fig. 4: Percentage of seedling injury induced by Gamma rays, EMS and SA in *Trigonella foenum-graecum* L.

Chromosomal abnormalities at metaphase-1: The frequency of PMC'S showing stickiness at metaphase-1 (Table 4, Plate 2, Fig. 7) ranged from 1.13% to 2.88% in case of gamma rays and from 0.96% to 2.58% in case of EMS and from 1.07% to 1.96% for SA treatments.

The frequency of univalents at metaphase-1 (Plate 2, Fig. 9) was highest at 400Gy (2.16%) followed by SA (1.78%) and EMS (1.66%) at 0.4% respectively.

Precocious separation or early disjunction of chromosomes was also observed at metaphase-1 in some PMC'S (Plate 2, Fig 8).The maximum frequency of precocious segregation was observed at 400Gy (1.96%) followed by EMS (1.84%) and SA (1.60%) at 0.4% respectively.

Table 4: Frequency of Meiotic abnormalities induced by Gamma rays, EMS and Sodium azide in *Trigonella foenum-graecum* L.

	At metaphase				At anaphase and telophase				
Mutagen	**Total no. of PMC'S Scanned**	**Stickiness**	**Univalents**	**Precocious Segregation**	**Laggards**	**Bridges**	**Disturbed polarity**	**Cytomixis**	**Total abnormalities (%)**
Control	547	-	-	-	-	-	-	-	-
Gamma rays									
100Gy	530	1.13	0.94	0.75	0.56	0.94	1.32	0.37	6.01
200Gy	563	1.95	1.42	1.06	0.89	1.42	1.78	0.53	9.05
300Gy	547	2.19	1.83	1.64	1.46	2.01	1.83	0.91	11.87
400Gy	556	2.88	2.16	1.98	1.80	2.70	2.34	1.26	15.12
EMS									
0.1%	520	0.96	0.76	0.38	0.57	0.76	0.96	0.19	4.58
0.2%	560	1.42	1.07	0.89	0.71	1.25	1.60	0.53	7.47
0.3%	534	1.68	1.49	1.31	0.93	1.87	2.05	0.74	10.07
0.4%	541	2.58	1.66	1.84	1.47	2.03	2.21	1.29	13.08
Sodium azide									
0.1%	557	1.07	0.72	0.54	0.36	0.54	0.36	0.18	3.77
0.2%	569	1.23	0.88	0.70	0.53	0.88	0.35	0.88	5.45
0.3%	543	1.84	1.29	1.10	0.74	1.47	0.55	0.92	7.91
0.4%	561	1.96	1.78	1.60	1.25	1.42	1.07	1.42	10.50

Abnormalities at anaphase and telophase

The chromosome abnormalities frequently observed at anaphase and telophase included laggards, bridges, disturbed polarity and cytomixis.

The frequency of laggards ranged from 1─4 or sometimes more per PMC (Plate 2 & 3, Figs 10-13). Bivalent laggards were also observed in some cells. The highest frequency of laggards was noticed in 400Gy (1.80%) followed by EMS at 0.4% (1.47%) and 0.4% SA (1.25%). Bridges were commonly observed at anaphase and telophase stages (Plate 3, Figs 14-17). The frequency of bridges was maximum at 400Gy (2.70%) followed by EMS at 0.4% (2.03%) and SA at 0.3% (1.47%). Disturbed polarity of chromosomes at telophase was observed in some PMC's of treated population (Plates 3 & 4, Figs 18-19). Among the three mutagens maximum frequency was induced by gamma rays at 400Gy (2.34%). Cytomixis i.e. cytoplasmic connection between two or more than two PMC's was observed in some PMC's induced by mutagenic treatments which may lead to the transfer of genetic material (Plate 4, Figs 20-24). Among the mutagenic treatments maximum frequency was induced by 0.4% SA (1.42%) followed by 0.4% EMS (1.29%) and 400Gy gamma rays (1.26%).

The overall percentage of meiotic aberrations showed an increasing trend with dose/conc. of the mutagen. Maximum frequency of meiotic aberrations was recorded at 400Gy (15.12%) followed by 0.4% EMS (13.04) and 0.4% SA (10.50%). In general gamma rays induced greater percentage of chemical aberrations followed by EMS and SA in the present study (Fig 5). The order of effectiveness was gamma rays>EMS>SA.

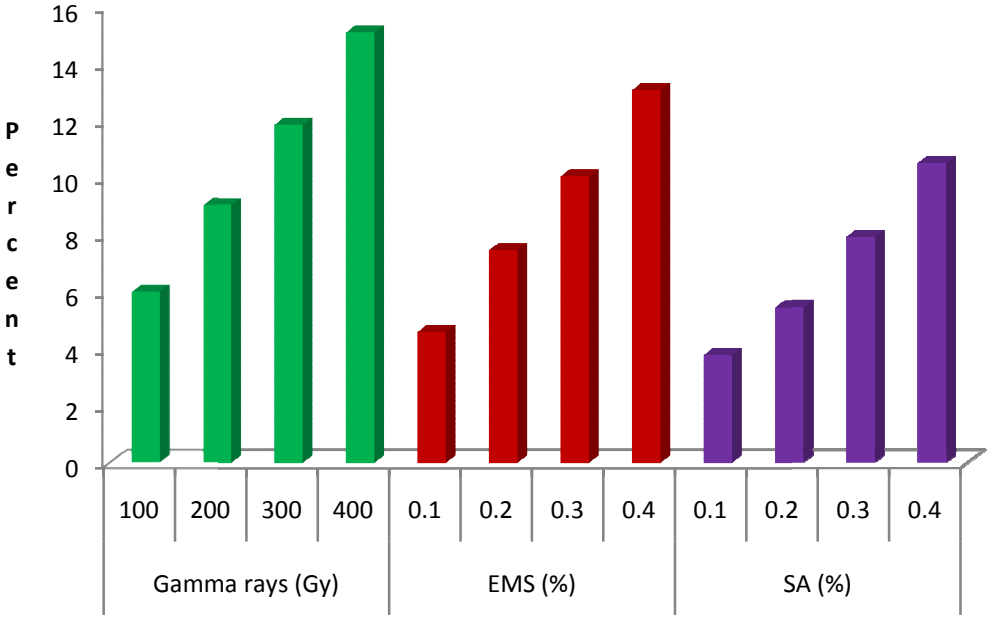


Fig. 5. Percentage of meiotic abnormalities induced by Gamma rays, EMS and SA in *Trigonella foenum-graecum* L.

4.1.6. Studies on quantitative characters

Five quantitative traits viz., plant height, number of pods per plant, pod length, number of seeds per pod and seed yield were taken into consideration for evaluating the effect of gamma rays, EMS and SA in M_1 generation of fenugreek. Data recorded on all these quantitative traits are presented in Tables 5-8.

i) Plant height (cm)

Mean plant height of control ranged from 35.9cm to 41.6cm with the mean value of 38.68±1.67cm. In mutagenic treatments the maximum plant height was recorded at 0.2% SA (41.67cm) and 0.2% EMS (40.08).

The variability measured in terms of coefficient of variation increased in all the mutagenic treatments as compared to control (4.32%). Maximum coefficient of variation was recorded for 400Gy (9.29%). Pooled mean values have shown that mean plant height was maximum in SA (38.62cm) followed by EMS

(37.75cm) and gamma rays (37.65cm). In other words gamma rays were most effective in reducing mean plant height followed by EMS and SA (Table 5).

Table 5: Effect of Gamma rays, EMS and Sodium azide on plant height (cm) in M_1 generation of *Trigonella foenum-graecum* L.

Treatments	Range (cm)	Mean ± SD	CV (%)
Control	35.9 ─ 41.6	38.68±1.67	4.32
Gamma rays			
100Gy	36.4 ─ 42.9	40.72±2.13	5.23
200Gy`	33.2 ─ 41.5	38.95±2.36	6.05
300Gy	32.4 ─ 40.3	36.16±2.55	7.05
400Gy	31.3 ─ 41.9	34.78±3.23	9.29
Pooled mean	**33.3 ─ 41.6**	**37.65±2.50**	**6.90**
EMS			
0.1 %	34.6 ─ 40.8	38.11±2.06	5.40
0.2 %	35.3 ─ 43.8	41.08±2.15	5.23
0.3 %	33.4 ─ 42.7	36.95±2.94	7.96
0.4 %	32.1 ─ 40.1	34.87±2.35	6.74
Pooled mean	**33.8 ─ 41.8**	**37.75±2.37**	**6.33**
Sodium azide			
0.1 %	34.2 ─ 42.7	40.55±2.07	5.10
0.2 %	35.8 ─ 44.6	41.67±2.34	5.61
0.3 %	33.1 ─ 40.7	37.85±2.75	7.26
0.4 %	31.3 ─ 39.2	34.41±2.45	7.12
Pooled mean	**33.6 ─ 41.8**	**38.62±2.40**	**6.27**

ii) Number of pods/plant

In untreated plants mean number of pods per plant ranged from 12 – 18 with the mean value of 15.07±1.42. In case of gamma rays and EMS mean number of pods per plant showed increase (16.47±2.26 and 17.27±1.59) at 100Gy and 200Gy and (17.6±1.96 and 15.6±1.92) at 0.1% and 0.2% respectively. However, in case of SA the mean number of pods / plant decreased in all the concentrations as compared to control except at 0.2% SA where a slight increase in mean number of pods / plant was recorded (15.13±1.92).

Coefficient of variation increased in all the treatments except 200Gy in case of gamma rays (9.21%) as compared to control (9.42%). From the overall mean

values it is revealed that SA was most effective in reducing the mean number of pods per plant followed by EMS and gamma rays (Table 6).

iii) Pod length (cm)

Pod length in control ranged between 6.9cm ─ 12.1cmwith the mean value of (9.53±1.78cm). The lower treatments of all mutagens increased the mean pod length, maximum pod length being observed at 0.1% EMS (11.12±2.7cm) followed by 0.2% EMS (10.77±2.82cm) and 100Gy gamma rays (10.37±2.50cm). Higher treatments of all mutagens significantly reduced the pod length, maximum reduction being observed at 0.4% SA (7.66±2.14cm) (Table 7).

A significant amount of variability was induced in all mutagenic treatments. The highest values of CV were recorded as 32.55% (400Gy) and 31.78% (0.4% EMS) as compared to 18.67% in control. Pooled mean values indicate that EMS was more effective in inducing maximum variability.

Table 6: Effect of Gamma rays, EMS and Sodium azide on number of pods/plant in M_1 generation of *Trigonella foenum-graecum* L.

Treatments	Range	Mean ± SD	CV (%)
Control	12 ─ 18	15.07±1.42	9.42
Gamma rays			
100Gy	13 ─ 19	16.47±2.26	13.72
200Gy`	13 ─ 19	17.27±1.59	9.21
300Gy	11 ─ 17	14.73±1.83	12.42
400Gy	10 ─ 15	12.87±1.59	12.43
Pooled mean	**11.7 ─ 17.5**	**15.33±1.82**	**11.94**
EMS			
0.1%	12 ─ 20	17.6±1.96	11.14
0.2%	11 ─ 18	15.6±1.92	12.31
0.3%	11 ─ 17	13.8±1.74	12.61
0.4%	10 ─ 16	12.87±1.85	14.37
Pooled mean	**11.0 ─ 17.7**	**14.97±1.87**	**12.61**
Sodium azide			
0.1%	11 ─ 17	14.93±1.79	11.98
0.2%	11 ─ 18	15.13±1.92	12.69
0.3%	10 ─ 16	13.93±1.53	10.98
0.4%	9 ─ 15	12.26±1.67	13.62
Pooled mean	**10.2 ─ 16.5**	**14.06±1.73**	**12.32**

Table 7: Effect of Gamma rays, EMS and Sodium azide on Pod length (cm) in M_1 generation of *Trigonella foenum-graecum* L.

Treatment	Range (cm)	Mean ± SD	CV (%)
Control	6.9 ─ 12.1	9.53±1.78	18.67
Gamma rays			
100Gy	5.8 ─ 13.4	10.37±2.50	24.10
200Gy`	5.4 ─ 13.5	9.42±2.42	25.69
300Gy	4.3 ─ 12.9	8.6±2.74	31.60
400Gy	4.2 ─ 11.2	7.7±2.51	32.55
Pooled mean	**4.9 ─ 12.7**	**9.0±2.54**	**28.48**
EMS			
0.1%	5.3 ─ 14.7	11.12±2.71	24.37
0.2%	5.5 ─ 14.6	10.77±2.82	26.18
0.3%	4.2 ─ 14.4	9.26±2.94	31.75
0.4%	4.3 ─ 11.4	7.68±2.44	31.78
Pooled mean	**4.8 ─ 13.7**	**9.70±2.73**	**28.52**
Sodium azide			
0.1%	4.9 ─ 12.8	9.01±2.40	26.64
0.2%	4.5 ─ 13.8	10.14±2.65	26.13
0.3%	3.9 ─ 12.7	8.92±2.77	31.05
0.4%	3.7 ─ 11.3	7.66±2.14	27.94
Pooled mean	**4.2 ─ 12.6**	**8.93±2.49**	**27.94**

iv) Number of seeds per pod

Number of seeds per pod in control populations ranged between 11.9 – 15.7 with the mean value of 13.48±1.19. The lower treatments of all the mutagens had stimulatory effect. The maximum number of seeds per pod was observed at 100Gy (15.22±1.37) and 0.1% EMS (15.2±1.33). Higher treatments of all the mutagens reduced the mean number of seeds per pod, maximum reduction being observed at 400Gy (11.97±1.64). Higher coefficient of variability was induced in all mutagenic treatments. The maximum value of CV was recorded as 13.89% (0.4% SA) and 13.70% (400Gy) as compared to 8.83% in Control.

From the pooled mean values it is revealed that EMS treatment was more effective in increasing the mean number of seeds per pod followed by gamma rays and SA (Table 8).

v) Seed yield per plant (g)

Seed yield in Control ranged from 2.19 − 5.37g with the mean value of 3.62±0.99g. Lower treatments of all mutagens increased the seed yield whereas, a significant reduction was observed at higher treatments. Maximum seed yield per plant was recorded at 0.2% EMS (5.05±1.62g) followed by 0.2% SA (4.20±1.51g) and 100Gy (4.02±1.15g). Lowest seed yield was recorded at 0.4% SA (2.87±0.85g).

All the mutagenic treatments induced significant variability for this trait. Maximum CV was recorded at 0.3% SA (37.50%) followed by 0.4% EMS (36.73%). Pooled mean values have shown that SA was most effective in inducing maximum variability (Table 9).

Comparative effect of all the three mutagens on various quantitative characters (Table 10) have shown that EMS in general had stimulatory effect in terms of pod length, seeds per pod and seed yield per plant (g) whereas SA and gamma rays increased plant height and pods per plant respectively.

Table 8: Effect of Gamma rays, EMS and Sodium azide on number of seeds per pod in M_1 generation of *Trigonella foenum-graecum* L.

Treatment	Range	Mean±SD	CV (%)
Control	11.9 ─ 15.7	13.48±1.19	8.83
Gamma rays			
100Gy	11.9 ─ 17.3	15.22±1.37	9.00
200Gy`	12.4 ─ 17.2	14.83±1.55	10.45
300Gy	10.5 ─ 15.3	12.89±1.59	12.33
400Gy	10.2 ─ 16.1	11.97±1.64	13.70
Pooled mean	**11.2 ─ 16.4**	**13.73±1.54**	**11.37**
EMS			
0.1%	13.1 ─ 17.3	15.21±1.33	8.74
0.2%	12.8 ─ 17.1	14.78±1.48	10.01
0.3%	11.1 ─ 16.3	13.82±1.45	10.49
0.4%	10.1 ─ 15.5	12.74±1.55	12.17
Pooled mean	**11.7 ─ 16.5**	**14.13±1.45**	**10.35**
Sodium azide			
0.1%	10.9 ─ 15.9	13.21±1.36	10.29
0.2%	12.4 ─ 17.2	14.39±1.33	9.24
0.3%	11.5 ─ 16.8	13.90±1.49	10.72
0.4%	9.5 ─ 15.5	12.24±1.70	13.89
Pooled mean	**11.0 ─ 16.3**	**13.43±1.47**	**11.03**

Table 9: Effect of Gamma rays, EMS and Sodium azide on seed yield (g) per Plant in M_1 generation of *Trigonella foenum-graecum* L.

Treatment	Range	Mean ± SD	CV (%)
Control	2.19 ─ 5.37	3.62±0.99	27.35
Gamma rays			
100Gy	2.11 ─ 5.63	4.02±1.15	28.35
200Gy`	1.98 ─ 5.89	3.83±1.12	29.24
300Gy	1.87 ─ 5.43	3.50±1.27	36.28
400Gy	1.75 ─ 5.13	2.98±1.07	35.91
Pooled mean	**1.92 ─ 5.52**	**3.58±1.15**	**32.51**
EMS			
0.1%	1.85 ─ 5.93	4.12±1.20	29.13
0.2%	2.15 ─ 7.94	5.05±1.62	32.08
0.3%	1.89 ─ 6.15	3.95±1.24	31.39
0.4%	1.26 ─ 4.32	2.94±1.08	36.73
Pooled mean	**1.78 ─ 6.08**	**4.01±1.28**	**32.33**
Sodium azide			
0.1%	1.69 ─ 4.76	3.16±0.92	29.11
0.2%	1.72 ─ 6.54	4.20±1.51	35.95
0.3%	1.23 ─ 4.94	3.28±1.23	37.50
0.4%	1.31 ─ 4.43	2.87±0.85	29.62
Pooled mean	**1.48 ─ 5.16**	**3.38±1.13**	**33.04**

Table 10: Comparative effeklct of mutagens on various quantitative traits in M_1 generation of *Trigonella foenum graecum* L.

Character	Control		Gamma rays		EMS		Sodium azide	
	Mean ± SD	**CV (%)**	**Mean ± SD**	**CV (%)**	**Mean ± SD**	**CV (%)**	**Mean ± SD**	**CV (%)**
Plant height(cm)	38.68 ± 1.67	4.32	37.65 ± 2.50	6.90	37.75 ± 2.37	6.33	38.62 ± 2.40	6.27
Pods / plant	15.07 ± 1.42	9.42	15.33 ± 1.82	11.94	14.97 ± 1.87	12.61	14.06 ± 1.73	12.32
Pod length (cm)	9.53 ± 2.27	18.67	9.04 ± 2.54	28.48	9.70 ± 2.73	28.52	8.93 ± 2.49	27.94
Seeds /pod	13.48 ± 1.19	8.83	13.73 ± 1.54	11.37	14.13 ± 1.45	10.35	13.43 ± 1.47	11.03
Seed yield (g)	3.62 ± 0.99	27. 35	3.58 ± 1.15	32.51	4.01 ± 1.28	32.33	3.38 ± 1.13	33.04

4.2. Studies on M_2 generation

4.2.1: Morphological mutations

Careful screening of control as well as treated populations was undertaken to identify the response of fenugreek to different mutagenic treatments. Various types of morphological mutations (Macromutations) were observed at different stages of growth in the M_2 generation. Data on frequency of morphological mutations induced by gamma rays, EMS and SA is presented in the Table 11 and Plates 5-8. The frequency of mutant type was estimated as number of mutants out of total number of M_2 plants scored (Table 11). The morphological mutations identified were grouped on the basis of the trait affected. The analysis of the frequency of mutations induced by physical and chemical mutagens had shown variations in the mutation spectrum. A brief description of these morphological mutants is given below.

4.2.1.1. Chlorophyll mutants

On the basis of intensity of pigmentation at seedling stage, two types of chlorophyll mutants were obtained in M_2 generation. These included: chlorina and xantha. A brief description of these chlorophyll mutants is given below.

Chlorina: These were characterized by the presence of light green to yellowish patches on some parts of leaf. Some of them died within 20 days. However, a few survived up to maturity (Plate 5; Figs b-d).

Xantha: These mutants were characterized by bright yellow colour of the seedlings. Such mutants survived for 10-20 days only (Plate 5; Fig a). A perusal of Table 11 reveals that among the three mutagenic treatments SA was most effective in producing maximum frequency of chlorophyll mutations. The order of effectiveness was SA> gamma rays> EMS.

4.2.1.2. Effect of mutagens on leaf morphology

a) Broad leaved mutants: These mutants were isolated from gamma ray treatments only. Foliage was dark green with broader or oblong leaves and enlarged pods (Plate 6; Fig b).

b) Small, narrow leaved mutants: These mutants were isolated from all the three mutagenic treatments with maximum frequency observed in EMS treatments. Mutants had small or narrow needle like long leaflets. These leaflets also had less chlorophyll intensity (Plate 6; Figs a & d.).

4.2.1.3. Effect of mutagens on plant height

a) Tall mutants: Plant height of these mutants ranged 55-63cm against 35-43cm in control and were isolated in all mutagens except 100Gy dose of gamma rays and 0.1% and 0.4% concentration of SA. The mutants produced less branches and the pod number was also reduced as compared to control (Plate 7; Fig. c).

b) Dwarf mutants: Plant height of these mutants ranged 21-25cm with profuse branching at the base. These were isolated in intermediate treatments of EMS and SA. In gamma rays these were isolated in 200Gy and 400Gy doses. Maximum frequency of these mutants was induced by EMS. The dwarf mutants were found fertile and set seeds (Plate 7; Fig b).

4.2.1.4. Effect of mutagens on pod

a) Small pod mutants: These mutants were associated with dwarfness. The length of these pods ranged between 2-5cm with 2-4 small seeds in each pod. Maximum frequency of small pods was observed in gamma rays (Plate 8; Figs a-b).

b) Double pod mutants: Isolated from all the three mutagenic treatments. Plants were tall with dark green foliage. Pod length had slightly increased

associated with increased number of seeds per pod. Maximum frequency of double pods was noted in EMS treatment (Plate 8; Figs. c-d).

4.2.1.5. High yielding mutant

Some high yielding mutants were isolated in 200Gy and 0.3% EMS only. These mutants showed excessive branching with increased number of pods and seeds per plant. The number of pods per plant in these mutants ranged from 18 to 32, whereas the number of seeds per plant ranged from 15.3 to 26.2 (Plate 7; Fig. d).

Overall the highest frequency of mutants was observed in EMS treatment (11.11%) followed by gamma rays (10.58%) and SA (7.14%).

4.2.2. Mutagenic effectiveness and efficiency

Mutagenic effectiveness was calculated to assess the frequency of mutations induced by each dose / conc. of the mutagens. The major trend pertaining to this parameter influenced by different mutagens can be understood through a critical perusal of Table 12.

In M_2 generation of fenugreek, the numerical values of effectiveness gradually reduced at the higher doses / conc. of all the three mutagens. In case of gamma rays the highest effectiveness value (0.146) could be seen at 100Gy. The effectiveness values decreased beyond 100Gy. Among EMS treatments effectiveness increased up to 0.2% but decreased at the higher treatments (0.3% and 0.4%). In case of SA the highest effectiveness value (2.158) could be seen at 0.2% and lowest value (0.537) at 0.4%.

Among the three mutagens EMS proved to be more effective in inducing mutations as compared to gamma rays and SA. The gamma rays were least effective in this regard. The order of mutagenic effectiveness was EMS followed by SA and gamma rays (EMS> SA> gamma rays).

Table - 11: Frequency of mutations affecting morphological characters in mutagen treated *Trigonella foenum-graecum* L.

Treatment	No. of plants studied	Chlorophyll mutants	Leaf mutants		Plant height mutants		Pod mutants		High yield mutants	Total	Percent of mutants
			Small	Broad	Tall	dwarf	small	Double pod			
Gamma rays											
100Gy	271	2(0.74)	–	1(0.36)	–	–	1(0.36)	–	–	4	1.46
200Gy	266	1(0.37)	–	–	2(0.75)	1(0.37)	–	1(0.37)	1(0.37)	6	2.23
300Gy	234	2(0.85)	1(0.43)	1(0.43)	1(0.43)	–	2(0.85)	1(0.43)	–	8	3.42
400Gy	230	2(0.87)	–	–	2(0.87)	1(0.43)	–	3(1.30)	–	8	3.47
EMS											
0.1%	274	2(0.73)	1(0.36)	–	1(0.36)	–	–	–	–	4	1.45
0.2%	263	1(0.38)	–	–	2(0.76)	2(0.76)	1(0.38)	2(0.76)	–	8	3.04
0.3%	230	2(0.86)	–	–	2(0.86)	1(0.43)	1(0.43)	3(1.30)	1(0.43)	10	4.31
0.4%	215	1(0.46)	2(0.93)	–	1(0.46)	–	–	1(0.46)	–	5	2.31
SA											
0.1%	277	1(0.36)	–	–	–	–	–	–	–	1	0.36
0.2%	269	2(0.74)	1(0.37)	–	1(0.37)	1(0.37)	–	2(0.74)	–	7	2.59
0.3%	220	4(1.82)	–	–	1(0.45)	1(0.45)	–	1(0.45)	–	7	3.17
0.4%	231	1(0.43)	1(0.43)	–	–	–	1(0.43)	–	–	3	1.29

The mutagenic efficiency is the ratio of frequency of mutations induced in M_2 generation to various biological damages induced in M_1 generation. The mutagenic efficiency was calculated on the basis of following four different criteria, viz;

1) Injury (Mf /I)

2) Lethality (Mf/L)

3) Sterility (Mf/S)

4) Meiotic aberrations (Mf/M)

Table 12 presents the data on efficiency of mutagens in relation to various biological damages. The efficiency of mutagens showed variable trend depending on the criteria selected for its calculation and the degree of efficiency of various mutagens also showed variation.

Among the four different criteria selected the highest efficiency was recorded in terms of meiotic abnormalities followed by lethality as compared with that of injury and sterility.

In gamma rays efficiency increased with the enhancement of dose when estimated on the basis of meiotic abnormalities and sterility up to 300Gy but decreased at the 400Gy. Efficiency recorded in terms of lethality showed a variable trend. Higher values were observed at 100Gy (0.181) and 300Gy (0.173). On the basis of injury efficiency showed decreasing trend with increase in the dose. In case of EMS efficiency was more at the intermediate doses except when estimated on the injury basis where it decreased at the higher doses. Among SA treatments higher efficiency values were noted at the intermediate treatments except when estimated in terms of injury basis where higher value was recorded at 0.2% (0.121).

In general, EMS proved to be the most efficient and gamma rays to be the least for all the criteria used (Table 13).

Table 12: Mutagenic effectiveness and efficiency of gamma rays, EMS and SA on *Trigonella foenum-graecum* L.

Treatments	Lethality (% L)	Pollen sterility (%S)	Seedling injury (%I)	Chromosomal abnormalities (%M)	Percent M_2 plants mutated (Mf)	Mutagenic effectiveness Mf/dose	Mutagenic efficiency			
							Mf/L	Mf/S	Mf/I	Mf/M
Gamma rays										
100Gy	8.08	21.33	6.68	6.01	1.46	0.146	0.181	0.068	0.128	0.243
200Gy	20.93	31.24	26.37	9.05	2.23	0.111	0.106	0.071	0.084	0.246
300Gy	19.94	47.70	49.87	11.87	3.42	0.114	0.171	0.072	0.069	0.288
400Gy	33.92	59.93	74.29	15.12	3.47	0.087	0.102	0.058	0.047	0.229
EMS										
0.1 %	6.06	24.47	10.80	4.58	1.45	2.417	0.239	0.059	0.134	0.316
0.2 %	10.60	32.96	24.35	7.47	3.04	2.533	0.287	0.092	0.124	0.407
0.3 %	16.65	44.20	41.13	10.07	4.31	2.394	0.259	0.097	0.105	0.428
0.4 %	28.11	56.06	62.21	13.08	2.31	0.962	0.082	0.041	0.037	0.177
SA										
0.1 %	2.84	18.32	4.88	3.77	0.36	0.600	0.127	0.019	0.074	0.095
0.2 %	6.60	28.04	21.33	5.45	2.59	2.158	0.392	0.092	0.121	0.475
0.3 %	15.19	34.69	44.21	7.92	3.17	1.761	0.208	0.091	0.072	0.401
0.4 %	30.01	42.40	63.75	10.50	1.29	0.537	0.043	0.030	0.020	0.123

Table 13: Mutation frequency of gamma rays, EMS and SA in relation to biological effects such as lethality, sterility, injury and chromosomal abnormalities in *Trigonella foenum-graecum* L.

Mutagen	Mf/L	Mf/S	Mf/I	Mf/Me	Pooled mean
Gamma rays	0.1405	0.067	0.104	0.252	0.141
EMS	0.217	0.072	0.100	0.332	0.180
SA	0.192	0.058	0.072	0.273	0.148

4.2.3. Quantitative characters

In M_2 generation, the effect of gamma rays, EMS and SA treatments was studied on five quantitative traits, viz; plant height, number of pods/plant, pod length, number of seeds/pod and seed yield (g). To assess the extent of induced variation by different mutagenic treatments the data of these quantitative characters were subjected to statistical analysis.

1. Plant height (cm)

Data recorded on mean, SD and CV for plant height are presented in Table 14. The mean values for plant height showed both positive and negative shifts from the control values. Plant height was reduced in the higher treatments of all the mutagens. In case of gamma rays maximum reduction in plant height was observed at 400Gy (37.89cm). Among EMS and SA treatments significant reduction in plant height was noticed at 0.4% concentration (35.56cm and 34.41cm respectively). In case of EMS 0.2% and 0.4% were significantly different from each other (43.92^{c} and 35.56^{ab} respectively). Among SA

treatments 0.1% (40.86[b]), 0.2% (42.81[c]) and 0.4% (34.41[a]) showed significant differences. In general, maximum plant height was induced by gamma rays and minimum by SA.

Coefficient of variation showed increased values as compared to control. The extent of variability was high as compared to M_1 generation in the respective treatments. In general, SA induced maximum variability followed by EMS and gamma rays.

Table 14: Effect of Gamma rays, EMS and SA on plant height (cm) in M_2 generation of *Trigonella foenum-graecum* L.

Treatment	Range(cm)	Mean±SD	Shift in Mean	CV (%)
Control	35.40─43.40	40.25[abc]±2.27	0.00	5.64
Gamma rays				
100Gy	30.80─46.70	42.38[c]±3.99	+2.13	9.41
200Gy	28.20─49.90	43.36[c]±5.27	+2.98	12.19
300Gy	25.90─48.60	40.91[bc]±5.54	+0.69	13.53
400Gy	24.50─50.20	37.89[abc]±5.81	−2.35	15.33
EMS				
0.1%	29.30─46.60	41.56[bc]±4.69	+1.32	11.28
0.2%	27.90─49.90	43.92[c]±5.29	+3.74	12.04
0.3%	26.30─45.70	37.60[abc]±5.69	−2.64	15.13
0.4%	25.60─47.60	35.56[ab]±5.98	−4.68	16.82
Sodium azide				
0.1%	28.90─46.30	40.86[b]±4.33	+0.68	10.60
0.2%	27.70─49.30	42.81[c]±5.52	+2.57	12.89
0.3%	26.80─46.90	38.85[abc]±6.19	−1.40	15.93
0.4%	23.10─47.60	34.41[a]±5.98	−5.84	17.38

2. Number of pods per plant

The data recorded on number of pods per plant in M_2 generation is presented in Table 15.

Mean shifted significantly in positive as well as negative directions in all the mutagenic treatments with a few exceptions. In case of gamma rays highest number of pods per plant was noticed in 200Gy (22.73). In case of both EMS and SA maximum mean values were recorded at 0.2% treatments (23.13 and 21.20 respectively). Among the SA treatments insignificant shift in mean value was noticed in 0.3% concentration (+ 0.06). In general, higher dose treatments of all the mutagens reduced the number of pods per plant. In gamma ray doses significant difference was recorded for 200Gy treatments. Among EMS treatments significant differences were shown by 0.1%, 0.2% and 0.3% concentrations. In case of SA 0.4% concentration varied significantly from control.

Coefficient of variation recorded higher values in all the mutagenic treatments. Maximum CV value was noticed in 0.4% EMS (22.29%).

Among the three mutagenic treatments EMS proved to be more effective in inducing the maximum number of pods per plant followed by gamma rays and SA.

Table 15: Effect of mutagens on number of pods / plant in M_2 generation of *Trigonella foenum-graecum* L.

Treatments	Range	Mean±SD	Shift in Mean	CV (%)
Control	15 − 20	17.73^{bc}±1.44	0.00	8.12
Gamma rays				
100Gy	11 – 23	20.53^{cd}±2.85	+2.80	13.88
200Gy	11 – 25	22.73^{d}±3.47	+5.00*	15.27
300Gy	10 – 25	19.87^{cd}±3.62	+2.13	15.05
400Gy	11 − 21	15.73^{ab}±3.17	−2.00	20.15
EMS				
0.1%	14 − 25	22.60^{d}±2.92	+4.86*	12.92
0.2%	12 − 26	23.13^{d}±3.72	+5.40*	16.08
0.3%	10 − 24	20.20^{cd}±3.93	+2.46	19.45
0.4%	10 − 22	13.73^{a}±3.06	−4.00*	22.29
Sodium azide				
0.1%	13 – 22	18.53^{bc}±3.11	+0.80	16.78
0.2%	12 – 23	21.20^{cd}±2.83	+3.46	13.35
0.3%	12 – 22	17.80^{bc}±3.36	+0.06	18.88
0.4%	10 − 19	12.86^{a}±2.56	−4.86*	19.91

3. Pod length (cm)

The data recorded for pod length in different mutagenic treatments and control are presented in Table 16.

The mean shifted in positive and negative direction in the treated populations. Except 300Gy dose a significant increase in pod length was observed in lower dose treatments of all the three mutagenic treatments. The most effective treatments in this regard were 0.2% SA and 0.2% EMS (14.28 and 13.11 respectively). Similarly, 0.4% EMS and SA and 400Gy gamma rays proved to be most effective in reducing the pod length. In gamma rays except 100Gy dose all the other treatments showed significant differences from each other. Among EMS treatments only 0.2% and 0.4% were significantly different. In

case of SA significant differences were observed between 0.2%, and 0.4%; 0.1% and 0.4%.

A significant amount of variability was induced by the mutagenic treatments. The highest CV among the gamma ray treatments was recorded at 400Gy (38.61%). Among EMS treatments, maximum CV was recorded at 0.4% EMS (37.86%). In case of SA treatments 0.4% recorded the highest CV (39.39%). In general, maximum CV was recorded in SA followed by EMS and gamma rays.

4. Number of seeds per pod

The data recorded on mean number of seeds per pod is presented in Table 17. Mean values recorded showed both positive and negative shifts in all the mutagenic treatments. There was significant increase in mean number of seeds per pod at lower dose treatments. 300Gy dose was most effective in this regard (18.01). Higher dose treatments induced considerable reduction in mean number of seeds per pod which was more pronounced in 0.4% SA treatment (8.58) as compared to control (13.69). Significant differences were noticed among 100Gy, 300Gy and 400Gy in case of gamma rays. 0.4% EMS treatment differed significantly from 0.1%, 0.2% and 0.3%. Among SA 0.2%, 0.3% and 0.4% differed significantly from one another. Coefficient of variation increased with increase in dose / concentration. Among the three mutagenic treatments maximum CV value was observed in 0.4% SA treatment (28.55). In general, gamma rays were most effective in inducing the maximum number of seeds per pod followed by SA and EMS.

Table16: Effect of Gamma rays, EMS and Sodium azide on pod length (cm) in M_2 generation of *Trigonella foenum-graecum L.*

Treatment	Range(cm)	Mean±SD	Shift in Mean	CV (%)
Control	5.8 − 13.4	10.98^{abc}±2.02	0.00	18.31
Gamma rays				
100Gy	5.1 − 14.9	12.02^{bc}±3.35	+0.96	27.87
200Gy	3.8 − 15.9	13.03^{bc}±3.92	+2.05	30.08
300Gy	3.5 − 16.7	14.15^{c}±4.11	+3.16	29.04
400Gy	3.9 − 12.6	7.33^{a}±2.83	−3.64	38.61
EMS				
0.1%	4.2 − 14.8	11.73^{bc}±3.48	+0.75	29.67
0.2%	4.8 − 15.9	13.11^{bc}±4.06	+2.12	30.97
0.3 %3	3.7 − 13.9	10.17^{abc}±3.37	−0.80	33.14
0.4 %	4.1 − 13.2	6.92^{a}±2.62	−4.06	37.86
Sodium azide				
0.1%	3.9 − 15.8	12.16^{bc}±4.01	+1.18	32.98
0.2%	3.3 − 16.9	14.28^{c}±4.25	+3.30	29.76
0.3%	3.9 − 13.9	9.78^{ab}±3.12	−1.20	31.90
0.4%	4.9 − 14.1	7.21^{a}±2.84	−3.76	39.39

Table 17: Effect of Gamma rays, EMS and Sodium azide on number of seeds per Pod in M_2 generation of *Trigonella foenum-graecum* L.

Treatments	Range	Mean±SD	Shift in Mean	CV (%)
Control	12.1 ─ 17.1	13.69^{bcd}±1.44	0.00	10.52
Gamma rays				
100Gy	11.4 ─ 17.8	14.71^{cde}±2.09	+1.02	14.21
200Gy	10.2 ─18.7	16.24^{def}±2.69	+2.54	16.56
300Gy	9.4 ─ 20.7	18.01^{f}±3.34	+4.31*	18.55
400Gy	8.3 ─ 15.3	10.62^{ab}±2.82	−3.02	26.43
EMS				
0.1%	10.6 ─ 18.2	14.52^{cde}±2.68	+0.83	14.52
0.2%	9.4 ─ 18.4	15.67^{cdef}±2.70	+1.98	17.23
0.3%	8.2 ─ 17.5	13.24^{bcd}±2.89	−0.45	21.83
0.4%	6.4 ─ 13.5	9.35^{a}±2.18	−4.34*	23.31
Sodium azide				
0.1%	10.3 ─ 18.9	15.60^{cdef}±3.02	+1.90	19.36
0.2%	9.8 ─ 20.4	17.30^{ef}±3.19	+3.60*	18.44
0.3%	8.4 ─ 16.9	12.76^{bc}±2.77	−0.92	21.71
0.4%	6.2 ─ 13.3	8.58^{a}±2.45	−5.11*	28.55

5. Seed yield per plant (g)

The data recorded for seed yield per plant in M_2generation is presented in the Table 18.

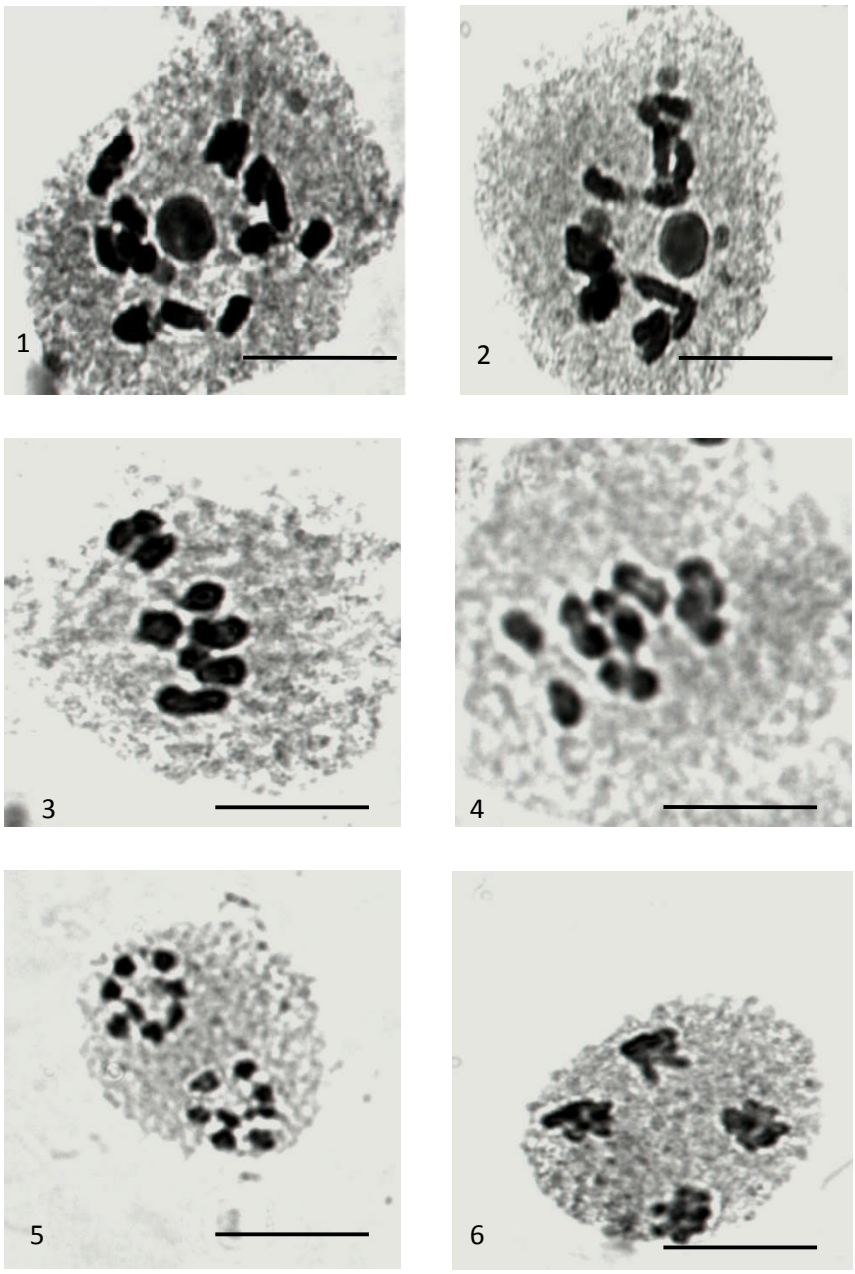
Mean shifted significantly in both positive and negative directions with a few exceptions. Mean values recorded for seed yield per plant showed increased values initially but decreased at the higher doses/concentrations. In case of gamma rays maximum mean value was recorded at 300Gy (9.39). Among EMS treatments highest mean value was observed at 0.2% (8.29). 0.2% (9.23) proved to be most effective in case of SA treatments.

Among the three different mutagenic treatments the lowest value for seed yield per plant was observed in 0.4% SA (2.98) and the highest value in 300Gy gamma rays (9.39). In general, maximum values for seed yield per plant were observed in gamma rays followed by SA and EMS. Significant differences were noticed between 100Gy, 400Gy; 200Gy, 400Gy and 300Gy, 400Gy in gamma radiations. Among EMS treatments significant variation was recorded between 0.2% and 0.4% concentrations only. In case of SA significant differences were found between 0.1% and 0.4%; 0.2% and 0.3% and 0.2% and 0.4%.

From the Table 18, it is evident that considerable amount of variability was created by the mutagenic treatments for seed yield per plant. In case of gamma rays maximum CV was noticed in 400Gy doses (43.81). In case of EMS and SA treatments the highest coefficient of variation was observed in 0.4% (45.13% and 43.29% respectively). In general, maximum variability was induced by EMS followed by gamma rays and SA treatments.

Table 18: Effect of Gamma rays, EMS and Sodium azide on Seed yield per Plant in M_2 generation of *Trigonella foenum-graecum* L.

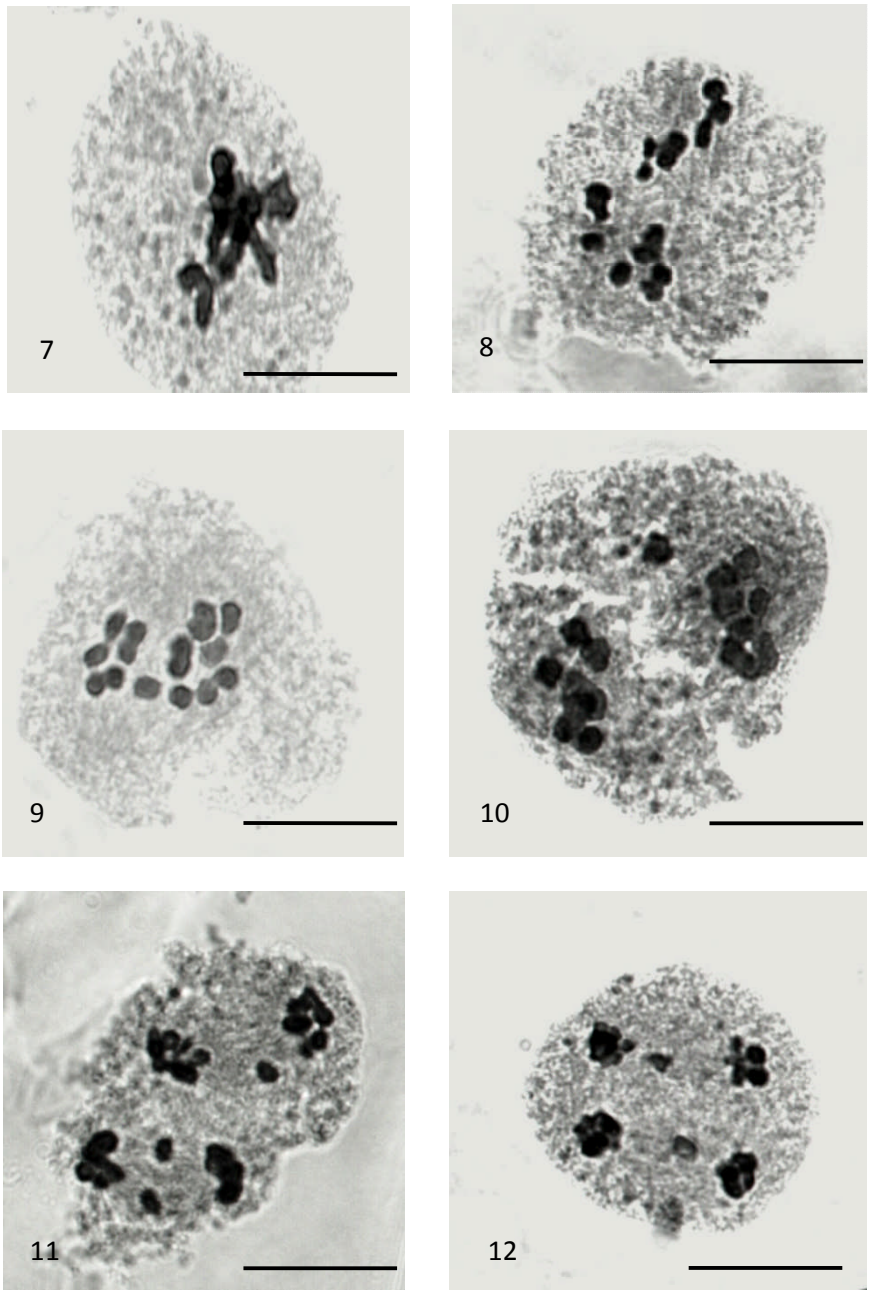
Treatment	Range	Mean ± SD	Shift in Mean	CV (%)
Control	3.1 ─ 7.0	5.61^{abcd} ±1.50	0.00	26.73
Gamma rays				
100Gy	2.5 ─ 09.8	7.06^{bcdef}±2.58	+1.45	36.54
200Gy	3.2 ─ 12.7	8.98^{ef}±3.69	$+3.37^{*}$	41.09
300Gy	3.1 ─ 12.9	9.39^{f}±3.68	+3.78*	39.19
400Gy	1.2 ─ 06.4	3.15^{a}±1.38	−2.46	43.81
EMS				
0.1%	3.1 ─ 9.3	6.43^{bcdef}±2.25	+0.81	34.99
0.2%	2.9 ─ 11.5	8.29^{def}±3.10	−2.67	37.39
0.3%	2.8 ─ 9.5	5.90^{abcde}±2.65	−0.28	44.91
0.4%	1.5 ─ 7.7	3.90^{ab}±1.76	−1.71	45.13
Sodium azide				
0.1%	3.7 ─ 11.4	7.83^{cdef}±2.99	+2.21	38.19
0.2%	3.1 ─ 12.6	9.23^{f}±3.46	+3.62*	37.49
0.3%	1.7 ─ 7.3	4.76^{abc}±1.92	−0.84	40.34
0.4%	1.5 ─ 6.0	2.98^{a}±1.29	−2.63	43.29



Scale = 10µm

Plate 1: PMCs showing normal meiosis (Control)

Figs. 1&2	PMCs showing 8 bivalents at diakinesis
Figs. 3&4	PMCs with 8 bivalents at metaphase-I
Fig. 5	PMC showing normal distribution of chromosomes at anaphase-I
Fig. 6	PMC showing 4 daughter nuclei at telophase-II.



Scale = 10µm

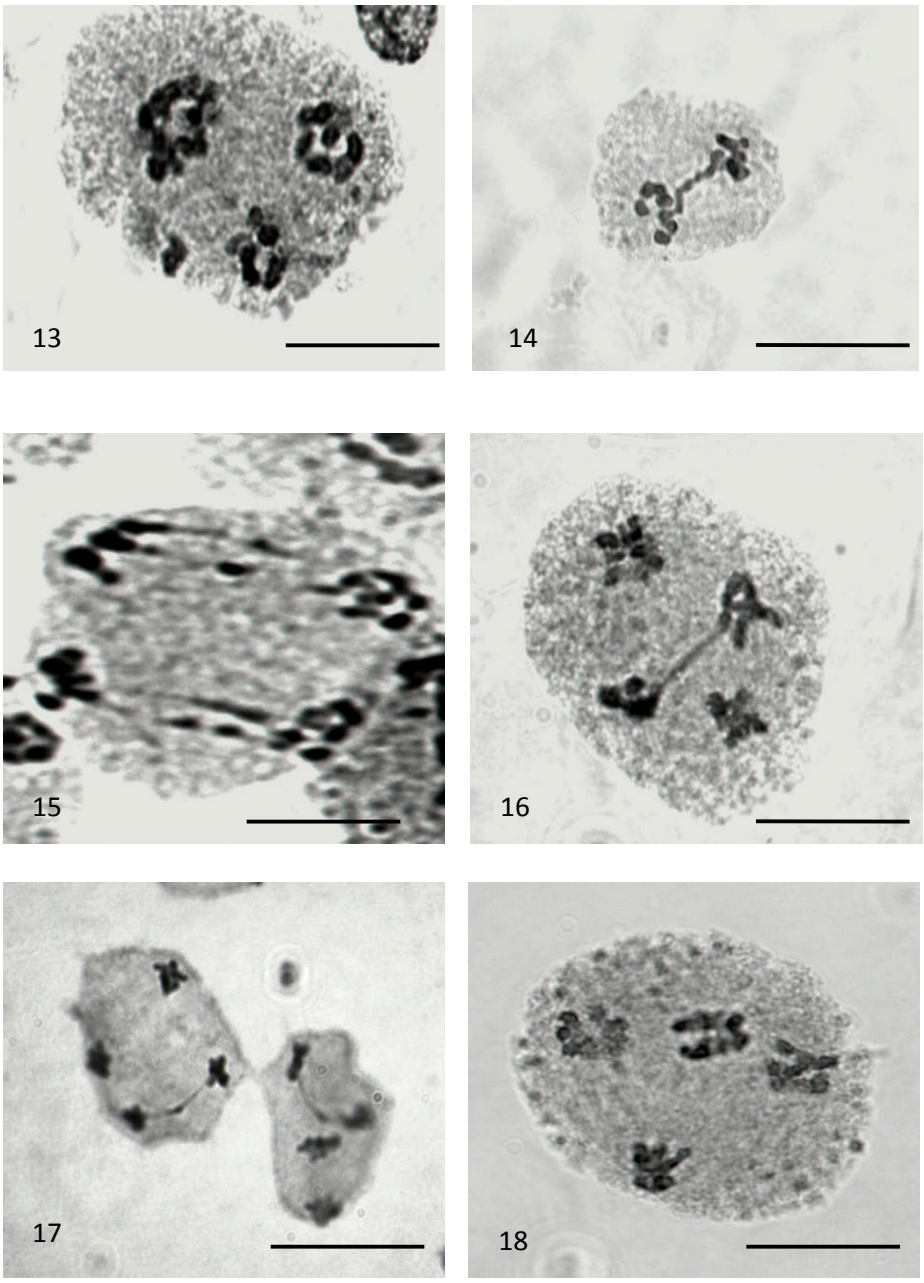
Plate 2: Chromosomal abnormalities induced by gamma rays, EMS and SA

Figs.7 PMCs showing stickiness of chromosomes at metaphase-I.

Fig. 8 PMC showing precocious separation of a bivalent at metaphase-I.

Figs. 9 PMC showing $6^{II} + 4^{I}$ at metaphase-I.

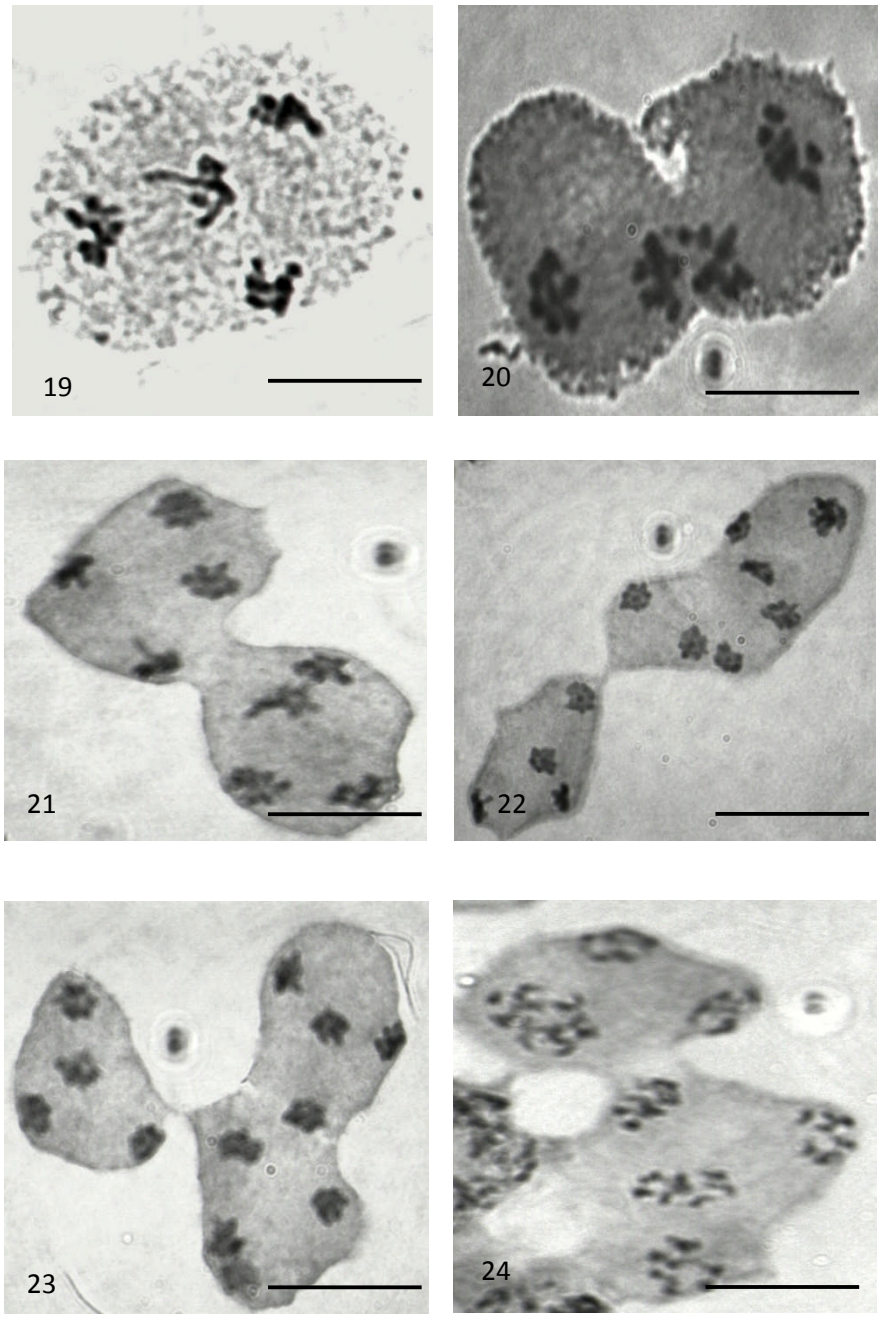
Figs. 10-12 PMCs showing lagging chromosomes at anaphase-I and anaphase-II



Scale = 10µm

Plate 3: Chromosomal abnormalities induced by gamma rays, EMS and SA

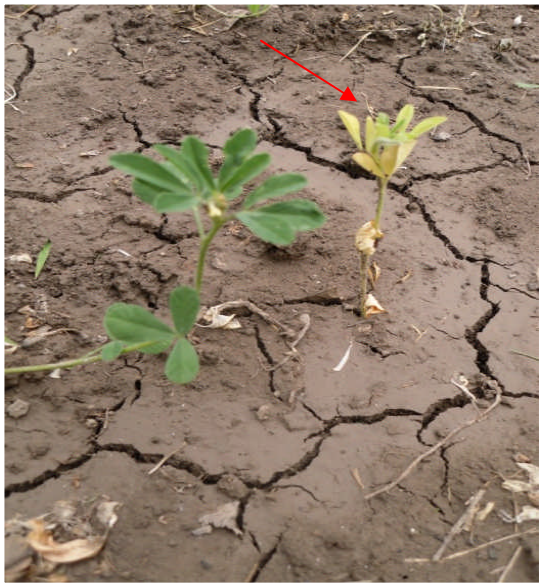
Fig 13 PMC showing lagging chromosome at anaphase-II
Fig 14 PMC showing chromosome bridge at anaphase-I
Figs.15-16 PMCs showing chromosome bridges at anaphase-II
Fig 17 PMC showing chromosome bridge at telophase-II
Fig 18 PMC showing disturbed polarity at telophase-II



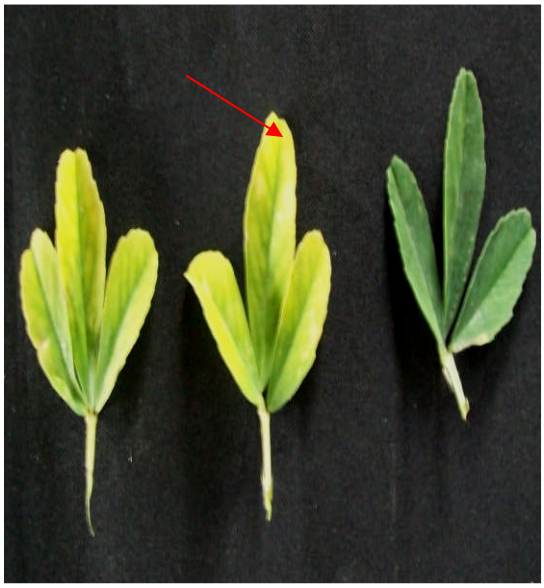
Scale = 10µm

Plate 4: Chromosomal abnormalities induced by gamma rays, EMS and SA

Fig 19 PMC showing disturbed polarity at telophase-II
Figs.20-24 PMCs showing cytomixis at telophase stages



(a)



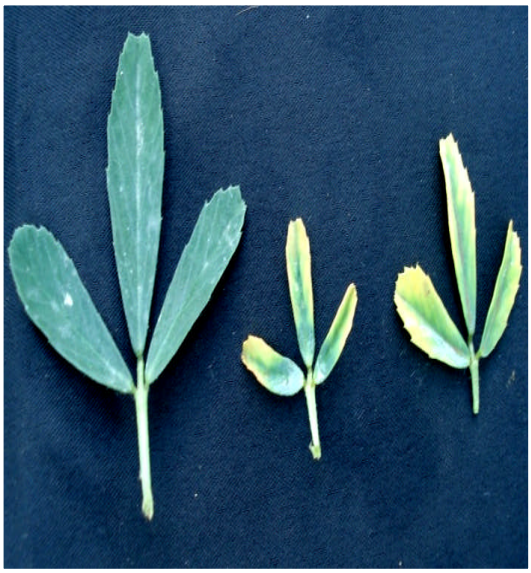
(b)

(c)

(d)

Plate 5: Chlorophyll mutants

Fig. a Showing xantha mutant
Fig. b Showing chlorina mutant (Control on extreme right)
Fig. c Showing chlorina mutant in comparison to Control seedling.
Fig. d Showing chlorina mutant



(a)

(b)

(c)

(d)

Plate 6: Leaf mutants

Fig. a Showing small narrow leaf mutants (Control on left side)
Fig. b Showing broad leaf mutants
Fig. c Showing normal leaves
Fig. d Showing long narrow needle shaped leaves

(a)

(b)

(c)

(d)

Plate 7: Height and high yielding mutants

Fig a Control
Fig b Dwarf mutant with small sized leaflets
Fig c Tall mutant with less branches and pods
Fig d Mutant with increased number of primary branches

Plate 8: Pod mutants

Figs. a-b Showing small pod mutants as compared to Control
Figs. c-d Showing double pod mutants

Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. Mutagenic agents such as, radiation and certain chemicals, can be used to induce mutations and generate variations from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety by way of inducing micromutations in addition to the visible macromutations. It offers the possibility of inducing desired attributes that either cannot be found naturally or have been lost.

Generally, the criteria such as germination, injury, lethality, sterility, chromosomal aberrations in M_1 generation, chlorophyll and viable mutation frequency in M_2 generation are used to assess the superiority of mutagens (Reddy *et al*., 1992; Thakur and Sethi, 1995; Kharakwal, 1998a, 1998b; Kumar and Dubey, 1998; Khan, 1999).

The present study was undertaken to determine the individual effect of gamma rays, EMS and SA in terms of seed germination, seedling height, plant survival at maturity, pollen sterility and meiotic aberrations in the populations emerging

from treated seeds along with controls in the local variety of *Trigonella foenum-graecum* L.

Seed germination, seedling height and plant survival decreased with the increase in doses / conc. of mutagens in the present study. However, the extent of decrease showed variation among different mutagens. In general gamma rays proved to be most effective in reducing seed germination, seedling height and plant survival.

Many workers have reported the adverse effects of physical and chemical mutagens on various biological parameters (Ravikesavan *et al*., 1995; Jabeen and Mirza, 2004; Kumar and Mishra, 2004; Kon *et al*., 2007; Lal *et al*., 2009; Dhakshanamoorthy *et al.*, 2010). These workers have observed dose dependent reduction of the above mentioned biological parameters.

Several workers have attempted to explain the causes responsible for inhibition of seed germination. Reduction in seed germination in mutagenic treatments is 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 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. Kumagai *et al*. (2000) in *Arabidopsis thaliana* and *Raphanus sativus* and Kovacs and

Keresztes (2002) in golden delicious and empire apples have ascribed the inhibition of seed germination and seedling growth to the formation of free radicals in irradiated seeds while Micco *et al*. (2011) have correlated it with abnormalities in mitotic cycles and in metabolic pathways of the cells. 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 is 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 M_1 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 with height reduction, 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).

Stimulating effects of low doses of ionizing radiation as observed in the present study have also been reported in chickpea (Haq *et al*., 1992; Khan and Wani, 2005), Winged bean (Bai and Sunil, 1993), *Bambusa arundinacea* (Lokesha *et al*., 1994), Soybean (De la *et al*., 2000) and rice (Wang *et al*., 1993; Cheema and Atta, 2003). Cepero *et al*. (2001) observed maximum stimulating effect of low doses on seedling height of *Leucocephala* cv. Cunningham. The lower doses of gamma radiation are concluded to produce a stimulus on the growth of the aerial as well as the underground parts. Khanna and Maherchandani (1981) measured peroxidases activity in chickpea seedlings raised after gamma irradiation and observed increased activity at lower gamma irradiation doses. Higher doses resulted in decreased activity. They reported that gamma radiation apparently causes damage to the tissues by producing H_2O_2 and organic peroxy radicals and peroxidase is the internal mechanism for removal of these radicals. The increase in enzyme activity at lower doses could be a response of the tissue to the increase in peroxides. At higher doses whole of the cellular metabolism is grossly impaired resulting in lower enzyme activity.

Ya Ping *et al*. (1996) reported EMS induced chromosomal aberrations of root tip cells and adverse effects on seedling height, root length, root number and peroxidase activity in *Coix lacryma-jobi*. The reduction in root length with increasing EMS concentration has been reported in chickpea (Haq *et al*., 1992), *Vicia hirsuta* (Kumari, 1994) and redgram (Potdukhe, 2004).

Srivastava *et al*. (2011) in wheat suggested that the reduction in seedling survival is due to the hindrance 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 such as Afsari Awan *et al.* (1980) in rice, Jayabalan and Rao (1987) in *lycopersicon esculentum*, Mahna *et al.* (1989) in *Vigna mungo,* Kumar and Dubey (1998a,b) in *Lathyrus sativus* and Lal *et al.* (2009) in blackgram. These workers have observed dose dependent decrease in plant survival. The reduction in seedling survival is 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).

In the present investigation, the pollen sterility among all the mutagenic treatments shows gradual increase with respect to the increase in dose / conc. The treatment of EMS and gamma rays were found to be more effective to produce maximum pollen sterility as compared to SA. In the present study, the increase in pollen sterility as a consequence of mutagenesis is in accordance with the findings in wild and cultivated urdbean and mungbeans (Ignacimuthu and Babu,1989), in chickpea (Wani, 2001), in mungbean (Tah, 2006; Lal *et al*., 2009) and in cowpea (Kumar *et al*., 2009). The high sterility 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. This is in agreement with many workers (Mathusamy and Jayabalan, 2002; Khan and Wani, 2005; Mensah *et al.*, 2007. Kumar and Rai (2006) in soybean reported that a decrease in pollen viability can be attributed to abnormal meiosis forming abnormal or unequal gametes. 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. This is in agreement with findings of Abel

(1970) and Vanhof and Harder (1995). Contrary to this, Sato and Gaul (1967) in barley reported a high sterility coupled with low frequency of meiotic abnormalities after EMS treatment. This was attributed to small undetectable deletions or gene mutations.

The observations on biological parameters in the present study revealed that gamma rays were more superior to EMS and SA in reducing seed germination and plant survival. However, EMS in turn was more effective than gamma rays and SA in inducing pollen sterility. The high percentage of pollen sterility in EMS treatments as compared to gamma rays is in conformity with the earlier reports of Dixit and Dubey (1986). Lal *et al*. (2009) have reported that gamma rays are more effective in inducing pollen sterility as compared to SA.

In general, during the present study the reduction in seed germination, plant survival and pollen fertility was more at the higher dose / concentration levels, which may be due to the fact that the fenugreek was more sensitive at these concentrations and therefore, the genic, chromosomal and physiological disturbances were more at these concentrations. The more toxic effects of gamma rays as compared to EMS and SA are due to the fact that the sensitivity of the crop / genotype varies with the mutagen type and depends upon the genetic architecture of the crop and the mutagens employed besides, the amount of DNA, its replication time in the initial stages and degree of heterochromatin.

Cytological analysis with respect to 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. In the present investigation, a vast array of meiotic aberrations was induced by gamma rays, EMS and SA in fenugreek. Meiotic

abnormalities increased with an increase in dose / conc. of the mutagens. All the three mutagenic treatments induced different types of meiotic aberrations and the percentage of anomalies differed showing that different mutagens have different mutagenic potential for fenugreek. 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 (Kumar and Rai, 2007).

Different types of chromosomal abnormalities observed during the present investigation have also 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, 1998; Verma *et al*., 1999; Dhamayanthi and Reddy, 2000; Khan *et al*., 2009; Kumar and Verma, 2011).

In the present study, the most frequent chromosomal aberrations encountered in all the three treatments was stickiness of chromosomes. Stickiness could be due to depolymerization of nucleic acid caused by mutagenic treatments or due to partial dissociation of nucleo proteins and alterations in their pattern of organization (Evans, 1962). Jayabalan and Rao (1987) suggested that stickiness might be due to disturbances in cytochemically balanced reactions. However, it seems most probable that some kind of a gene mutation leads to incorrect coding of some non- histone proteins involved in chromosome organization. When affected, these proteins lead to chromosome clumping. It may also be possible that the mutagen itself reacts with the histone proteins and brings about a change in the surface property of chromosomes due to improper folding of DNA, thereby causing them to clump or stick (Gaulden, 1987).

The occurrence of univalents at metaphase has been reported in various plants like barley (Kumar and Singh, 2003) and broadbean (Bhat *et al*., 2005). Mutagen induced structural change in chromosomes and mutations might be responsible for the failure of pairing among homologous chromosomes and

hence the presence of univalents. Further, the disturbances in the pairing mechanism were attributed to the presence of chromosome breakage in the PMC's of plants raised from treated seeds. Some of the bivalents disjuncted early and presumably this happened due to genic differences. Anis and Wani (1997) reported that such chromosomal divergences, in the form of precocious movement are pointed towards structural differentiation of homologous pair. Precocious separation of chromosomes at metaphase observed during the present investigation might have resulted due to disturbed homology for chromosome pairing or disturbed spindle mechanism. Similar findings were reported by Khan *et al.* (2009). Precocious separation was also observed in tomato by Bose and Saha (1970) who concluded that the bivalents separating precociously seemed to be a result of desynapsis.

The laggards observed during the present study might be due to delayed terminalization, stickiness of chromosomal ends or because of failure of chromosome movement (Permjit and Grover, 1985; Jayabalan and Rao, 1987; Sobeir *et al*., 1989; Bhat *et al*., 2006). Acentric fragments or laggards may result in the formation of micronuclei at telophase II and ultimately variation in the number and size of pollen grains (Bhat *et al*., 2007). Tarar and Dyansagar (1980) reported that the occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result spindle fibres failed to carry the respective chromosomes to the Polar Regions and consequently lagging chromosomes appeared.

Bridges were also observed at anaphase and telophase stages in the present study. According to Saylor and Smith (1996), the formation of bridges can be due to failure of chiasmata in a bivalent to terminalize as a result the chromosomes get stretched between the poles. Bridge formation at the anaphase may be ascribed to interlocking of bivalents (Bhattacharjee, 1953). In the present study, bridge formation can be attributed to the general stickiness of chromosomes at metaphase stage or breakage and reunion of chromosomes

which is in conformity with other workers (Aamer and Farah, 1985; Ahmad, 1993).

In the present case, disturbed polarity observed at telophase could be due to spindle disturbance which has also been reported in several plants like *Vicia faba* (Gulfishan *et al*., 2010) and chickpea (Sharma and Kumar, 2004).

Cytomixis is a phenomenon of transmigration of chromatin material from one cell to an adjoining cell through cytoplasmic channels. The factors proposed to cause cytomixis are the influence of genes (Kaul and Nirmala, 1991), formation of abnormal cell wall during premeiotic divisions (Kamra, 1960), action of chemical agents (Sinha, 1988), effect of gamma radiation (Amma *et al*., 1990) and fixation effects (Haroun, 1995; Heslop - Harrison, 1966). In a few PMC's deviation of chromosome number from the normal has been attributed to the cytomixis. Cytomixis between and among different stages of meiosis was earlier reported by Maria De Souza and Pagliarini (1997) and Bhat *et al.* (2005). It is considered to be a source of production of aneuploidy and polyploidy gametes (Koul, 1990; Yen *et al*., 1993; Bhat *et al.*, 2006).

Most of the workers have in general concluded that gamma rays were more effective than chemical mutagens in causing chromosomal abnormalities (Van Harten, 1998 and Wani, 2000). Similar results have been obtained in the present study.

In the present investigation, studies conducted on various quantitative traits in M_1 generation have revealed that no appreciable change was induced in the pooled mean values by different mutagens with a few exceptions. However, the mutagenic effect was clearly evident at different concentrations of all the three mutagens leading to reduction in plant height, number of pods per plant, length of pods, number of seeds per pod and seed yield at higher dose treatments and stimulation in the mean values of these traits in some lower dose treatments. Similar findings were reported in previous works of Tripathi and Dubey

(1990); Kumar *et al*. (2009); Naik *et al*. (2009) and Dhakshanamoorthy *et al*. (2010). The reason that no appreciable change was noticed in pooled mean values for quantitative characters is due to the fact that data were recorded on normal looking plants only excluding macromutational variants.

The inhibitory effect on plant height at higher doses / conc. can be attributed to the inhibition of growth due to low rate rate of cell division, decreased amylase activity and increased peroxidase activity (Cherry and Lessman, 1967). Reduction in pod number at higher treatments may be due to a probable inhibitory action of enzymes, changes in the enzyme activity and toxicity of the mutagens (Blinks, 1952). Similarly the reduction caused by mutagens on seed yield per plant can be attributed to high seed sterility and reduced pod number as caused by physiological and biochemical disturbances in the development of plants (Prabhakaran, 1992). The decline in yield could also be probably due to indirect influence of altered yield contributing components.

Macromutants obtained in the present study were either distinguished in the seedling stage and were referred as chlorophyll mutants or at mature stage which were classified as morphological mutants.

The scoring of chlorophyll mutations in M_2 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). The induction of chlorophyll mutations by physical and chemical mutagens has been reported in chickpea (Kalia *et al.*, 1981; 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).

In the present study, two different types of chlorophyll mutations were isolated viz., chlorina and xantha. 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). Mutations in these chlorophyll genes may induce chlorophyll mutations. Among the three mutagenic treatments, SA proved to be most effective than gamma rays and EMS in inducing chlorophyll mutations. Similar observations were reported by Ando and Montalvan (2001) in rice, Lal *et al*. (2009) in blackgram and Shirshat *et al*. (2010) in horsegram. The frequency of chlorophyll mutations were dose independent in the present study. Similar results were obtained by Reddy and Rao (1988) in rice, Khan (1979) in mungbean. 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).

The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness, but also on its mutagenic efficiency, efficient mutagenesis being the product of the minimum desirable changes accompanied by the least possible undesirable changes. Effectiveness usually means the rate of point mutations relative to dose, where as efficiency refers to the rate of point mutations relative to other biological effects induced by the mutagen and is considered a measure of damage (Konzak *et al*., 1965). 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.

Effectiveness and efficiency of mutagens have been worked out by a number of workers (Shah *et al*., 2008; Girija and Dhanavel, 2009; Shirsat *et al*., 2010).

Some researchers found that chemicals are more effective and efficient in inducing mutations than gamma rays (Solanki, 2005; Rekha and Langer, 2007; Basu *et al*., 2008; Dhanavel *et al*., 2008; Ganapathy *et al*., 2008; Wani, 2009).

In the present study, the degree of effectiveness and efficiency varied among different mutagens. In general lower or intermediate doses proved to be most effective in inducing mutations. The decrease in effectiveness at higher dose / treatments may be attributed to the failure in proportional increase of mutation frequency induced at higher treatments. Similar findings were obtained by Singh and Chaturvedi (1980) in *Vigna radiata*, Wani (2000) and Parveen (2006) in chickpea.

Mutagenic efficiency calculated on the basis of lethality, sterility, injury and meiotic aberrations with respect to induced morphological mutations in M_2 population basis showed variation depending upon the criterion selected for its estimation. In general, the intermediate dose treatments proved to be most efficient on the basis of all the criteria used. Similar conclusions have been drawn by several workers (Sudha, 1990; Reddy and Annadurai, 1991; Solanki and Sharma, 1994). The higher efficiency obtained at lower and intermediate doses of mutagens might be due to the fact that the lethality, injury, sterility etc increases with mutagen concentration at a rate faster than the frequency of mutations (Blixt, 1964).

Mutagenic efficiency seemed to vary with respect to biological criteria selected. In general, mutation rate based on meiotic aberrations (Mf / Me) was highest followed by lethality (Mf / L), whereas, it was lowest in case of sterility (Mf / S). EMS proved to be efficient followed by SA. Gamma rays were least efficient. Variations obtained on the basis of criteria used in the mutagenic efficiency have also been reported by Kumar and Ratnam (2010) in sunflower; Bhosle and Kothekar (2010) in clusterbean; Dixit and Dubey (1986) in lentil.

In M_2 population, different types of morphological mutations affecting plant height, leaf and pod size were isolated. The frequency of these morphological mutants showed variation among different mutagens used. EMS proved to be more effective in inducing macromutations. In general, maximum frequency of morphological mutations was induced by intermediate treatments of EMS and SA, whereas; in gamma rays frequency of morphological mutations was maximum at higher doses. The more frequent induction of certain mutation types by a particular mutagen may be attributed to the fact that the genes controlling these characters may be more responsive to alkylating agents/ 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. Differences in the frequency of induced morphological mutations have been reported earlier (Tripathi and Dubey, 1992; Vandana and Dubey, 1994). Solanki and Sharma (1999) and Kharakwal (2001) reported that the chemical mutagens, particularly alkylating agents are more effective than ionizing radiations in inducing morphological mutations. 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).The higher mutagenic sensitivity of gamma rays has been reported in soybean by Waghmare and Mehra (1998) as compared to EMS. Various investigations suggest that the possible cause of these macromutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations (Singh *et al*., 1980).

A number of mutants including early maturing mutants with a determinate growth habit, high seed yield, seed quality and adaptation to a short growing season have earlier been reported in fenugreek (Basu *et al*., 2008). 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). The absence of chlorophyll and morphological mutation types in M_1 and their appearance in M_2 generation might be attributed to one of these two assumptions: 1) 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. 2) 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).

Double pod mutants obtained in the present study are of special interest since these mutants resulted in improvement in seed yield. Double or twin pods are an indication of high diosgenin content in the seed (Petropoulos, 2002).

Improvement of any crop depends on the kind and magnitude of genetic variability existed in the population. Mutagenesis has proved to be a potential tool to be employed for crop improvement. Particularly, induction of micromutations in the polygenic system, controlling the quantitative characters is important for crop improvement. In recent years, the role of mutation breeding in increasing the variability for quantitative characters has been proved beyond doubt (Sharma *et al*., 1990; Mensah and Akomeah, 1992; Kumar *et al*, 1995; Srivastava and Singh,1996; Rajput *et al*., 2001; Ramesh *etal*., 2001; Sakin and Yildrin, 2004; Khan *et al*., 2006; Basu *et al* ., 2008; Wani, 2011).

In the present investigation, data on five quantitative characters viz., plant height, number of pods per plant, pod length, number of seeds per plant and seed yield were analyzed to evaluate the extent of induced variability in M_2 generation of fenugreek. The mean shifted both in positive and negative direction for all the quantitative characters in the present study. The positive shift was more pronounced at the lower or intermediate dose treatments, whereas negative shift was observed at higher dose treatments. The changes in the mean values after mutagenic treatments has been reported earlier in many pulse crops such as urdbean (Deepalakshmi and Kumar, 2003), mungbean (Wani *et al*., 2005; Tah, 2006; Arulabalachandran and Mullainathan, 2009) and lentil (Singh *et al*., 2006). Singh *et al*. (2001) reported that the increase in mean values could be due to occurrence of polygenic mutations with cumulative effects.

In the present study, the coefficient of variation (CV) was invariably higher in mutagen treated populations than in control for all the five quantitative characters studied. This could be due to induced genetic changes and release of polygenic variability in all the treated populations. The magnitude of variability was different in treated populations for different characters indicating the variable degree of induced changes in different dose treatments of mutagens. The increased variability coupled with increased mean values of these characters in comparison to control offers a unique possibility of creation of new germplasm for crop improvement. A similar view has been reported by various authors from time to time (Konzak, 1987; Kumar *et al*., 2004; Mensah *et al*., 2005). Frey (1969) has reported that the mutagen derived variability for quantitative characters in a crop plant is heritable and the response to selection is good. The similar results were also reported earlier by Khan and Siddiqui (1993, 1997), Kumar and Mishra (2004) and Singh (2006). Induction of greater variability in polygenic traits might be due to increased mutations and recombination (Singh *et al*., 2001).

In the present study the plant height showed both positive and negative shift in mean. Similarly, there was wider range in the treated as compared to the control population. Variability increased irrespective of increase or decrease in the mean values. Scossiroli (1965); Gaul (1966); Gill *et al*. (1974) and Sakin (2002) reported a decrease in mean values and an increase in the variability of M_2 generations. Increase in variability accompanied with a reduction of the mean values indicated that major part of the induced genetic variability is negative (Borojevic, 1991).

The number of pods per plant increased in most of the treatments, however, a significant reduction was observed at highest dose treatments of all the mutagens in the M_2 generation. Increase and decrease in number of pods per plant in M_2 generation have been reported by many workers (Abdalla and Hussein, 1977; Upadhyaya and Singh, 1979; Waghmare and Mehra, 2000; Kozgar *et al*., 2011) after treatments with physical and chemical mutagens. Reduction of mean in mutagenic populations might be due to induction of more mutations in negative direction and increase in mean could be attributed to induction of more positive mutations in the polygenes governing the character (Singh and Rao, 2008).

The pooled mean values for pod length have shown that overall mean values were almost same as that of control in EMS and SA treatments, whereas a slight increase in pooled mean values was observed in gamma ray doses. The lower dose treatments had stimulatory effect on pod length; whereas higher dose treatments decreased the mean pod length in all mutagen treated populations. Changes in the mean pod length by mutagens have been observed earlier (Khan, 1982; Singh and Rao, 2008).

The yield, as such, is a complex manifestation of large number of genes involved in physicochemical processes of the plant system. Induced mutations can contribute to the physiological efficiency of the plant for grain yield by

generation of more favourable correlations between various yield components (Waghmare and Mehra, 2000). In the present investigation a positive correlation was observed between seed yield and many other quantitative traits like number of pods per plant, pod length and mean number of seeds per plant. Similar findings have been reported by many workers (Khan, 1982; Vijayalakshmi *et al*., 2000; Wani, 2000; Khan *et al*., 2004 and Khan and Wani, 2006).

The present investigation was conducted to study the mutagenic effect of gamma rays, EMS and SA in the local variety of fenugreek (*Trigonella foenum-graecum* L.).

Fenugreek seeds were treated with four doses and concentrations of each gamma rays (100Gy, 200Gy, 300Gy & 400Gy), EMS (0.1%, 0.2%, 0.3% & 0.4%) and SA (0.1%, 0.2%, 0.3% & 0.4%) respectively. M_1 and M_2 generations were raised during summer seasons of years 2010 and 2011 respectively at the Kashmir University Botanical Garden.

The main objective of the study was to induce the genetic variability in quantitative traits and to isolate the promising mutants associated with increase in yield potential of the crop. The other parameters of the study included: 1. Biological damage in M_1 generation. 2. Effectiveness and efficiency of the mutagens and 3. Spectrum and frequency of macromutations.

The significant findings are summarized as follows:

6.1. M_1 generation

The mutagenic effect studied on M_1 parameters included seed germination, seedling height, plant survival, pollen fertility, meiotic studies and various quantitative traits.

a) Seed germination, seedling growth, plant survival and pollen fertility decreased with an increase in mutagenic treatment.

b) Chromosomal abnormalities increased with an increase in mutagenic treatment. Various meiotic aberrations induced by mutagens included stickiness, univalents, precocious separation of chromosomes, laggards, bridges, disturbed polarity and cytomixis.

c) Gamma rays proved to be most effective in causing maximum biological damage. The order of effectiveness was gamma rays > EMS > SA.

d) Studies on various quantitative parameters showed the inhibitory effect of higher treatments and stimulatory effect of lower or intermediate treatments in M_1 generation.

e) The mean values for various quantitative traits decreased at higher treatments, but stimulatory effects were noticed at some lower treatments.

f) A significant amount of variability was induced in the treated populations as compared to Control.

6.2. M_2 generation

a) A broad spectrum of morphological mutations were isolated in the M_2 generation. Intermediate treatments of EMS and SA induced maximum frequency of mutations where as gamma rays showed dose dependent increase of mutations.

b) The most promising macromutations included tall, dwarf, broad leaved and double podded mutants.

c) The mutagenic effectiveness measured on the basis of frequency of morphological mutations divided by dose of the mutagen revealed EMS to be most effective followed by SA in causing mutations. Gamma rays were least effective in this regard.

d) The mutagenic efficiency calculated on the basis of meiotic aberrations (Mf/Me) was generally higher followed by lethality (Mf/L) as compared with that based on injury (Mf/I) and sterility (Mf/S).

e) Intermediate dose treatments of mutagens proved to be most efficient on the basis of all criteria used.

f) The mean values of different quantitative traits showed positive and negative shifts in M_2 generation.

g) Coefficient of variability was further increased in treated populations as compared to Controls in M_2 generation. However, CV showed dose dependent increase in all the mutagenic treatments.

h) Increase in pods per plant and number of seeds per pod played significant role in enhancing the seed yield per plant in treated populations.

The present findings lead to the following conclusions.

1) Maximum frequency of mutations can be achieved in local variety of fenugreek by exposing it to the lower doses and concentrations of gamma rays (below 400Gy) and EMS and SA (below 0.4%) respectively.

2) Increase in mean values compared with increased variability in M_2 generation especially for yield contributing traits suggests scope for further selections in subsequent generations.

3) A positive correlation was observed between seed yield and many other quantitative traits like number of pods per plant, pod length and mean number of seeds per pod.

It is suggested that the mutants isolated in the present investigation will be of great utility in cultivation of fenugreek.

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