Chapter-5
DISCUSSION

The breeding potential of a crop is to exploit the existing variability through selection or created variability. Mutation breeding technique is the best method to enlarge the genetically conditioned variability of a species within a short time and has played a significant role in the development of many crop varieties (Micke, 1988).

Since the discovery of induced mutation (Muller, 1927; Stadler, 1928), which occurs at a higher rate than that which occur in nature, the breeder is no longer limited to the source of natural mutations. Moreover, the spontaneous and induced mutations do not differ qualitatively from each other and the whole range of natural genetic variability could be reproduced by means of induced mutations and by recombination following crossing. Any mutation could be induced that has occurred naturally and probably many of which have either never occurred or have been lost from natural population. By applying appropriate selection technique such mutants with suitable agronomic traits could be retain rather than that be dependent upon those that have survived natural and primitive selection (Brock, 1971).

As genetic variability is essential for any crop improvement programme, the creation and rearrangement of genetic variability become central base of crop breeding. Experimentally, induced mutations provide an important source of variability (Singh et al., 2000).

In the past, mutation research was limited to the study of macro-mutations. Such mutations are often associated with
reduced fertility and viability due to pleiotropic effect of the mutant gene or simultaneous mutations in the linked or associated genes. The occurrence of negative effects are the greatest barrier for the direct use of macro-mutations in plant breeding. However, the undesirable defects can be eliminated by transferring them in a new genetic background (Gottschalk and Wolf, 1983).

Serious attempts have been made in late 50's for the improvement of crop plants through induced polygenic variability. In India, 43 varieties of cereal crops, 38 of grain legumes, 23 of oil seeds, 13 of fibre crop and 10 of millets that were developed by mutagenesis, have been released/approved for cultivation (Kharakwal, 1996).

Micro-mutations occur more frequently and generally have less pronounced pleiotropic effects on fertility and vitality. Moreover, such mutations can be easily handled and subjected to selection in a definite direction. Keeping in view the usefulness of mutation breeding, the present investigation was undertaken to explore the possibility of inducing desirable macro and micro-mutations for various agronomic traits which can be utilized in breeding programme.

In the present investigation the immediate effect of mutagenic treatments in M₁ generation were estimated in two species of Trigonella viz. Trigonella foenum-graecum L. and Trigonella corniculata L. through different biological parameters such as seed germination, plant survival at maturity, pollen sterility and meiotic aberrations in treated as well as control populations. Generally, all these biological parameters in M₁
generation and chlorophyll and viable mutation frequency in $M_2$
are used to assess the superiority of mutagens (Bhamburkar and
Bhalla, 1985; Reddy et al., 1992; Thakur and Sethi, 1995;
Kharakwal, 1998a, 1998b; Kumar and Dubey 1998c; Khan,
1999).

Seed germination and plant survival decreased with the
increase in mutagenic treatments in the present investigation.
However, the extent of decrease differed both among different
mutagens as well as between the two species. Number of reports
have indicated significant reduction in germination percentage
after mutagenic treatment (Grover and Tejpaul, 1979; Mahapatra,
1983; Adamska et al., 1995; Pandey et al., 1996; Kumar and
Mani, 1997; Arumugam et al., 1997; Khan et al. 1998; Anis et
al., 1999). Most of these workers have observed a dose
dependent reduction in germination.

Several workers attempted to explain the cause responsible
for inhibition of germination. Kalia (1984) reported that the
inhibition of germination in chemical treatments may be due to
damage to the enzyme system involved in repair mechanism or
due to the production of toxic substances in the cell. Griffith
and Johnson (1962) and Srivastava (1979) considered the
reduction in germination percentage to be due to weakening and
disturbances of growth processes.

As a result of mutagenic treatment, plant survival decreased
appreciably with increasing dose of mutagens in both the species
of *Trigonella*. Dose dependent reduction in plant survival was
also reported by Chekalin (1968); Bhadra (1982) and Siddiq and
Swaminathan (1968). Decrease in seedling survival may be
attributed to a series of events occurring at the cellular level which affect the macromolecules and bring about a physiological imbalance in the cell as a consequence of exposure to chemical mutagens. However, more lethality at higher doses of chemical mutagen may be because of physiological toxicity of the mutagen or production of toxic intermediate substances in metabolic pathways due to blockage or mutation (Waghmare, 1993). Reduction in survival could also be due to inhibition of enzymes or growth substances or inability of cells to utilize growth substances (Riley, 1954).

In the present study varying degree of pollen sterility was induced in all the mutagenic treatments in both the species, although, some 1-2% pollen sterility was also observed in the control plants. Also, the magnitude of sterility was considerably high and increased with the increase in mutagenic concentrations. Similar reports have been made earlier by Ganguli and Bhaduri (1980), Prasad and Das (1980b), Bhadra (1982), Nadarajan et al. (1983) and Kumar and Dubey (1994, 1998a, 1998b).

The high sterility observed in the treated populations may be attributed to the vast array of meiotic aberrations that were induced by the chemical mutagens leading to aberrant pollen mother cells and ultimately in the inactivation of pollen grains. This is in agreement with many workers (Rana and Swaminathan, 1964; Sinha and Godward, 1972a; Ramanna, 1974).

Among the three mutagens DMS proved to be most effective in inducing pollen sterility. Failure of homologous pairing during
meiosis could be the main cause of high pollen sterility. Sterility may also be caused due to the physiological damage produced by the hydrolytic products of the alkylating chemicals (Kumar and Mani, 1997). Gaul (1970) has suggested that the chromosomal aberrations are probably the major effect of all mutagenically induced pollen sterility. The actual reason of sterility caused by these chemical mutagens may be a gene mutation or more probably "invisible deficiencies".

Studies on some biological parameters viz. germination, survival and sterility revealed that much of the inhibitory effects were reduced in M2 generation, although the higher treatments of all mutagens still retained the adverse effects, such a recovery mechanism in M2 generation has also been reported by different workers (Katiyar, 1978; Jayabalan and Rao, 1987a; Subba Rao, 1988).

For almost all biological parameters, *Trigonella corniculata* showed comparatively more biological damage than *Trigonella foenum-graecum*, indicating its greater sensitivity to the chemical mutagens. Similar varietal sensitivity has been reported in *Lathyrus sativus* (Nerkar, 1976), microsperma lentil (Sharma and Sharma, 1981), groundnut (Venkatachalam and Jayabalan, 1995) and in black gram (Khan, 1999). It is concluded that varieties with a large assortment of recessive characters show greater sensitivity than varieties with dominant characters (Gelin et al., 1958; Blixt, 1970).

Mutations can be beneficially utilized for tailoring better varieties of crop plants. But in general, ionizing radiations and chemical mutagens effects a wide range of chromosomal
alterations resulting into abnormal behaviour during meiosis, leading to various degree of sterility. Further, the cytological abnormalities during meiosis has also been regarded as one of the dependable parameters for estimating mutagenic sensitivity of a species (Dhamyanthi and Reddy, 2000).

Cytogenetic studies are also important for obtaining information regarding the role and effect of various mutagens and elucidating the response of various genotypes to a particular mutagen (Reddy and Annadurai, 1992). In this context, cytological investigations appear rewarding as they deal with the primary genetic material, the chromosome and more appropriately the DNA which, controls the phenotypes. The best approach would be to consider the chromosomes as the source of genetic informations necessary for the development of phenotypes (Katiyar, 1978).

The pollen mother cells undergoing meiosis attain this stage after many mitotic cycles. At the same time recovery mechanism can start operating in the elimination of lethal and sub-lethals through natural selection right from the initiation of germination following mutagenic treatment (Sinha and Godward, 1969). Yet some aberrations persist and affect the viability of gametes and subsequently the fertility of plant.

A vast array of meiotic aberrations were induced with SA, MH and DMS in the present investigation. Meiotic abnormalities increased with the increase in dose/concentration of the mutagen. Although, the type of abnormalities induced were more or less common in both species, but the frequency of aberrations were comparatively more in T.corniculata, indicating

**Stickiness of chromosomes** was the most common abnormality observed in the present investigation. Chromosomes clumped into one, two or many groups due to stickiness at metaphase causing difficulty in normal disjunction of chromosomes. These results are in agreement with Sinha and Godward (1972b); Katiyar (1978), Tarar and Dnyansagar (1980) and Mitra and Bhowmik (1996), who also reported stickiness as the most common abnormality and grouping of different bivalents due to stickiness. Stickiness could be due to a partial dissociation of the nucleoproteins and alterations in their pattern.
of organization (Anis and Sharma, 1997) or due to the
depolymerisation of nucleic acid caused by mutagenic treatment
(Tarar and Dnyansagar, 1980). Darlington and La Cour (1945)
observed this feature in Allium and Trillium and suggested that
there was a reduction of correctly polymerized nucleic acid on
the chromosomes producing characteristic errors of spiralization
which combined with superimposed excess of non-polymerized
nucleic acid to cause surface stickiness.*

The frequency of univalents ranged from 2-5 per PMC and
these were later found as laggards at anaphase and telophase
stages. Univalents seem to arise from partial or lack of
homologous chromosome pairing or due to cryptic structural
changes in some of the chromosomes which restrict pairing. Rao
and Laxmi (1980) attributed univalent formation to the partial
and complete lack of homologous chromosome pairing. Further,
the disturbances in the pairing was ascribed to the presence of
chromosome breakage in the PMCs of plants raised from treated
seeds. Some of the univalents disjuncted early and presumably
this happened due to genic differences. Such chromosomal
divergences in the form of precocious movement is pointed
towards structural differentiation of homologous pair (Anis and
Wani, 1997). Mitra and Bhowmik (1996) reported that non-
pairing and early separation of chromosomes at meiosis may
result in the formation of univalents. Zeerak (1992a) concluded
that mutagen induced structural changes in chromosomes and
gene mutations might be responsible for the failure of pairing
among homologous chromosomes and hence the presence of
univalents."

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Different types of multivalent associations (Tri, tetra and chain of bivalents) as observed in the present investigation have also been reported by many workers. Alteration in chromosome associations which composed of uni, tri, tetra and multivalents were possibly the outcome of non or irregular pairing of chromosomes or due to translocations (Katiyar 1978). According to Zeerak (1992a) multivalent formation can be attributed to irregular pairing and breakage followed by translocations and inversions.

The laggards observed during the present investigation may be due to delayed terminalization, stickiness of chromosomes ends or because of failure of chromosomal movement (Jayabalan and Rao, 1987b; Soheir et al., 1989). Schulz and Shaeffer (1980) concluded that lagging chromosomes and their presence as univalents may result in aneuploidy.

Bridges without fragments at anaphase and telophase stages were frequently observed in the present investigation. Bridges often break as the dyads move further apart in the late anaphase. The presence of chromosome bridges without fragments may be due to restitution or the fragments getting entangled or attached with normal chromatids of chromosomes (Tarar and Dnyansagar, 1980). PMCs with a single bridge without acentric fragment in Anaphase I is formed by two sister chromatids of a broken chromosome which has undergone fusion during interphase at the time of duplication (Mc Clintock, 1938).

In the present investigation, bridge formation can be attributed to the general stickiness of chromosomes at metaphase stage or breakage and reunion of chromosomes or chromatids.
Similar bridges are reported by many workers after irradiation and chemical treatments (Tarar and Dnyansagar, 1980; Reddy et al., 1992; Reddy and Annadurai, 1992; Zeerak, 1992a; Anis and Sharma 1997). The presence of the chromosome bridge was common at anaphase I, this may be due to the paracentric inversion (Tarar and Dnyansagar, 1980).

Movement of bivalents towards poles at Anaphase due to non-disjunction of homologous chromosomes at metaphase as observed during the present study was due to stickiness of chromosomes and could result in unequal distribution of chromosomes in the daughter nuclei (Anis and Wani, 1997).

Abnormalities, such as lagging chromosomes and unequal separation of chromosomes especially the last one would lead to the production of aneuploid gametes and thus of aneuploid plants in the next generation. Such plants (aneuploids) are of immense importance in fundamental as well as applied research in crop improvement. It may be noted that, in case of unequal separation at AI, the most frequent separation was 9:7 chromosomes. A functioning gamete with 9 chromosomes will produce a trisomic upon union with a normal gamete (Wani, 2000). Mitra and Bhowmik (1996) reported that unequal separation of chromosomes was caused by spindle irregularities.

Micronuclei generally arise from fragments and lagging chromosomes which failed to reach the poles and get included in the daughter nuclei (Kumar and Dubey 1998c).

Cytomixis refers to the migration of chromatin/chromosomes from one cell into the cytoplasm of another cell through cytoplasmic channels. Cytomixis has been reported to occur
mainly during prophase I when the callose wall that surrounds the microsporocytes is not yet fully formed. The probable causes of cytomixis are fixation effects (Heslop-Harrison, 1966; Haroun, 1995), pathological conditions (Bobak and Herich, 1978), altered physiological controls (Bell, 1964), changes in gene control (Omara, 1976). The process is considered to be a source of production of aneuploid and polyploid gametes (Koul, 1990; Yen et al., 1993). Cytomixis between and among different stages of meiosis was also reported by Maria de Souza and Pagliarini (1997) in Centella asiatica.

In the present study, DMS proved to be most effective in inducing the maximum frequency of aberrations followed by MH and SA. Similar results with other alkylating agents like EMS were reported by Reddy and Annadurai (1992), Singh et al. (1989) and Roy (1989) in lentil; Dhamyanthi and Reddy (2000) in Capsicum annuum.

Mann (1977) suggested that alkylating agents react with several nucleophilic centres in a cell, with DNA being the primary site of alkylation. The alkylated DNA separates and leave the DNA depurinated; resulting in gaps in the DNA molecule. These gaps may initiate same exchange process at this stage, giving rise to various types of observed aberrations.

Among different stages of meiosis, the frequency of meiotic aberrations was maximum at metaphase stage in the present study. Similar observations were also reported by Mitra and Bhowmik (1996) in Nigella sativa and Kumar and Dubey (1998c) in Lathyrus sativus L.
Studies on various quantitative traits in $M_1$ generation have revealed that no appreciable change was caused among different polygenic traits as indicated by the pooled mean values of different mutagens, although the mutagenic effect was clearly evident at different concentrations of all mutagens by reduction in plant height, number of branches, number of pods per plant, number of cluster per plant, number of pods per cluster, number of seeds per pod, 1000 seed weight (g) and total plant yield at higher treatments and stimulation in the mean values of these traits in some lower treatments.

A decrease in plant height, number of branches, number of pods per plant, seeds per pod and seed yield are common features of mutagenic treatments in various crops (Sree Ramulu, 1974; Sharma and Sharma, 1982; Laxmi and Gupta, 1983; Khan, 1984; Sarkar and Sharma, 1989; Tripathi and Dubey, 1990; Jain and Agrawal, 1993; Kumar and Dubey, 1994, 1998a, 1998b; Anis et al., 1999).

The coefficient of variation increased for almost all polygenic traits in the treated populations as compared to control in $M_1$ generation. Similar increase in CV for most of the quantitative traits in $M_1$ was also reported earlier (Sahai and Dalal, 1974; Sharma and Sharma 1982; Khan; 1984).

Since no appreciable change was observed within pooled mean values for various quantitative characters, it could be due to the fact that in the present study macro-mutational variants were excluded from the assessment of mean in $M_1$ and data were recorded on normal looking plants only.
The chlorophyll mutation rate in \( M_2 \) has been used as a preliminary index for estimating relative genetic effects of mutagenic treatments and mutability of the variety (Gustafsson, 1951; Gustafsson and Von Wettstein, 1956). It is assumed that the changes in green colour of plant are related with point mutations, intragenic changes or small deletions. However, complete fertility of heterozygotes (obtained by crossing chlorophyll mutants with normal) indicates that chlorophyll mutations are genic. Chlorophyll development seems to be controlled by many genes located on several chromosomes (Goud, 1967a) which could be adjacent to centromere and proximal segments of chromosomes (Swaminathan, 1964; 1965) Mutations in these chlorophyll genes may induce chlorophyll mutations.

The mutation frequency and spectrum of mutations have been reported to be controlled by the nature of mutagens as well as genotypic architecture of the varieties (Sidorova, 1966). Despite the fact that different frequencies of similar mutations are induced by different mutagens, the chief limiting factor in the induction and recovery of mutations is the genetic constitution of the experimental material (Gregory, 1965).

In general, all mutagenic treatments induced fairly high frequency of chlorophyll mutations with four different types of chlorophyll mutants viz. albina, xantha, chlorina and maculata, in the present investigation. Though, the chlorophyll mutation frequency initially increased with the increase in dose of the mutagen, a slight decrease in mutation frequency was observed at higher treatments of all mutagens with a few exceptions. Similar claims of obtaining high frequency of chlorophyll mutations with medium or lower doses of mutagen were made
by Srivastava et al. (1973); Nadarajan et al. (1982); Kharakwal (1998b); Mitra and Bhowmik (1999). However, dose dependent increase in mutation frequency as reported by Sarkar (1985); Kulshreshtha and Singh (1983) and Das and Kundagrami (2000) are in contradiction with the results obtained in the present study. The conflicting results may be due to differential response of genotypes in relation to differences in dose range of the mutagen used.

It seems that strong mutagens reach their saturation point even at lower doses in the highly mutable genotypes and further increase in dose does not add to the mutation frequency. With the increase in dose beyond a point, the strong mutagen became more toxic than the higher doses of relatively weaker mutagen (Filippetti et al., 1977).

The decrease in mutation frequency at higher doses may be attributed to chromosomal aberrations or saturation in the mutational events which may result in the elimination of mutant cells during the growth (Brock, 1965).

The occurrence of chlorophyll mutations in the present investigation showed predominance of xantha followed by chlorina and maculata in both the species, suggesting that genes for xanthophyll development are readily available for mutagenic action. Albina type was found in the least frequency. Similar observations were made by Reddy and Annadurai (1992) in lentil.

Mutagen dependent specificity for various chlorophyll mutation type was found to be an interesting phenomenon. Certain mutations appeared more frequently whereas, some chlorophyll mutations did not appear in the population. The
relative pattern of mutagenic specificity of this kind has been demonstrated by several workers (Blixt et al., 1963; Nerkar, 1970; Hussein et al., 1974; Sharma, 1977; Chekalin, 1977; Waghmare, 1993).

Based on the extent of chlorophyll mutation frequency and spectrum, *T. corniculata* found to be more sensitive as compare to *T. foenum-graecum* and showed a wider spectrum of chlorophyll mutations. Similar varietal sensitivity has been reported in *Lathyrus sativus* (Prasad and Das, 1980c), chickpea (Kharakwal, 1998b), urdbean (Singh et al., 1999), grasspea (Das and Kundagami, 2000).

The usefulness of a mutagen depends both on its mutagenic effectiveness and efficiency, efficient mutagenesis being the production of maximum desirable changes accompanied by the least possible undesirable changes. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagen. Mutagenic efficiency is an indicative of the proportion of mutations against associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility, induced by the mutagen in question (Konzak et al., 1965; Nilan, 1967).

efficiency of various mutagens and concluded that alkylating agents are more effective and efficient in inducing mutations. Furthermore, the lower doses of mutagen were more efficient and effective as compared to the higher doses.

A common observation in the present investigation revealed that the degree of effectiveness and efficiency varied among different mutagens as also between the two species. In general, *T. corniculata* showed greater mutagenic response as compare to *T. foenum-graecum*. Similar differences in mutagenic response has also been reported by Sharma and Sharma (1981), Kharakwal (1998a) and Khan (1999).

In general, lower or intermediate treatments proved to be most effective and efficient in inducing mutations in the present investigation. This confirms, the findings of Kharakwal (1998a) in chickpea. The higher efficiency of lower concentrations of a mutagenic agent is due to the fact that the biological damage (seedling injury, lethality, and sterility) increases with the increase in dose at a faster rate than the mutations (Konzak *et al*., 1965).

In the present investigation, SA proved to be most effective and efficient among the different mutagen on the basis of lethality and meiotic aberrations. Similar observations were recorded by Konzak *et al.* (1965) and Nilan *et al.* (1973) also. In contrary to this, on the basis of lethality, DMS proved to be most efficient mutagen.

Mutagenic efficiency seemed to vary with respect to lethality, sterility and meiotic aberrations. In general, mutation rate based on meiotic aberration (Mf/M) was highest, followed by lethality
(Mf/L) whereas, it was lowest in case of sterility (Mf/S). Among the different mutagens, SA showed highest mutation rate followed by DMS. MH was least efficient. Thakur and Sethi (1995) also reported SA as a most effective and efficient mutagen compared to other mutagens in barley. Such variations in the mutagenic efficiency based on different criteria used, has also been reported by Sharma and Sharma (1981) in lentil, Dixit and Dubey (1986) in lentil, Geetha and Vaidyanathan (1997) in Soybean, Bhattacharjee et al. (1998) in Catharanthus roseus.

The decrease in effectiveness at higher treatments may be attributed to the failure in proportionate increase of mutation frequency with increase in dose/concentration of mutagens. Similar results were obtained by Gupta and Yashvir (1975) in Setaria italica, Nerkar (1977) in Lathyrus sativus and Singh and Chaturvedi (1980) in Vigna radiata.

Besides chlorophyll mutations, several morphological mutants, exhibiting change in their morphological features were isolated in the screening of M2 populations. Some of the morphological mutations viz. mutations affecting growth habit and mutation affecting pods and seed characteristics appeared more frequently than others. The occurrence of certain mutations in high frequency may be because of the genes governing these characters are probably more sensitive to mutagenic treatments.

Nilan (1967) concluded that different mutagens and treatment procedures may also change the relative proportion of different mutation types. Differences in the frequency of various morphological mutations have been reported in the published literature (Sharma, 1977; Sarkar, 1985; Dixit and Dubey, 1986;
The frequency of these morphological mutants differed among different mutagens as also between the two species. The genetic differences in the cultivars under reference for inducing spectrum and frequency of mutants have also been observed in Bengal gram (Nerkar and Mote, 1978), lentil (Sharma and Sharma, 1981), pigeonpea (Rao and Reddy, 1984) and in urdbean (Singh et al., 1999).

The most striking mutants in the present investigation include tall, dwarf, bushy, broad leaf, pod size and bold seeded mutants. All these mutants were confirmed true breeding in the M₃ generation. Similarly, Verma and Singh (1984), Pandey and Raghuvanshi (1988), Singh et al. (1978), Singh and Yadav (1991), Singh (1996), Singh et al. (1999) reported mutations for plant type, branching pattern, leaf morphology, peduncle length, pod length, seed colour and boldness etc.

The possible cause of these macro-mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations (Singh et al., 1980). Several workers have reported that these viable mutations were monogenic and recessive in nature controlled by one or more recessive genes (Singh and yadav, 1982; Singh et al.; 1987).

The bold seeded mutants isolated in the present investigation are of special interest, since the mutant showed considerable improvement in yield. Cytological observations of these mutants revealed 8 bivalents (2n=16) at metaphase although some meiotic aberrations like stickiness, precocious
separation were also met with. The normal cytological behaviour of mutants may indicate their genetic nature however, cryptic structural changes in the chromosome can not be denied.

In general, the morphological mutants induced in the present study included agronomically desirable features which may possibly be utilized in the future breeding programmes. Besides to test the genetic nature of the trait concerned the segregation pattern of these mutants will be known after crossing them with their respective parents in the future study.

Mutagenesis has proved to be a handy tool to enhance natural mutational rate and thereby enlarging the genetic variability and increasing the scope for obtaining desired selections. Particularly, induction of micro mutations in the polygenic system, controlling the quantitative characters is important for crop improvement.

The components of yield such as plant height, number of branches, number of pods per plant, seeds per pod and seed weight have a complex inheritance. They are known to be governed by polygenes, each of which has a small effect. Gregory (1956) was of the view that normal appearing plants in the mutagenised populations may be variously mutated with a large number of small individually inconsequential changes which on the whole form a sound basis for artificial and natural selection.

Studies which underline the importance of polygenic mutations in plant breeding have been summarised by many workers (Scossiroli, 1965; Gaul, 1965; Brock, 1965; Gregory 1956, 1965). From the work already reported by several authors, especially in self-pollinated crops (Rawlings et al., 1958; Bhatia
and Swaminathan, 1962; Gaul, 1965; Scossiroli et al., 1966; Borojevic and Borojevic, 1972; Chaturvedi and Singh, 1980; Khan, 1984; Sharma, 1986), It is now quite clear that polygenic mutations result in the release of considerable variability in mutagen treated populations. In recent years the role of mutation breeding in increasing the variability for quantitative characters has been proved beyond doubt (Mehetre et al., 1990; Ignacimuthu and Babu, 1993; Srivastava and Singh, 1993; Solanki and Sharma, 1999b; Tickoo and Chandra, 1999; Waghmare and Mehra, 2000).

The degree of success in the genetic improvement of particular trait in crop plants depends on the magnitude of genetic parameters and the breeding methodology adopted. Since, most of the economically important characters are influenced by environment, estimates of genetic parameters like genotypic coefficient of variation, heritability and genetic advance in percent of means are needed to formulate suitable breeding procedures and to see the possibilities upto which a particular trait could be improved. The increase in genetic variability and heritability estimates, due to mutagenic treatments indicates the induction of micro-mutations governing the quantitative parameters. On the other hand, increased heritability in M₃ generation also indicates that significant gains could be expected from selection.

In the present investigation, data on different quantitative characters viz. plant height, number of branches per plant, number of pods per plant, number of clusters per plant, number of pods per cluster, number of seeds per pod, 1000 seed weight and total plant yield were analysed to assess the extent of
induced variability in M₂ and M₃ generation in two different species of Trigonella viz., T. foenum-graecum and T. corniculata.

The mean shifted in positive and negative direction for all quantitative traits in the present investigation. The positive shift was more pronounced at lower or intermediate treatments whereas, negative shift in the mean values was observed at higher treatments. Moreover, there was further increase in the mean values towards positive direction especially for yield and yield contributing traits in M₃ generation as compared to M₂ for most of the mutagenic treatments. The increase in mean values coupled with increased variability in M₃ as compared to M₂ especially for pods per plant, 1000 seed weight and plant yield suggest scope for further selection in M₃ populations.

Scossiroli et al. (1966) also found increase in the mean values of quantitative characters in M₂ and M₃ generation in Triticum and explained this change as a consequence of elimination of "bad" genes.

By using physical and chemical mutagens many workers have found mean values decreasing significantly for most of the characters in M₂ generation (Brock, 1965; Virk et al., 1978; Tickoo and Chandra, 1999). They attributed the decline to either physiological damage, caused chiefly by chemical mutagens or chromosomal aberrations, caused mainly by irradiations. These physiological disturbances get eliminated progressively in the subsequent generation. Brock (1965) observed that random mutations in characters with definite selection history, shift the treatment means away from the control mean, in the direction opposite to the previous selection history. Contrarily, it is
proposed that random mutations bring about unidirectional changes in the mean values of almost all the quantitative characters of interest to the plant breeder (Gaul and Aastveit, 1966).

Some of the workers argued that the induced genetic changes are unidirectional in effect and are of negative sign (Oka et al., 1958; Sakai and Suzuki, 1964). Small changes in quantitative characters which are genetically uncorrected with other changes of great magnitude will be symmetrically distributed, when the changes of great magnitude are removed from the populations by selection (Brock, 1966; Stucker et al., 1968).

Another group of workers believe that mean remains almost unchanged although there is increase in variance due to mutagenic treatments indicating bidirectional mutations (Rao, 1974; Upadhyaya and Singh, 1979). It is generally believed that in a population selected for high mean, the induced mutations would reduce the mean since the desirable genes could be mutated to undesirable ones. This may be true only when the proportion of favourable genes is more than unfavourable genes so that possibility of unfavourable mutations is more. The mean performance of a population having equal proportion of favourable and unfavourable genes would remain unchanged. Since, mutations in plus and minus direction will be equally likely (Upadhyaya and Singh, 1979).

The approximation of the mean of mutated lines to those of control can also be explained on the basis of Gregory's model (Gregory, 1968), when the number of plus and minus effects are
essentially equal if the results in the individual changes are exceedingly small. Shift in mean values in both positive and negative direction after mutagenic treatment has been reported by many workers (Sinha and Joshi, 1986; Mehetre et al., 1996; Singh et al., 2000 and Waghmare and Mehra, 2000).

Plant height decreased considerably in most of the mutagenic treatments in M₂ generation whereas, some lower and intermediate treatments of SA and MH showed stimulatory effect. Plant height decreased significantly at higher treatments of all mutagens in both the species. The occurrence of mutations with equal frequencies towards positive and negative directions may be considered as an important reason to justify the tendency of positive and negative shifts in the mean values for plant height (Gregory, 1965). In the treated populations significant decrease in plant height has been reported in Triticum (Sinha and Joshi, 1986), mungbean (Mehetre et al., 1990), grasspea (Waghmare and Mehra, 2000) after treatment with physical and chemical mutagens. Reduction in plant height may be attributed to the fixation of genes controlling reduced plant height (Satyanarayn et al., 1993). Singh et al. (2000) reported increase in plant height whereas, Waghmare and Mehra (2000) reported increase as well as decrease in mutagenised population.

The number of branches per plant increased in most of the treatments however, a significant reduction was observed at higher treatments of all mutagens in M₂ generation in both species. The mean number of branches per plant increased further in M₃ for all treatments, indicating, mutations in positive direction for this trait. The results are in agreement with other
workers (Mehetre et al., 1990; Singh et al., 2000; Waghmare and Mehra, 2000) who also reported increase in mean number of branches after treatments with physical and chemical mutagens.

It is interesting to note that the treatments which showed increase in number of branches also showed increase in number of pods per plant, suggesting close correlation between these two traits. Both these traits, increased further in M₃ generation. It indicates that the induced variability for these traits was in positive direction and the selection was effective in M₂ generation.

The decrease in number of pods per plant at higher treatments in M₂ generation is probably due to high sterility that still existed in these treatments. However, occurrence of polygenic mutation towards positive and negative direction can not be denied in such cases. All the selected treatments showed significant improvement in pods per plant in M₃ in both the species. It should be noted here that increase in mean number of pods per plant in M₃ was associated with increase in genetic parameters in most of the treatments. Such associations of increased mean with high variability suggest scope for further selection in M₃. Similar results were obtained in mungbean (Tickoo and Chandra, 1999).

Some workers reported an increase in number of pods per plant (Ignacimuthu and Babu, 1992; Singh et al., 2000) others reported decrease in pods per plant (Mehetre et al., 1990; Srivastava and Singh, 1993; Tickoo and Chandra, 1999) while still others reported both increase and decrease in number of pods per plant in M₂ generation (Abdalla and Hussein, 1977;
Upadhyaya and Singh, 1979; Waghmare and Mehra, 2000) after treatment with different physical and chemical mutagens.

The different treatments of the mutagens do not make much dent in the mean pod length in any of the species. It seems to be very stable character. However, large and small podded mutants were isolated with certain mutagenic treatments. It was also observed that these large podded mutants could not contribute towards seed yield as the total number of pods per plant was much less in comparison to control.

A significant increase as well as decrease was observed in mean pod length in M2 generation in both species. Similar results were obtained by Khan (1985a, 1985b) in mungbean. Almost all the selected treatments in M3 showed a significant increase in pod length in *T. foenum-graecum* whereas in *T. corniculata*, both significant increase as well as decrease was noticed in selected treatments.

The average number of seeds per pod reduced in the higher treatments of all mutagens however, a significant increase in overall population mean was achieved in some lower and intermediate treatments in M2 generation. Most of the selected treatments showed significant improvement in M3 generation. This improvement was associated with increase in variability from M2 to M3 generation indicating that selection in M2 was effective and that this trait could be improved through further selection.

Increase or decrease in seeds per pod due to mutagenic treatments has been reported earlier (Khan, 1984; Tickoo and Chandra, 1999; Singh et al., 2000). Waghmare and Mehra, (2000) concluded that increase in seeds per pod in M3 is probably
because the individuals carrying sterility due to chromosomal aberrations were more frequent in M₂ and get eliminated in M₃ generation.

The 1000 seed weight increased significantly in lower and intermediate treatments of almost all mutagens in M₂ generation whereas in M₃ generation, 1000 seed weight increased significantly in almost all the selected treatments. This character has been reported to be governed by less number of genes unlike other polygenic traits (Ghose et al., 1960).

Increase in seed weight after mutagenic treatment has been reported in Triticale (Singh and Joshi, 1986), Lathyrus sativus (Sinha and Chaturvedi, 1990), mungbean (Khan, 1984), and urdbean (Singh et al., 2000). Decrease in seed weight in both M₂ and M₃ generation has been reported in mungbean (Tickoo and Chandra, 1999) and Lathyrus sativus (Waghmare and Mehra, 2000).

Plant yield is a complex character and is influenced by many other quantitative characters, like branches per plant, number of pods per plant, seeds per pod and 1000 seed weight. The plant yield as such, is a complex manifestation of large number of genes involved in physio-chemical processes of the plant system. The plant yield was increased significantly in lower and intermediate treatments of all the mutagens in M₂ generation whereas, a significant increase for this trait was noticed in all selected treatments in M₃ generation. Increase in seed yield is probably due to the increase in other yield contributing traits especially pods per plant and 1000 seed weight in these treatments. Increase in seed yield in the present
investigation could be attributed to effective selection adopted for various yield contributing traits in M2 and subsequent generation.

Increase or decrease in plant yield following treatments with different mutagens has been reported by many workers (Khan, 1988; Singh, 1988). The increase in yield may be due to elimination of detrimental effects after selfing and also selection operated against alleles for reducing values (Waghmare, 1993). Singh (1988) concluded that increase in mean seed yield in M3 over the M2 and control could be a result of directed selection for yield exercised in M2 generation. Tickoo and Chandra (1999) observed decrease in seed yield in M3 generation in mungbean.

Decrease in seed yield following mutagenic treatments in M2 and M3 generation was reported by other workers also (Rajput and Siddiqui, 1983; Sarkar, 1985; Srivastava and Singh, 1993 and Waghmare and Mehra, 2000). The reduction in mean yield can be attributed to higher frequency of mutations with negative effects for yield contributing traits. Singh et al. (2000) observed significant increase as well as decrease for plant yield in M2 generation in urdbean.

In general, the mean values in M3 for all yield and its contributing traits were at par with the control in all selected treatments, which can be attributed to two reasons. Firstly, the selection applied to select normal looking plants in M1 and also the selection of high yielding plants in M2 could lead to elimination of plants carrying gross chromosomal abnormalities. Secondly the gradual recovery from the non-genetic mutagen damage could also attribute significantly to this improvement (Sharma and Sharma, 1982).
A more close look on the data for various quantitative characters revealed that lower treatments of SA and DMS, and intermediate treatments of MH induced not only maximum variability but also positive mutations were induced for almost all quantitative traits under study. Further, the selection was effective in M₂ especially for yield and yield contributing traits where the mean values increased significantly from M₂ to M₃ generation. No linear relationship was observed between the dose and mean values for all polygenic traits and species differences in terms of mutagenic response were clearly evident. Such differences have also been reported by most of the workers discussed earlier.

As stated earlier a wide range of variability was induced by all mutagenic treatments in both species. The amount of induced variability however, varied not only among different treatments but also from trait to trait. No linear relationship was observed between the doses and the induced variability for the character investigated. Among the different mutagens DMS and SA induced greater variability than MH in both species. In M₂ generation, the highest coefficient of variation was recorded for branches per plant followed by pods per plant and seeds per pod in \textit{T. foenum-graecum} whereas, in \textit{T. corniculata} highest coefficient of variation was recorded in seeds per pod followed by branches per plant and pod length. Khan (1988) reported that polygenic mutations governing the quantitative characters were the cause of induced variability.

The induced phenotypic variation in M₃ generation, although high among treated population, does not reveal the relative amounts of heritable (genetic) and non-heritable variation. This
was ascertained with the help of some genetic parameters such as genotypic coefficient of variation (GCV), heritability in broad sense ($h^2$) and genetic advance (GA) as a percent of mean. The estimates of genotypic coefficient of variation and heritability of various quantitative characters are essential (Falconer, 1960; Kaul and Bhan, 1974; Khan, 1989). Since they indicate the degree of stability to the environmental fluctuations and the potential transmissibility of a character from parent to offspring and from generation to generation. It is clearly evident from the data that considerable amount of genotypic coefficient of variation was induced in all the selected treatments, for different polygenic traits in both species. In general, the highest genotypic coefficient of variation was recorded for pods per plant followed by branches per plant and seeds per pod in *T. foenum-graecum* whereas, in *T. corniculata*, highest genotypic coefficient of variation was recorded for 1000 seed weight followed by branches per plant and clusters per plant. The amount of genotypic coefficient of variation (GCV) was comparable for rest of the yield contributing traits whereas, plant height recorded comparatively lower values of GCV in both species.

Factors responsible for such increase are considered to be favourable for mutation work in polygenic systems (Sharma and Sharma, 1982). The studies in different crops have shown that the genetic variability is subsequently enlarged for various quantitative characters in M$_2$ and subsequent generation after mutagenic treatment (Goud, 1967b; Gaul *et al.*, 1969; Rao and Siddiq, 1977). No dose dependent increase was observed for genotypic coefficient of variation for the characters investigated. This may be due to additional uncontrolled environmental

Heritability is one of the important way to estimate the heritable portion of the total variation induced. Besides, the most important function of heritability in the genetic studies of quantitative characters, is its predictive role expressing the reliability of phenotypic value as a guide to the breeding value. The heritability is a property of not only a character but also of the population and the environmental circumstances to which the individuals are subjected to. Since the value of heritability depends on the magnitude of all the components of variance, a change in any one of these may affect it.

Heritability, in the control populations was low for all polygenic traits. However, a wide range of heritability was observed in the treated populations. In general, heritability was medium to high for most of the polygenic traits. Different workers have different opinions regarding the range or amount of heritability for various quantitative traits. Nevertheless a wide range of heritability induced by physical and chemical mutagens has been reported by different workers, Upadhyaya and Singh (1979) in soybean, Sharma and Sharma (1982) in lentil, Khan (1984) in mung bean, Ignacimuthu and Babu (1993) in pigeon pea etc. The disparity in results could be because heritability is a property not only of a character but also of the population, environment and the circumstances to which the genotype is subjected to (Falconer, 1960; Kaul, 1980).
Kaul and Bhan (1974) suggested that all the genetic components are influenced by gene frequencies (which differ from population to population according to the history of the population) and by the environmental variations, since more variable conditions reduce heritability or more uniform conditions increase it. The high estimates of heritability in the quantitative characters have been found to be useful in selecting suitable types based on their phenotypic performance.

A rational approach towards the improvement of any crop plant involves selection. A selection within the base population and utilisation of selected material would produce germplasm and that will result in the desired ideotypes. Johnson et al. (1955) advocated that heritability estimates along with genetic advance is usually more helpful than heritability value alone in predicting the resultant effect of selection. This is probably because heritability estimates are subjected to genotype-environmental interactions (Lin et al., 1979). Genetic advance is indicative of the expected genetic progress for a particular trait under suitable selection procedure and consequently carries much significance in self-pollinated crop.

The estimated value of genetic advance in percentage of mean, were of high magnitude for all the yield contributing traits. The value differed in different mutagenic treatments and also from trait to trait. In general, genetic advance was high for pods per plant followed by branches per plant and seeds per pod in T. foenum-graecum whereas, in T. corniculata, it was highest for 1000 seed weight followed by branches per plant and clusters per plant. Increase in heritability and genetic advance in the treated population is mainly due to increase in genetic
component i.e. the induced genetic changes for the quantitative characters. From the plant breeding point of view, this should mean a higher response to selection (Sharma and Sharma, 1982).

The higher values of heritability and genetic advance (GA) also suggested that mutations have mostly occurred at the loci having additive effects (Lawrence, 1965). Increase in heritability coupled with increase in genetic advance has also been reported earlier (Sharma and Sharma, 1982; Khan, 1984; Ignacimuthu and Babu, 1992; Lokesh and Veeresh, 1993).

In general, results about yield and yield components are quite encouraging, since they possess sufficient high value of heritability and genetic advance.

The high yielding variants isolated in M2 generation showed considerable improvement in yield and yield related traits in M3 generation also. The increase in pods per plant and 1000 seed weight were probably the main reasons of increase in seed yield per plant among these mutant lines. It appears that these two traits are highly correlated with yield in *Trigonella* spp. Among different mutant lines isolated in the present study, the maximum plant height was recorded as 51.62cm in case of *T. corniculata* D as compared to 48.09cm in control. Similarly, the highest branches per plant were recorded in case of *T. corniculata* E (8.73) as compared to 6.63 in control. In case of *T. foenum-graecum* the highest number of pods were observed in *T. foenum-graecum* C (22.80) as compared to 12.50 in control. Whereas, in *T. corniculata*, the highest clusters per plant were noticed in *T. corniculata* E (80.13) as compared to 65.53 in
control. The maximum, 1000 seeds weight (10.58 g) and seed yield per plant (2.40 g) were recorded in *T. foenum-graecum* A and *T. foenum-graecum* C respectively. All the mutant lines isolated in the present investigation showed considerable increase in coefficient of variation as compared to the controls for almost all quantitative traits under study, thus suggesting possibilities of selecting better high yielding types in the future generation.