DISCUSSION
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The present discussion is mainly concerned with the effect of chemical mutagens, methyl methane sulphonate (MMS), ethyl methane sulphate (EMS) and cadmium nitrate Cd(NO\textsubscript{3})\textsubscript{2} and lead nitrate Pb(NO\textsubscript{3})\textsubscript{2} on seed germination, morphology, plant survival, pollen fertility, growth and yield as well as chromosomes in M\textsubscript{1}, M\textsubscript{2} and M\textsubscript{3} generations in *Trigonella foenum graecum* L. The probable reasons regarding the cyto-morphological variations induced by mutagens have been discussed.

1. MORPHOLOGICAL STUDIES

A. SEED GERMINATION AND BIOLOGICAL DAMAGE

Seed germination is an important parameter to estimate the effect of mutagens on plants. Germination of seeds after breaking dormancy period is a process of resumption of active metabolism manifested in visible growth. Inhibition in seed germination, after the treatment of seeds with different mutagens is a convenient technique for studying their effects of mutagens in plants. In the present investigation the seed germination gradually decreased with increasing concentrations of all four mutagens. Seed germination was highly affected by Cd(NO\textsubscript{3})\textsubscript{2} followed by Pb(NO\textsubscript{3})\textsubscript{2}, MMS and EMS.

The reduced germination due to inhibitory effect of chemical mutagens (MMS and EMS) and heavy metals [Cd(NO\textsubscript{3})\textsubscript{2} and Pb(NO\textsubscript{3})\textsubscript{2}] as observed in the present investigation in *Trigonella foenum graecum* L. has also been reported in same plant by several workers such as (Siddiqui *et al.*, 2007, 2008; Jabee *et al.*, 2008; and Choudhary *et al.*, 2012) and other plants such as, *Lathyrus sativus* L. (Kumar and Dubey, 1998a, b); *Nigella sativa* (Mitra and Bhowmik, 1998); *Vigna radiate* (Khan *et al.*, 1998); *Plantago ovate* (Lal and Sharma, 2000); *Cicer arietinum* (Jabee and
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Ansari, 2005); *Vicia faba* (Agarwal and Ansari, 2001; Khan et al., 2006a; Bhat et al., 2007); *Zea mays* (Kumar and Rai, 2007); *Helianthus annuus* (Khursheed et al., 2008); *Cichorium intybus* (Ahmad et al., 2009); *Solanum melongena* (Aruna et al., 2010); *Triognella foenum graecum* (Siddiqui et al., 2007, 2008; Jabee et al., 2008); *Vigna mungo* L. (Goyal and Khan, 2010). Heavy metals have also reduced the germination in other plants such as *Phaseolus vulgaris* (Bhardwaj et al., 2009; Cavusoglu, 2009); Grass pea (Kumar and Tripathi, 2007a, b); *Capsicum annuum* L. (Kumar and Gupta, 2008) and *Barbunia* (Cavusoglu and Yalcin, 2010).

Favret (1963) reported that delayed seed germination caused by various mutagens may be due to the depression in the rate of mitotic proliferations. The denatured DNA caused by mutagenic treatment, after sometime, may be repaired resulting in the activation of biological processes involved in germination and thus delayed germination has been observed (Hutterman et al., 1978). In treated populations the delayed germination might be due to chromosomal aberrations/delay in DNA synthesis/delayed metabolic processes.

The seed germination was inhibited parallel to the increasing concentrations of mutagens and anti-parallel to the germination percentage. Moreover Cd(NO$_3$)$_2$ exhibited more inhibitory effect on germination followed by Pb(NO$_3$)$_2$, MMS and EMS. Several explanations have been given by different workers regarding the inhibition in seed germination. Krishna *et al.*, (1984) suggested that inhibition in germination may be due to interaction between mutagens and the seed cell system. It may also be due to toxicity of mutagens followed by mutational changes at genic or chromosomal level, because the reduction in germination corresponds with the increasing chromosomal aberrations. Falque, (1994) considered that occurrence of
seeds without completely developed embryos may be one of the reasons for reduction in germination. Endogenous growth regulators play an important role in seed germination and there exists a balance between promoters and inhibitors, and that any disturbance in this balance results in the reduction in seed germination (Aman, 1986). According to Khan et al., (2007) reduction in seed germination in mutagenic treatment may be due to chromosomal deletion. Delay or inhibition of physiological and biological processes is necessary for seed germination which includes enzyme activity (Kurobane et al., 1979), hormonal imbalance (Chrispeeds and Varner, 1976) and inhibition of mitotic process (Ananthaswamy et al., 1971) may also be responsible. Datta and Biswas, (1985) are also of the opinion that inhibition in seed germination following heavy metal treatment may be due to disturbance of physiological process and induction of chromosomal aberrations leading to mitotic arrest and cell death.

Plant survival was the highest in EMS and least in heavy metal Cd(NO₃)₂. It was directly related to germination and inversely to the inhibition percentage. Since EMS and MMS caused less damage/inhibition, the survival was higher in these mutagens. Reduction in plant survival with increasing doses of mutagens as reported in Trigonella is also supported by earlier reports in Triticale by EMS and gamma rays treatment (Reddy and Gupta, 1989, Edwin and Reddy, 1993); in Lathyrus sativus (khesari) by EMS and gamma rays (Kumar and Dubey, 1998a); Capsicum annum (chilli pepper) by gamma rays and EMS (Dhamayanthi and Reddy, 2000); Triticum by industrial chemical agents (Kalia et al., 2001); Capsicum annum and sunflower by caffeine (Kumar and Tripathi, 2004, Khursheed et al., 2008); Nigella and Triticum by heavy metal treatment (El-Ghamery et al., 2003).
Reduction in survival following mutagenic treatments may be due to various factors such as cytogenetic damage and physiological disturbances (Sato and Gaul, 1967), changes in the metabolic activity of cells (Natarajan and Shivashankar, 1965), disturbances in balance between promoters and inhibitors of growth regulators (Meherchandani, 1975).

Biological damages were higher in higher concentrations of mutagens. The greater sensitivity at higher concentrations of mutagens may be due to disturbances in genetical as well as physiological processes leading to cell death (Sree Ramulu, 1972).

**B. FREQUENCY OF VARIATIONS/MUTATIONS**

In the present study, the maximum frequency of variations was induced in Cd(NO₃)₂ followed by Pb(NO₃)₂, MMS and EMS in M₁ generation in *Trigonella* and that the frequency of variations was more in M₁ than the mutations in M₂ and M₃ generations. It means that most of the variations occurred as adopted dominant characters in M₁ but in M₂ and M₃ the adopted characters were eliminated and the recessive mutations appeared along with few homozygous dominant mutations.

Earlier reports by Jabeen, (2002) in *Cicer arietinum*; Solanki and Sharma, (2002) in *Lens culinaris* also showed a similar concentration dependent increase in the frequency of mutations. However the frequencies of mutations in M₂, obtained after selfing the M₁ variants, were comparatively lower and finally in M₃ their frequency was the lowest, possibly due to ceasing toxic effects of mutagens and repair in DNA damage in successive generations and possible segregation of recessive genes.

**C. POLLEN FERTILITY/STERILITY**

The negative effect of mutagens on pollen fertility was the highest in
Cd(NO₃)₂ followed by Pb(NO₃)₂, MMS and EMS. The fertility was comparatively lower in the first generation in all mutagenic treatments but gradually increased in the subsequent generations and the most prominent recovery being in MMS in M₃. Similar adverse effect on pollen fertility has also been reported in many plants, such as mungbean (Ignacimuthu and Babu, 1992); lentil (Reddy and Annadurai, 1992); chilli (Dhamayanthi and Reddy, 2000); cowpea (Singh et al., 2006); cotton (Sheidai and Dezfolian, 2008); chicory (Khan et al., 2009a) and in Trigonella foenum graecum (Choudhary et al., 2012) etc.

This may be due to cumulative effects of various meiotic aberrations (Jabeen and Ansari, 2005; Khan et al., 2009a). Chromosomal anomalies, as discussed later, like univalents, multivalents, stickiness, laggards, bridges, micronuclei etc. are closely associated with pollen sterility in mutagen treated populations (Reddy and Rao, 1981, 1982; Singh, 1992; Anis and Wani, 1997; Kumar and Tripathi, 2004; Kumar and Rai, 2007b; Cali, 2008; Jabe et al., 2008) and the accumulation of more and more chromosomal abnormalities greatly affected microsporogenesis leading to the formation of non-viable gametes, which considerably reduced plant fertility (Kumar and Rai, 2007b). The fact that meiotic abnormalities are responsible for pollen sterility has also been supported by Sinha and Godward, (1972), Koul (1993), Pagliarini and Pereira, (1992), Zeerak, (1992), Pagliarini et al., (1993), Consolaro et al., (1996), Taschetto and Pagliarini, (2004), Khan et al., (2009a) etc. According to Reddi (1977) the pollen sterility was the result of interchange of segments between non-homologous chromosomes. Low chiasma frequency may be one of the causes of low pollen fertility, because chiasmata are responsible for the maintenance of the bivalents which permit normal chromosome segregation and this process ensures
pollen fertility (Defani Scoarize et al., 1995a; Pagliarini, 1990; Consolaro et al., 1996). Srivastava and Kapoor, (2008) reported that spindle related aberrations like tripolarity, multipolarity and non-orientation may cause the formation of unbalanced and sterile gametes affecting the plant fertility.

The present study has shown that pollen grains produced as a result of mutagenic treatments allow the production of chromosome-altered plants, mostly without any loss of vigour, which facilitate the perpetuation of the induced chromosome variations. The pollen sterility was much less in M3 generation as compared to M1 and M2, indicating that some sorts of recovery mechanism have operated in the intervening period. These findings were in support with earlier observations of Viccini and Carvalho, (2002), Ansari and Ali, (2009), Khan et al., (2009b).

D. VARIATIONS IN COTYLEDONARY AND VEGETATIVE LEAVES

Variations in cotyledonary and vegetative leaves were the common effects of the mutagens. The frequency of these variations was more in M1 than the mutations in M2 and M3, because most of the variations that occurred as adopted characters/variants in M1 were eliminated in further generations and the recessive mutations appeared along with few homozygous dominant mutations. The variations in the cotyledonary/vegetative leaves such as increase or decrease in size, shape, number and thickness, fusion of margins, blunt/obtuse/notched leaf apices, decreased angle between cotyledonary leaves, were observed. Similar abnormalities have also been reported by Krishna et al., (1984) in Chloris gayana Kunth; Murray and Wilson, (1991) in Medicago trunctata; Vandana and Dubey, (1988), Vandana, (1992) and Kumar et al., (1993) in Vicia faba; Jain and Agarwal, (1993) in Trigonella; Salam, (1990) and Zeerak, (1998) in Solanum melongena L; Tabassum, (2002) in

The production of abnormal leaf types in treated populations may be attributed to the disturbances of internal growth regulators like IAA induced by the action of mutagens or may be sequel to primary or secondary effects of free radicals caused by the mutagens (Lea, 1955).

Hagen and Gunckel, (1958) suggested that concomitant increase in the free amino acid contents in leaves may cause the formation of abnormal leaves. It may also be either due to the disturbance in metabolic activities after mutagenic treatments (Devreux and Scarascia-Mugnozza, 1964) or due to chromosomal aberrations (Venkateshwarlu et al., 1988).

Blixt, (1972) considered that leaf aberrations seemed to be due to actual mutation process. According to Dubinin, (1976) several enzymes are involved in mutation, these enzymes participate in the whole process at various stages and many of the potential lesions are converted into mutations as a result of enzymatic activity. Ansari and Ali, (2009) suggested that occurrence of these leaf mutants may be correlated with the increasing meiotic anomalies at higher doses of mutagens. The disturbances in metabolic activities due to mutagenic treatments may also be one of the important factors responsible for such anomalies in plants.

E. PLANT HEIGHT

Average height of plants generally decreased with the increasing concentrations of mutagens, but it was found that in lower concentrations of EMS it increased significantly over control in M1 as well as in successive generations. Overall the
maximum retarding effect on height in $M_1$, $M_2$ and $M_3$ generations was caused by $\text{Cd(NO}_3\text{)}_2$ followed by $\text{Pb(NO}_3\text{)}_2$, MMS and higher concentration of EMS. Higher CVs in $\text{Cd(NO}_3\text{)}_2$ and other treated populations showed more variability in height and providing greater chances for the selection of desirable mutants. Reduction in plant height followed by mutagenic treatments was also observed by many workers such as Jain and Agarwal,(1993) and Choudhary et al., (2012) in Trigonella; Nabipour et al., (2004)in *Helianthus*; Jabeen and Mirza, (2004)in *Capsicum*; Das et al., (2004)in *Vigna*; Stamo et al., (2007)in *Triticum*; Omar et al., (2008) in *Capsicum* etc.

There have been many theories put forward by many workers regarding the reduction in plant height. Salam, (1990) concluded that the reduction in seedling growth may be due to the gross injury caused at cellular level, either due to gene controlled biochemical process and/or acute chromosomal aberrations. Inhibition of cell division along with the chromosomal damages may be one of the chief reasons of reduced seedling growth (Gray and Read, 1950; Thoday, 1954; Sparrow et al., 1961; Arumugam et al., 1997). Uneven damage to meristematic cells as a result of genetic injuries and physiological disturbances also causes reduction in seedling and plant growth (Ansari and Siddiqui, 1996). Arumugam et al., (1997) considered that the reduction in seedling height after mutagenic treatments is generally due to the inhibition of mitotic proliferation and variation in auxin level. According to Tabassum, (2002) the chromosomes carrying various genes responsible for the life process and expression are one of the most sensitive elements and the damage to any part of these vital and tiny elements are bound to go a long way to bring about several morphological and growth abnormalities in the plant or plant organs. Kumar and Tripathi, (2008)are also of the opinion that the reduction in plant height can be
attributed to chromosomal abnormalities after the treatment of mutagenic chemical. Moreover chromosomal breakage during mitotic division and inhibition of DNA synthesis, have also been implicated as the cause of reduced plant growth because chromosomal aberrations and height reduction in higher concentration of mutagens were positively correlated.

Moreover the average height in the lower concentrations of EMS (0.10%) in M1, M2 and M3 generations increased significantly over control showing the enhancing effect even in M3 generation in which the taller and higher yielding mutants had been obtained. Similar enhancing effects have also been reported by Anis and Wani, (1997) in *Trigonella* by caffeine; Sawan *et al.*, (2000) in *Gossypium* by kinetin and Jabeen, (2002) in *Cicer* by DES. Moreover the reduction in height sometimes proves to be better because some dwarf and semi dwarf mutants selected in M3 were high yielding (Mutant code: A1, A2, G1, G2, Q).

**F. YIELD**

Yield is a very important parameter in mutation breeding, because ultimately the plant breeder wants to improve the yield along with other characters. A crop plant can be improved in productivity and adaptation to environment only when more genetic variabilities for the specific traits are available in the treated population. Although, generally the yield decreased with the increasing concentrations of Cd(NO$_3$)$_2$, Pb(NO$_3$)$_2$, MMS and higher concentrations of EMS but a significant increase in yield per plant was observed in the lower concentration of EMS and in some isolated cases of MMS.

Reduction in yield might have occurred due to disturbances in meiosis which affected the frequency of normal microspores and megaspores and hence the fruit set.
was directly affected. The reason for reduction in yield in higher concentrations of MMS, Pb(NO₃)₂ and Cd(NO₃)₂ may be due to their highly genotoxic nature, which might have resulted in physiological disturbances, chromosomal damage, failure or restricted pairing, delay in DNA synthesis and/or disturbed spindle formation and high pollen sterility. However, pollen sterility appeared to be more responsible, because the yield was found to decrease under the condition of high pollen sterility (Lakshmi et al., 1988). Decrease in yield may also be indirectly related to decrease in the number of branches and number of pods per plant due to the toxic effects of mutagens, which generally occurred in higher concentrations of mutagens.


Contrary to other three mutagens the number of branches and pods per plant, number of seeds per pod, 1000 seed weight, and total yield per plants increased significantly in the lowest concentration of EMS resultanty some positive mutants have been obtained.

The increase in the yield in lower doses of mutagens have earlier been recorded in
a number of crops such as wheat and triticale (Viswanathan et al., 1994); *Vicia faba* (Vandana and Dubey, 1988 and Khan *et al.*, 2005a, b, 2006a, b); *Vigna radiata* (mungbean) (Khan *et al.*, 1999); *Lens culinaris* (Verma *et al.*, 1999); *Triticum aestivum* (Kalia *et al.*, 2000); *Trigonella foenum graecum* (Jabee *et al.*, 2007); *Helianthus annuus* (Khursheed *et al.*, 2009) etc. The reason for the increased yield in lower doses/concentrations may be attributed to the enhancing effect (Jahagirdar, 1975; Kothekar, 1983) and growth regulatory effect of mutagen (Audus, 1961).

Some positive mutants showing higher yield have been isolated in M2 and M3 generations. However, the increase in mean values for quantitative traits could be due to the occurrence of polygenic mutations with cumulative effects (Singh *et al.*, 2000).

**G. VIABLE MUTANTS**

All the mutants observed in M2 generation were grown in M3 generation to test their true breeding nature. Twenty eight different morphological mutants in M3 generation were isolated on the basis of their cyto-morphological characters. Out of these mutants, tall, dwarf, high yielding, bushy, profusely branched semi-dwarf, sterile and seeds mutants are agronomically important and can be used in cross breeding programs or directly as mutant varieties.

**(a) CHARACTERIZATION OF MUTANTS**

**(i) TALL MUTANTS**

Tall mutants exhibited significant increase in height with significantly increased in number of pods and yield per plant as compare to control. Tall mutants with increased number and size of pods and improved yield were isolated in present investigation. Such mutants have also been previously reported by Patil and Bawankar, (2000); Begum *et al.*, (1995); Santhoslal and Pavithran, (1997); Manjaya
and Nandanvar, (2007); Kharkwal, (2000); Kumar et al., (2007) and Tambe and Apparao, (2009). According to Jana, (1962) the tallness of tall mutants is apparently due to increase in the number and length of internodes.

(ii) DWARF MUTANTS

Significant decrease in height is the main characteristic of this type of mutants. Dwarf mutants have also been previously reported by Rao and Reddy, (1983); Ravi and Bala, (1983); Nadarajan et al., (1985); Dhari and Wadia, (2005); Auti, (2005); Kirtane, (2002), Kumar et al., (2007). Reduction in the height of the dwarf mutants is ascribed to different reasons by different authors, such as due to shortening of internodes, inhibition of auxin synthesis (Suss, 1966); genetic loss due to chromosomal aberration (Evans and Sparrow, 1961); interference with the synthesis of new DNA (Pele and Howard, 1955); delay and loss of proliferation capacity and cell death (Evans, 1965); inhibition of phytohormone responsible for normal growth (Tarar and Dnyansagar, 1974), destruction of growth, inhibition of the apical meristem or partial failure of the internodes to elongate (Rao et al., 1983). Any one or all these factors may be responsible for the observed reduction in plant height in the dwarf mutants. The induced dwarfness is definitely a desirable agronomic trait of importance because they are lodging resistant and easy for cultural operations.

(iii) HIGH YIELDING BUSHY MUTANTS

Some bushy mutants obtained in the present investigation showed compact habit. They were profusely branched and bushy in appearance, considerable increase in pod number and seed yield per plant as compared to control. High yielding mutants have also been reported by Srivastava and Singh, (1996), Pawar and Wanjari, (1994) in pigeonpea; Auti, (2005), Tickoo and Chandra, (1999), Kharkwal, (2000), Singh and
Kumar, (2005), in mothbean; Manjaya and Nandanvar, (2007) and Tambe and Apparao, (2009) in Soybean. Bushy mutants isolated in the present study, however, became the advantage of narrow leaves, because the sunlight reached to all plant parts increasing its sink potential at the time of flowering and reproductive phase. Excellence in yield performance and its component characteristics were also contributed by the plant architecture, forming the optimum angle between leaflets and stems and contributing to higher photosynthetic efficiency (Naik *et al*., 2002).

**(iv) STERILE MUTANTS**

Some sterile mutants were also observed in the present study. Lamprecht, (1958) reported that when a gene, responsible for certain vegetative character, mutates to an allele foreign to the species, in that situation, some of the genes responsible for transforming vegetative structures into floral organs are unable to function, leading to non-flowering condition. Similar sterile mutants have been reported earlier by Saxena *et al*., (1984) and Gahukar *et al*., (1996) in pigeonpea; Subramanian, (1980) in *Vigna trilobata*; Panchbhaye, (1997), Jayakumar and Selvaraj, (2003) in sunflower; and Tambe, (2009) in soybean. Vanniarajan *et al*., (1993) and Manapure and Patil, (1997) in blackgram; Apparao, (2005) in chickpea; Ahire *et al*., (2005), Karthika and Lakshmi, (2006) and Manjaya and Nandanwar, (2007) in soybean have also reported the sterile mutants. Sterile mutants with full green leaves were obtained from gamma rays treatments (Kumar *et al*., 2007) in blackgram.

**(v) PROFUSELY BRANCHED SEMI-DWARF MUTANTS**

Profusely branched semi-dwarf mutants obtained in the present investigation was a novel mutant showing two fold increase in the number of branches per plant, threefold increase in number of pods per plant and the mean yield per plant almost...
twice-thrice as compared to that of control plants. Similar types of vegetable mutants were also reported earlier by Faris et al., (1987), Saxena, (2008) and Jain et al., (1981) in pigeonpea. Such mutations can be considered as evolutionary conversion of the plant habit genes carrying substantial polygenic significance. Since both leaves and seeds are in present in the crop, this was considered to be significant achievement.

(vi) SEED MUTANTS

Genetic variability in the size of the seeds was also observed in *Trigonella*, as a result of mutagenic treatments. Seed mutants observed in the present investigation showed size and colour variation. In the present investigation brown, light brown, green, black, black shaded, black spotted, reddish-brown, greenish-brown seed coat with different shapes were observed. Similar seed shape and coat colour mutants were also reported by Biradar, (2004) and Shinde, (2007) in pigeonpea; Patil, (2009) in cowpea. Similar seed size variation was reported earlier by Sudharani, (1990), Singh and Raghuvanshi, (1991) in blackgram; Reddy and Reddy, (1972) in rice; Malik and Mary, (1973) in rye and Stapute and Suradkar, (2011) in groundnut. Singh and Yadav, (1991) also reported different seed variants viz., bold seed, light black seeds, dull green, shining green, light brown and yellow coloured mutants in green gram induced with gamma radiation. Seed shape, size and coat colour seem to be under the control of polygenes. Disruption of any one of these genes might manifest in the form of seed mutations. The observed mutations in seed coat colour, size and shape of the seeds in *Trigonella* might be due the disruption of one or few genes controlling these characters. All the mutants mentioned above seem to be the most efficient for further improvement of the *Trigonella* and there is scope for utilizing these characters in future breeding programme.
H. PROTEIN CONTENT

Increase as well as decrease in protein content in some mutants has also been demonstrated by Ignacimuthu and Babu, (1989) in *Vigna radiata* and *Vigna mungo* using EMS and gamma rays as mutagens. Induction of substantial variations in seed protein content was also reported in earlier studies by Pavadai *et al.*, (2010) and Kavithamani *et al.*, (2010) in soybean; Kozgar *et al.*, (2010) in *Vigna radiata* and *Vigna mungo* and Bhat *et al.*, (2012) in *Trigonella foenum graecum*. Induced mutants with altered protein contents have also been reported by Harsulkar, (1994), Shen *et al.*, (2008), Sagade, (2008) in urdbean and Mundhe, (2008), Yathaputanon *et al.*, (2009) in soybean; Satpute and Dhulgande, (2010) in pea; Devi and Mullainathan, (2012) in blackgram. It has also been reported that protein production is directly linked with the quality of seeds, better the quality of seed more is the protein production. Therefore, in the case of increased protein content the seed quality also increased. It may also be due to interaction of genes and environment (Singh *et al.*, 1990). According to Gottschalk and Muller, (1970), the improvement made in protein content and composition through genetic manipulation is a permanent improvement. In recent years it has been very much accepted that the protein synthesis in seeds is regulated by a series of specific genes and changes in proteins are produced due to genetic alterations in most of the events.

I. MUTAGENIC EFFECTIVENESS AND EFFICIENCY

Mutagenic effectiveness is an index of the response of a genotype to the increasing concentrations/doses of the mutagens in M₂ generation. The selection of effective and efficient mutagen is very essential to recover a high frequency and spectrum of desirable mutations (Solanki and Sharma, 1994).
In the present experiment the mutagenic effectiveness was the highest in the lowest concentration but decreased with the increasing concentrations of mutagens. The order of mutagens based on effectiveness was Cd(NO\textsubscript{3})\textsubscript{2}>Pb(NO\textsubscript{3})\textsubscript{2}>EMS>MMS. Moreover mutation rate was not proportional to the increase in concentrations of mutagens, rather it was found to be inversely proportional due to elimination of affected seedlings/plants during the growth. Similar results were obtained by Mitra and Bhowmik, (1999) in *Lens culinaris*; Waghmare and Mehra, (2001) in *Vigna radiata*; Sharma *et al.*, (2005) in *Vigna mungo* (L.) Hepper. Kharkwal, (2001) considered that chemical mutagens showed higher effectiveness than physical mutagens.

Efficiency of a mutagenic agent is of complex nature, as it depends not only on the reactivity of the agent with the material and on its applicability to the biological system, but also on the degree to which physiological damage (Mp/I), and pollen sterility (Mp/S) are induced in addition to mutation. Since sterility depends directly on chromosomal behaviour, the chromosomal aberrations are also responsible for mutagenic efficiency. According to Girja and Dhanavel, (2009) it gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagenic agent.

Similarly the mutagenic efficiency with respect to inhibition and sterility decreased with increasing concentrations of mutagens in *Trigonella*. The efficiency with regard to inhibition in germination and pollen sterility was the highest in EMS, followed by MMS, Cd(NO\textsubscript{3})\textsubscript{2} and Pb(NO\textsubscript{3})\textsubscript{2}. The decline in mutagenic effectiveness at higher concentrations were due to elimination of highly affected seedlings or plants at an early stage. Higher efficiency of a mutagen indicates relatively less biological damage.
in relation to mutation induced (Solanki and Sharma, 1994). This may be taken as an established fact for almost all situations.

**J. MEIOTIC STUDIES**

Meiotic study is very important aspect in mutation breeding experiment. The mutagens which cause morphological and cytological abnormalities generally act on DNA structure, which ultimately cause different types of meiotic irregularities. So by the meiotic studies we can observe the potentiality of mutagens. In the present study the changes in chromosomal behaviour induced via chemical mutagens have been extensively studied in order to assess the spectrum of chromosomal damages caused by these mutagens and their effect on morphological characters as well. Cytological studies also provide information regarding the response of various genotypes to a particular mutagen and provide greater chances for the selection of desired characters.

As a result of treatment of seeds with chemical mutagens and heavy metals, the plants showed varying degrees of meiotic abnormalities, like univalents, multivalents, precocious movement of chromosomes, stray chromosomes, stickiness, laggards, bridges, unequal separation, micronucleate and multinucleate conditions, disturbed polarity, cytomixis and reduction in chiasma frequency.

Generally the meiotic abnormalities increased with increasing concentration of chemical mutagens and heavy metals in the present study and this trend has been supported by many workers (Anis and Sharma, 1997; Jabee and Ansari, 2005; Bhat et al., 2007; Khan et al., 1998a, 2007b, c, 2009a; Jabee et al., 2008; Gulfishan et al., 2010 and Choudhary et al., 2012). The frequencies of chromosomal aberrations were higher in M₁ generation but gradually decreased in subsequent generations due to ceasing toxic effect of mutagens as well as DNA repair mechanism.
(i) UNIVALENTS

The univalents occurred uniformly at diakinesis and their frequency generally increased with the increasing concentrations of mutagens. Although it was dose dependent but did not occur in the lowest concentration of EMS. The frequencies were more in Cd(NO₃)₂ followed by Pb(NO₃)₂, MMS and EMS. The frequency of univalents was the highest in M₁ generation, but decreased in M₂ and M₃ generations.

The reason behind the formation of univalents may be due to induced heterology in homologous chromosomes, as a result of desynapsis or asynapsis. According to Kaul and Nirmala, (1993) and Reddi et al., (1999), where univalency is complete the asynapsis is presumed and where it is partial desynapsis is inferred. Resultantly the occurrence of univalents may be due to the induction of structural changes in chromosomes (Zeerak, 1992), failure of pairing or slipping off of the chiasmata followed by failure of chromosome movement (Singh, 1992), disturbed normal pairing of homologous chromosomes (Siddiqui and Ansari, 2005, Kumar et al., 2006), absence of crossing over at pachytene (Kumar and Rai, 2007a) and due to precocious chiasmata terminalisation (Sidhu, 2008) etc.

On the other hand the reduction in the frequency of univalents in M₂ generation was due to the fact that desynapsis or asynapsis did not occur due to ceasing effect of mutagens leading to normal pairing among bivalents or due to repair mechanism in case of DNA damage.

(ii) MULTIVALENTS

Multivalents occurred both at prophase and metaphase stages. Their frequency was higher at prophase, and more in Cd(NO₃)₂ followed by Pb(NO₃)₂, MMS and EMS. Moreover, their occurrence was the highest in M₁ generation, but decreased in
M$_2$ and M$_3$ generations in all four mutagens.


The multivalent formation has been reported due to the breakage in chromosomes caused by these mutagens and their reunion through reciprocal translocation. Chaghtai and Hasan, (1979) recorded the multivalents with increasing dosage of EMS, DES and MMS in *Lens esculenta* and suggested that translocation might have been produced due to terminal affinities of broken chromosomes.

The formation of multivalents may be attributed to the irregular pairing and breakage followed by translocation and inversion (Vandana et al., 1996), abnormal pairing and non-junction of bivalents (Siddiqui and Ansari, 2005), irregular breakage followed by irregular pairing, translocation and inversion (Zeerak, 1992; Katiyar, 1978; Anis and Wani, 1997 and Kumar and Gupta, 2009).

(iii) PRECOCIOUS MOVEMENT

Precocious movement of chromosomes was observed at metaphase-I and II stages. Their frequency was positively correlated with the concentrations of mutagens and was comparatively more in M$_1$ generation and highest in Cd(NO$_3$)$_2$ followed by Pb(NO$_3$)$_2$, MMS and EMS, but did not occur in the lowest concentrations of Cd(NO$_3$)$_2$ and Pb(NO$_3$)$_2$. Besides the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosomes at metaphase.

The precocious movement of chromosomes might have been caused by the early terminalization of chromosomes and/or movement of chromosomes ahead of the rest during anaphase (Premjit and Grover, 1985). It may also be due to the disturbed homology for chromosome pairing or disturbed spindle mechanism (Agarwal and Ansari, 2001; Amer and Ali, 1974; Umar and Singh, 2003; Kumar and Gupta, 2009).

(iv) **STRAY CHROMOSOMES**

Stray chromosomes occurred in all concentrations of Cd(NO₃)₂ followed by Pb(NO₃)₂, MMS and EMS at metaphase-I & II stages. It did not occur in the lowest concentration of MMS in M₁, M₂ and M₃ generations. They occurred uniformly in M₂ also but in lower frequencies. Stray chromosomes at metaphase-I seem to be caused by spindle dysfunction and clumping of chromosomes (Bhat *et al*., 2007). According to Khan *et al*. (2009a), along with precocious separation of univalents, the bivalents were also observed to move ahead to one pole resulting into unequal distribution of chromosomes or loss of a complete bivalent at later stage.

(v) **STICKINESS**

Chromosomal stickiness is characterized by clustering of chromosomes during
any phase of cell cycle and the number involved in comprising the stickiness varied from two to whole chromosome complement and failed to disjunct individually.

The stickiness among the chromosomes was present frequently at metaphase and rarely at anaphase. Generally the frequency increased with increasing concentrations of all mutagens and that it was the highest in M₁ generation, but decreased in subsequent generations.

Stickiness has been common meiotic abnormality reported by various workers, such as Pagliarini et al., (2000); Jabee and Ansari, (2005); Khan and Aslam, (2006); Kumar and Srivastava, (2006); Kumar and Tripathi, (2007); Chidambaram et al., (2008); Rai and Kumar, (2010); Gulfishan et al., (2011). According to Tarar and Dnyansagar,(1980); Kumar et al., (2003); Kumar and Tripathi, (2003); Jabee et al., (2008) the stickiness of chromosomes might have arisen as a result of depolymerisation of nucleic acid caused by mutagenic treatments. Myers et al., (1992); Kumar et al., (2003) and Kumar and Rai, (2007b) suggested that stickiness occurred due to improper folding of chromosome fibers and as a result there is intermingling of fibers, consequently the chromosomes become attached to each other by means of sub-chromatid bridges. It could also be due to the result of partial dissociation and altered pattern of organization of nucleoproteins. Rao and Lakshmi,(1980) hold the view that stickiness could be due to disturbances of cyto-chemically balanced reactions by the effects of alkylating agents. According to Chidambaram et al., (2008) stickiness appears as a result of disturbances in the nucleic acid metabolism in the cell. It may cause unequal division by the movement of whole bivalent towards one pole at anaphase due to the non-disjunction of homologous chromosomes.
(vi) LAGGARDS

Laggards were present in almost all treatments except in the lowest concentrations of mutagens. These exhibited increasing trend in the higher concentrations of mutagens in M1, but their frequency decreased in the subsequent generations. Occurrence of laggards as observed in the present study has also been reported previously by many workers, such as Siddiqui et al., (1982) in *Helianthus annuus* L. Singh et al., (1999) in *Vigna radiata*, Zeerak, (1992) in *Lycopersicon*; Iqbal and Dutta, (2007) in *Withania somnifera* L. Dun; Khan et al., (2009b) in *Cichorium intybus* L.; Rai and Kumar, (2010) in *Zea mays*; Gulfishan et al., (2011) in *Capsicum annuum*; and *Trigonella foenum graecum* (Choudhary et al., 2012) etc. The fragments, which appeared on the breakage of bridges, as a result of spindle fibers functioning to pull the chromosome towards poles, formed laggards (Kumar and Gupta, 2009). Asynaptic condition which results in abnormal meiosis in later stages may also lead to laggard formation (Sjodin, 1970). Kumar and Rai, (2007a, 2009) also have the opinion that laggards might have appeared due to improper spindle functioning.

(vii) BRIDGES

The single, double and multiple bridges occurred at anaphase-I and II and telophase-I and II stages in the treated populations. The frequency was generally higher at anaphase than telophase and increased with the increasing concentrations of all mutagens in M1 generation. The lower frequency at telophase was due to the fact that the bridges broke during separation of the chromosomes at telophase. In M2 generation the pattern was same but their frequencies were comparatively lower. The bridge formation may be due to sister chromatid exchange followed by delayed or

Singh and Khanna, (1988) suggested that anaphase bridges may be formed due to unequal exchange or dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosomes, which are pulled equally to both the poles at anaphase and a bridge is formed. El-Ghamery *et al.*, (2000) also hold the opinion that the presence of single and multiple bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage and reunion of these chromosomes. Agarwal and Ansari, (2001) suggested that chromosomal stickiness, subsequent failure of anaphase separation and unequal translocation or inversion of chromosome segments are the main reasons for the presence of chromosomal bridges during cell division. Bridges might have occurred as a result of delayed terminalisation, stickiness of chromosome ends and failure of chromosome movement (Das and Roy, 1989; Iqbal and Datta, 2007; Bipasha and Shella, 1992).

Kumar and Gupta, (2009) reported that gene mutation or direct action of mutagen on the target protein, responsible for chiasmata terminalisation during diakinesis at meiosis-I, cause some structural defects in the protein which lead to their improper
functioning, thus resulting into bridges.

(viii) UNEQUAL SEPARATION OF CHROMOSOMES

Unequal separation of chromosomes was found in higher concentrations of mutagens at anaphase and telophase-I/II stages owing to disturbances in spindle mechanism. Cadmium was found to induce unequal separation in more number of cells followed by others. Its presence has been also reported by Venkateswarlu et al., (1988) in *Catharanthus*; Tarar and Dnyansagar, (1980) in *Turnera*; Ahmad, (1993) in *Cicer arietinum*; Kaul and Nirmala, (1993) in *Pisum*; Singh, (2002) in *Hordeum vulgare*; Rai and Kumar, (2010) in *Zea mays* L; Gulfishan et al., (2011) in *Capsicum annuum* etc. Unequal separation might have resulted due to stickiness among affected chromosomes.

Kumar and Rai, (2007b) reported that unequal separation of chromosomes in meiosis-I and II might be the result of the non-oriented bivalent formation due to spindle dysfunction. It might have also occurred due to early or delayed separation of bivalents.

(ix) MULTINUCLEATE/ MICRONUCLEATE CONDITION

Multinucleate conditions (more than four nuclei) at telophase II stage were found in low frequency only at higher concentrations of all mutagens but cadmium was more effective to induce the same. Moreover their frequency decreased in subsequent generations. Occurrence of micronuclei (more than four smaller nuclei) was also reported by Reddi and Rao, (2000) in rice; Dryanova and Dimitrova, (2000) in *Triticale* callus; Singh, (2002) in *Hordeum vulgare*; Utsunomiya et al., (2002) in *Zea mays*; Cavusoglu et al., (2009) in *Phaseolus vulgaris*; Cavusoglu and Yalcin, (2010) in barbunia and Choudhary et al., (2012) in *Trigonella foenum graecum* etc.
There is a correlation between the occurrence of laggards due to failure of spindle formation and the formation of micronuclei at telophase. The laggards and non-oriented chromosomes when fail to reach the poles in time to be included in the main telophase nucleus, form micronuclei, leading to multinucleate condition (Reddy and Rao, 1982; Utsunomiya et al., 2002). This may also occur due to reorganization of the chromosomes between pro-metaphase and anaphase-II (Pozzobon and Schifino-Wittmann, 2006), coalescence or aggregation of chromatin materials into masses of various number and size (Kabir and Alam, 1986) and denature of spindles and delay in the formation of chromosome spindle complex (Cavusoglu and Yalcin, 2010) etc.

(x) DISTURBED POLARITY AND CYTOMIXIS

Disturbed polarity and cytomixis were observed in the present study and their frequency increased with increasing concentrations of mutagens.


Cytomixis was reported by many researchers in a wide range of flowering plant families (Bellucci et al., 2003; Lattoo et al., 2006; Boldrini and Pagliarini, 2006; Sheidai and Bagheri-Shabestarei, 2007; Singhal and Kumar, 2008; Zhen-Qiao Song and Xing-Feng Li, 2009). In recent years there is accumulating evidence that cytomixis is a normal genetically controlled phenomenon influenced by physiological and environmental factors (Bellucci et al., 2003; Lattoo et al., 2006; Boldrini and Pagliarini, 2006) rather than being due to fortuitous causes such as fixation,
mechanical injuries or pathological anomaly.

(xii) CHIASMATA FREQUENCY

Formation of chiasmata results due to homologous pairing of chromosomes and controls the degree of recombination. According to Rees and Dale, (1974) and Sheidai et al., (2006) the variation in chiasmata can be considered as a means for generating new forms of recombination which influences variability within natural populations in an adaptive way. Therefore, the attempts were aimed to induce variations in chiasmata per cell and per bivalent.

The chiasmata frequency at diakinesis was comparatively higher than metaphase in control as well as treated populations due to their terminalisation at later stage. The frequencies per cell and per bivalent at both stages were inversely proportional to the increasing concentrations of all mutagens. The maximum adverse effect on chiasmata was observed in Cd(NO₃)₂. Apparently it was caused due to increasing frequency of univalents, rod bivalents and multivalents, but particularly due to increased heterology induced by mutagens which directly affected the crossing over.


Reduction in chiasmata may be attributed to the mutations in the genes governing homologous pairing. This may also be referred to as cryptic structural changes in the chromosomes forming genetic differences and restricting the pairing with other homologous ones. Sadanandam and Subhash, (1985) attributed the reduction in chiasmata to the nature and potency of mutagens and also to the underlying factors,
such as complex structural changes or the change in the nature of gene responsible for chiasmata formation. It might lead to the failure of complete pairing.

**CONCLUSIONS**

In the present study cyto-morphological variations observed were an outcome of physio-biochemical disturbances induced at genic level by the action of mutagens along with their interactions with environment.

Several positive and negative mutants were screened in M₂ and M₃ generation. MMS showed linear concentration effect on different aspects of *Trigonella*, such as seed germination, growth and yield etc. EMS showed enhancing effect on these aspects in lower concentrations, but its effect in higher concentrations was similar to MMS and induced more variations leading to screening of mutants in M₂ and M₃ generations. In addition giant and high yielding mutants have been isolated in M₃ generation. Most deleterious effects was observed in Cd(NO₃)₂ which had caused maximum chromosomal abnormalities. However in M₃ generation the deleterious effect was minimized to a much extent possibly due to operation of some recovery mechanism and/or elimination of highly affected seedlings and plants. The morphological variations were more in Cd(NO₃)₂ treatments. In spite of their deleterious effects some positive mutants were established in M₃ generation viz. Semi dwarf and dwarf mutants with increased number of branches, leaves and pods, improved yielding mutants with bigger leaves and bold seeded mutants etc.

Maximum frequency of chromosomal aberrations were induced by Cd(NO₃)₂ at cytological level. The chromosomal abnormalities as a whole were dose dependent and increased along with the increasing concentrations of mutagens. The occurrence of many cytological irregularities clearly indicates that the mutagens have genotoxical
effect and the mutants which survived after going through these stages were genic mutants. Since leaves and seeds are equally important in *Trigonella*, the importance in these two characters was significant achievement.

From the result obtained in the present investigation, it can be concluded that all the mutagens are highly effective in inducing genetic variability with varying frequencies and significant alterations in cyto-morphological and biochemical parameters. The results obtained decisively demonstrate the usefulness and effective potential of the induced mutational approach in genetic improvement of *Trigonella foenum graecum* L. for recovering superior mutant plant types having enhanced yield, protein content. The present studies, with respect to isolation of viable mutants, have been remarkably successful. Some of these mutants have been found to be superior to their parent plant in several respects (height, branches, protein content and yield contributing traits). Some of the mutants isolated in the present investigation were exhibiting negative selection value and these might be useful only to the plant breeder in hybridization programmes. But a few mutants could be improved through selection by eliminating some of the undesirable characters, they can be used in breeding programmes aimed at genetic improvement of *Trigonella*.

The above results thus suggest that cyto-morphological diversities induced in the *Trigonella* via mutagenic application can be used for selection of better mutants with desirable characters, because more the variations greater will be the chances for the selection of better qualitative and quantitative characters and better chances for the adaptation of these mutants.