Chapter - 5
Discussion
Induced mutagenesis finds a prominent place in the augmentation and recreation of genetic variability which was lost by too rigid selection or narrow base of germplasm of a crop plant under improvement. The potentiality of mutations for this purpose, however, depends upon the efficiency of induction of mutations to be aimed at, efficiency of screening of the mutants and on the nature of induced mutation. The enhancement of mutation frequency and the alteration of mutation spectrum in a predictable manner remain the all times important aspects of mutation research. To ensure a speedy generation of variability for plant traits to be improved, a mutation breeder has to go through all basic events met in the methodology to ensure a reliable information about the mutagenic sensitivity of biological material and the extent of effectiveness and efficiency of a mutagen in question. Mutagens vary in their mode of action, effectiveness, efficiency and the spectrum of mutations induced. Similarly, genotypes show differential sensitivity towards mutagens even at varietal level.

Mungbean (Vigna radiata (L.) Wilczek), a self-pollinated crop, possesses low genetic variability. Crossing produces limited genetic variability. Therefore, mungbean was chosen as an experimental material for chemical mutagenesis.

The present study was planned to estimate the extent of induced genetic variability for nine quantitative traits and the biological damage induced by three chemical mutagens viz., EMS, HZ and SA. The
observations made during the course of present study have been discussed in this chapter.

5.1. Biological damage

The estimation of biological damage induced by chemical mutagens helps in determining the sensitivity of a biological material as well as the potency of a particular mutagen.

The study of biological damage in terms of percentage of seed germination, seedling growth depression, survival at maturity, meiotic aberrations and pollen fertility in M₁ generation, chlorophyll and morphological mutations frequency in M₂ generation, are generally used to evaluate the mutagenic sensitivity of the biological system under study.

In the present study, it was observed that EMS, HZ and SA bring about a reduction in seed germination, seedling growth, pollen fertility and survival at maturity. Such reductions, with an exception of survival, were found to be dose dependent. Earlier studies of Chaturvedi et al. (1983) in Vigna mungo, Kumar and Mani (1997) in Oryza sativa, Arumugam et al. (1997) in Hordeum vulgare, Hussein and Siddiqui (1997) in Solanum melongena, Gaikwad and Kothekar (2004) in Lens culinaris, Khan and Wani (2005a) in Vigna radiata and Barshile et al. (2006) in Cicer arietinum have shown a linear relationship between the mutagen dose applied and the parameters mentioned above. The survival of plants decreased appreciably in the mutagenic treatments in both the varieties of mungbean, however, there was no direct relationship between the concentrations of the mutagens and survival. These findings are contradictory to those reported by Bhadra (1982), Mahto et al. (1989), Solanki and Sharma (1992), Kumar and Dubey (1998a) and Barshile et al. (2006). The reduction in survival is attributed to
cytogenetic damage and/or physiological disturbances (Sato and Gaul, 1967; Ignacimuthu and Babu, 1988).

Seed germination decreased with the increasing concentrations of mutagens. However, the extent of decrease in seed germination differed both among various mutagens as well as between the two varieties. Seed germination was found to be affected more adversely in the different concentrations of SA. Reduction in germination in mutagenic treatments has been explained due to delay or inhibition of physiological and biological processes necessary for seed germination, which include enzyme activity (Kurobane et al., 1979), hormonal imbalances (Chrispeels and Varner, 1967) and inhibition of mitotic process (Ananthaswamy et al., 1971). Kleinhofs et al. (1978) suggested that azide’s mutagenic action in barley might depend upon either metabolism or the state of DNA, i.e. S phase, in the embryonic shoot cells of the seed. Usuf and Nair (1974) inferred that gamma irradiation interfered with the synthesis of enzymes and at the same time, accelerated, the degradation of existing enzymes involved in the formation of auxins and thus reduces the germination of seeds. High reduction in germination percentage in SA treatments may be due to weakening and disturbances of growth processes. Moreover, the greater lethality at the higher mutagenic concentrations was observed in both the varieties. The greater sensitivity at higher doses of mutagens has been attributed to various factors such as changes in the metabolic activity of the cells (Natarajan and Shiva Shankar, 1965), inhibitory effects of the mutagen (Sree Ramulu, 1972) and to disturbances of balance between promoters and inhibitors of growth regulators (Meherchandani, 1975).
The average seedling height decreased with increasing mutagen concentrations in both the varieties. Seedling injury was more drastic in SA treatments than in treatments with other chemical mutagens. The reduction in seedling height following mutagenic treatments is mainly due to the uneven damage to the meristematic cells as a consequence of genetic injury. The badly damaged cells would produce only a few cell progeny and growth will recur from those cells which are least damaged genetically. Variation in auxin level (Goud and Nayar, 1968), changes in the specific activity of several enzymes (Cherry et al., 1962; Reddy and Vidyavathi, 1985) and physiological injury in the seeds and seedlings (Ignacimuthu and Babu, 1988) were correlated with reduction in seedling height after mutagenic treatments.

Although some four percent pollen sterility occurred in control plants also, the magnitude of sterility increased with the mutagen concentration revealing a linear dependence of fertility on dose as found in Nigella sativa (Mitra and Bhowmik, 1998), Gossypium hirsutum (Muthusamy and Jayabalan, 2002), Hordeum vulgare (Kumar and Singh, 2003), Lens culinaris (Wani et al., 2004), Cicer arietinum (Barshile et al., 2006), Vigna radiata (Khan and Wani, 2006b) and Vigna mungo (Sharma et al., 2006). EMS and HZ treatments induced higher pollen sterility in both the varieties as compared to SA treatments. Among different mutagens, EMS caused more drastic effects on fertility. In most cases meiotic abnormalities are responsible for pollen sterility (Gaul, 1970; Sinha and Godward, 1972; Gottschalk and Klein, 1976; Reddy and Annadurai, 1992; Gupta et al., 1999; Dhamyanthi and Reddy, 2000; Kumar and Singh, 2003).
Ali and Siddiq (1999) reported that among the three mutagens; gamma rays, EMS and SA, the pollen fertility was affected the most with SA treatments in rice. Mustafa (1976) has also found that azide treatments induced high levels of sterility in M1 rice plants. Contrary to these findings, SA proved to be less toxic with regard to pollen sterility in mungbean. Since azide does not induce chromosomal aberrations in barley (Walther, 1976), its effect on sterility has been attributed to gene mutations (Nilan et al., 1976). The sterility induced by SA seems to be more genic and less chromosomal. The percentage of pollen sterility was relatively much less in M2 generation than in M1, indicating that some sort of recovery mechanism must be operating in the intervening period.

A comparison of the mutagenic effects on various biological parameters suggest that the var. NM-1 was more sensitive, while the var. PDM-11 was slightly resistant. Differences in mutagenic sensitivity between the varieties have also been reported earlier (Al-Rubeai and Godward, 1981; Rao and Rao, 1983; Khan and Siddiqui, 1995; Pandey et al., 1996; Gaikwad and Kothekar, 2004; Khan and Wani, 2005a). According to Sparrow et al. (1965) even a single gene difference induced significant changes in radiosensitivity. However, in the present study, the two mungbean varieties differ in their genome architecture. This is testified by the fact that the var. PDM-11 was developed through selection, while the var. NM-1 is a product of inter-varietal hybridization. Therefore, it is suggested that the varieties developed by inter-varietal hybridization are more sensitive than the varieties developed through selection. Khamankar (1984) working with tomato (Lycopersicon esculentum) reported that the rate of mutation was different.
with different mutagens at certain loci. Some of the gene loci affected by one mutagen were not necessarily affected by the other.

The seedlings of mungbean normally emerged with two juvenile cotyledonary leaves oriented opposite to each other on the axis. Chemical mutagens induced variations in the number of cotyledonary leaves in the two varieties of mungbean. The actual mechanism of the induction of such anomalies is still unknown. However, it is generally believed that disturbance in the proportion of growth hormones in the treated materials may be responsible for cotyledonary abnormalities. Grover and Tejpal (1982) held a similar opinion for the occurrence of the chromosomal aberrations. The presence of a single cotyledonary leaf in some seedlings may be due either to cytochemical disturbances or to the acute chromosomal aberrations leading to the death of leaf primordia or of the embryonal cell responsible for leaf development. The formation of an extra cotyledonary leaf, on the other hand, indicates the formation and involvement of additional leaf primordia or the embryonal cell.

Leaf shape is an important characteristic which finally governs the plant form. Most of the variations in the lamina shape was observed in the trifoliate leaves at the higher concentrations of the mutagens, which turned into bifoliate, tetrafoliate or pentafoliate with much variation in shape and size of lamina like lanceolate and lobed, were observed in both the varieties of mungbean. The M1 plants from EMS and HZ treated seeds showed considerably more leaf aberrations as compared to the M1 plants from SA treated seeds. This effect was postulated to be an indication of chromosome damage (Blixt, 1972). The lack of leaf aberrations in the SA treated M1 plants of *Pisum* is due to the unique specificity of azide as a mutagen that
produces intra-genic mutations without concomitant chromosome aberrations (Sander and Muehlbauer, 1977). The altered metabolism as a result of cellular damage may also be one of the reasons for variation in leaflets/leaf. Chaturvedi and Singh (1978), Singh (1980), Lamseejan et al. (1983), Dwivedi and Singh (1985), Singh and Sharma (1993), Khan and Siddiqui (1996) and Sangsiri et al. (2005) also succeeded in inducing leaf mutations in Vigna radiata similar to the present findings. Gamma rays induced multifoliate leaf mutants have been reported by Satyanarayana et al. (1989) in M2 generation of mungbean var. Pant Mung-2. The mutants were found to be controlled by a single recessive gene designated as mfl. Though mungbean is trifoliate in nature, increase in multifoliation will increase the biomass production, which could make a positive impact on seed yield, if the translocation activity in the genotypes were increased by genetic manipulation (Bhagat and Chakravarty, 1989).

5.2. Chlorophyll mutations

The chlorophyll mutation frequency is useful in assessing the potency of a mutagen. Hence scoring of chlorophyll mutations has proved to be a much dependable index for evaluating the genetic effects of the mutagenic treatments. The frequency of chlorophyll mutations was calculated on M1 progeny basis as well as on M2 seedling basis. Using both criteria, the highest M2 mutation frequency based on M1 progenies and M2 seedlings was induced by the highest concentration of EMS, HZ and SA. A linear relationship between chlorophyll mutations frequency and dose was observed in both the varieties of mungbean. Higher frequency of chlorophyll mutations with medium or lower doses of mutagen were reported by Srivastava et al. (1973) in Cicer arietinum and Nadarajan et al. (1982) in
Cajanus cajan, while several other reports indicate dose dependent increase in chlorophyll mutations frequency (Subramanian, 1980; Khan and Siddiqui, 1992a; Reddy et al., 1993; Kharkwal, 1998b; John, 1999; Das and Kundagrami, 2000; Barshile et al., 2006). Of the six types of chlorophyll mutants recorded in M2 generation, chlorina followed by xantha types were predominant in the two varieties. Some previous studies (Subramanian, 1980; Bahl and Gupta, 1982) revealed as many as 7-8 types of chlorophyll mutations. The conflicting reports on the spectrum of chlorophyll mutations in a single species may be due to genotypic differences between the varieties used by different workers. Arora and Kaul (1989) in Pisum sativum, Vanniarajan et al. (1993) in Vigna mungo and Kharkwal (1998b) and Khan et al. (2005b) in Cicer arietinum also reported that the chemical mutagens produced a high frequency of chlorina and xantha types of chlorophyll mutants. Occurrence of chlorina mutants in large number of crops have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids (Bevins et al., 1992).

The frequency and spectrum of chlorophyll mutations are both mutagen and genotype dependent in mungbean. Among the mutagens used in the present study, EMS induced the higher frequency and wider spectrum of chlorophyll mutations than HZ and SA. EMS is supposed to be specific to certain chromosomal regions (Goud, 1967) containing genes for chlorophyll development and has been reported to induce high frequency of chlorophyll mutations (Swaminathan et al., 1962). Recovery of a higher number of chlorina from HZ treated population is contrary to the contention of Reddy and Reddy (1972) that HZ induced more albina mutants. The frequency of
chlorophyll mutations induced by SA was much less as compared to EMS and HZ. Variable frequencies of azide induced chlorophyll deficient mutations have been reported with different treatment conditions (Sideris and Nilan, 1970; Kleinhofs et al., 1974). Awan et al. (1980) observed a high frequency of chlorophyll mutations in rice after sodium azide treatments. The disparity could be due to altered treatment conditions since they used 20 hours pre-soaked rice seeds for mutagen treatment. Pre-soaking enhances metabolic activity and initiates DNA synthesis and its subsequent replication. Both G and S phases of cell cycle are sensitive to mutagens (Arora and Kaul, 1989). Hence, both enhancement in frequency and types of mutations recovered after pre-soaking may be expected. The low chlorophyll mutations frequency in SA treatments may be due to the inhibition of catalase and peroxidase and an increase in peroxide concentration in the cell (Kleinhofs et al., 1978).

The higher number of mutant seedlings recorded after each treatment with the NM-1 variety compared to that of the PDM-11 variety is an indicative of the differential response of these two varieties to the mutagens. Inter-varietal differences with regard to mutation frequency confirm the findings of Singh et al. (1999) in Vigna mungo, Das and Kundagrami (2000) in Lathyrus sativus, Devi et al. (2002) in Vigna umbellata, Paul and Singh (2002) in Lens culinaris and Khan et al. (2005b) in Cicer arietinum.

A glance at the data presented in Tables 14 & 17 shows that in contrast to the high frequency of chlorophyll mutations, the morphological mutations rate was low. This result confirms the earlier findings of Swaminathan et al. (1962) in barley and wheat and Awan et al. (1980) in
rice, who demonstrated the high potency of chemical mutagens in inducing chlorophyll mutations.

5.3. Mutagenic effectiveness and efficiency

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 relates mutagen dose to the mutational events, while mutagenic efficiency shows the proportion of mutations in relation to the undesirable biological effects, such as, pollen sterility and seedling injury induced by the mutagen in question (Konzak et al., 1965). Mutagenic effectiveness and efficiency were estimated on the basis of the frequency of progenies segregating for chlorophyll mutations. In both the varieties of mungbean, the mutagenic effectiveness and efficiency of mutagenic treatments varied not only between treatments of a mutagen but also between the mutagens. The order of mutagenic effectiveness as determined on the basis of mutated plant progenies was HZ>SA and EMS. HZ was also reported to be an effective mutagen in maize (Chandrashekhar and Reddy, 1971); rice (Reddy et al., 1973), barley (Kak and Kaul, 1975) and sorghum (Reddy and Smith, 1984). Contrary to the earlier reports of various workers (Reddy, 1992; Kumar and Dubey, 1998a; Waghmare and Mehra, 2001), EMS proved to be less effective than HZ and SA. The difference in mutagen concentration and/or in the genotypic response seem to be the reasons for low values of its effectiveness. The higher effectiveness of SA over EMS has been demonstrated by Reddi and Suneetha (1992) in rice. All the three chemical mutagens were found to be effective at moderate concentrations. The decline in the mutagenic effectiveness recorded at higher
doses shows that the increase in mutation rate was not proportional to the increase in the doses of various mutagens.

The values of efficiency provide an idea of the extent and type of damage caused by a mutagen in question. The lowest efficiency was recorded in SA and the highest in EMS, HZ being intermediate. The efficiency was higher at moderate doses and decreased with the increase in dose of the mutagens indicating negative relationship. Sharma and Sharma (1979), Dixit and Dubey (1986b), Gautam et al. (1992) and Ratnam and Rao (1993) have reported that mutagenic efficiency increased with increase in dose of the mutagens but Konzak et al. (1965) in barley, Khan and Siddiqui (1993) in different varieties of mungbean and Khan (1999) in black gram reported higher mutagenic efficiency at lower doses. The higher efficiency of lower concentration of a mutagenic agent is due to the fact that the biological damage (seedling injury and pollen sterility) increases with the increase in dose at a faster rate than the mutations (Konzak et al., 1965).

The degree of mutagenic efficiency varied depending on the criteria selected to estimate the mutagenic efficiency. Based on seedling injury (Mp/I), the efficiency was generally higher compared with that based on sterility (Mp/S) for both EMS and HZ. This may be due to the fact that induced seedling injury was less for both the mutagens than the amount of pollen sterility. In SA treatments, the efficiency calculated on the basis of sterility was higher than seedling injury. This confirms the findings of Kumar and Dubey (1998a) in Lathyrus sativus.

5.4. Morphological mutations

Present study has proved fruitful in inducing a wide range of morphological mutations. Although most of them proved uneconomical,
nevertheless, some mutants recorded can be used as a source of many beneficial genes, in cross breeding programmes or for the improvement of some quantitative traits like yield. Various workers suggested that such mutants might be either a result of pleiotropic effects of mutated genes or chromosomal aberrations or gene mutations. The progenies of tall, dwarf, semi-dwarf, bushy, prostrate and yield mutants bred true for the altered traits in M₃ generation. Such mutant types were found to be under the influence of polygenes (Konzak et al., 1969; Marghitu, 1972; Shakoor et al., 1978; Reddy and Gupta, 1988). Some mutants, induced in the present study, show desirable characters from a breeder’s point of view and hold promise for isolating improved types from their progenies in later generations.

Morphological mutants, isolated in M₂ generation, differed not only in the two varieties of mungbean but also within variety in different mutagenic treatments, suggesting that the varieties responded differently to the dose and type of mutagens employed. Based on morphological mutation frequency, NM-1 was proved to be the most mutable variety, while PDM-11 showed least mutability. In both the varieties, EMS produced maximum mutation frequency followed by HZ and SA. The superiority of alkylating agents to induce the highest frequency of morphological mutations has been demonstrated by several workers (Desai and Bhatia, 1975; Tripathi and Dubey, 1992; Rao and Reddy, 1993; Vandana et al., 1994; Sharma and Kumar, 2003; Khan et al., 2004b). A high frequency and broad spectrum of morphological mutants induced by HZ in comparison to EMS has been reported in *Hordeum vulgare* (Kak and Kaul, 1975). Some of the morphological mutations, like yield and plant height, appeared more frequently than growth habit and growth period. Relative differences in the
mutability of genes for different traits have been observed, as some of the mutant types appeared with higher frequencies in some mutagens. For instance, dwarf mutants appeared more frequently with SA and HZ treatments, whereas EMS induced comparatively more mutations affecting growth habit than HZ and SA. The more frequent induction of certain mutation types by a particular mutagen may be attributed to the fact that the genes for these traits are probably more responsive to different mutagens with different modes of action. Nilan (1967) concluded that different mutagens and treatment procedures may also change the relative proportion of different mutation types.

Earliness is one of those characters of a crop that can be obtained reliably in mutation experiments. Lateness is less desired as a mutant character (Gottschalk and Wolf, 1983). A number of early maturing mutants were isolated at various concentrations in the two varieties of mungbean. In these mutants, earliness was combined with normal seed yield. However, a mutant strain of Vigna radiata, obtained in EMS treatments, superior to its mother variety under varying conditions of rainfall due to favourable combination of earliness, drought resistance and increased seed production was reported by Prasad (1976). Early mutants with unaltered agronomic characteristics like yield and growth habit were isolated in Glycine max and Phaseolus vulgaris (Tulmann Neto and Alves, 1997; Tulmann Neto et al., 1984, 1988). Semi-dwarf mutants accompanied with increased pod number and seed yield may be utilized for producing high yielding lines by breeding with desired genotype. Long pod and bold seeded mutant is a useful variation and can be exploited in increasing the number of seeds per pod and seed size leading to increased genetic potential of the yield. Singh (1996)
characterized the mutations obtained in *Vigna mungo* as gene mutations as there were no visible chromosomal changes associated with them. The present long pod and bold seeded mutants also fall in this category. This conclusion is supported by high pollen fertility in the mutants. Bushy mutants showed reduction in yield and yield components. Though these mutants may not be useful for direct commercial cultivation because of reduced yield, they may, however, be used in hybridization to transfer some of its useful traits to other high yielding varieties of mungbean. Though, it is not easy to eliminate the negative traits of the pleiotropic spectrum from the positive ones, the pleiotropic pattern of mutant gene can be altered to some extent by transferring it into a specific genotypic background (Sidorova, 1981). The *erectoides* gene of the barley *erectoides* mutants display a pleiotropic action influencing the number and length of culm and spike internodes. The single traits of this pleiotropic complex can be altered to some extent when the *ert* genes are transferred into specific genotypic backgrounds (Gaul and Grunewaldt, 1971). Pod shattering mutants were noticed in the var. NM-1. Verma (1969) reported a number of pod shattering mutants in *Phaseolus aureus* and found shattering of pod was monogenically dominant over non-shattering. The pod shattering habit seems to be understandable as several varieties of *Vigna radiata* infact shatter pods readily.

5.5. **Quantitative traits**

The nature and extent of genetic variability available within the species forms the basis of effective selection for economic traits under improvement. It is now an established fact that mutagen derived variability is heritable and response to selection is good. Particularly, induction of
micromutations in the polygenic system, controlling the quantitative traits is important for crop improvement. From the work already reported by several authors in various crops (Gregory, 1965; Swaminathan, 1969; Borojevic and Borojevic, 1972; Ignacimuthu and Babu, 1993; Khan et al., 1998; Joshi and Verma, 2004; Khan and Wani, 2005b; Singh et al., 2006) it is now quite clear that micromutations result in the release of considerable genetic variability in the mutagen treated population.

In some early studies on the use of mutations for quantitative traits improvement, it was found that the traits differ in their response to the mutagenic treatments. Variance level may be less responsive in one trait and highly responsive in other (Sharma, 1995). Moreover, the direction of polygenic mutations depends on the genotypic background of the material under study (Loesch, 1964). Thus the genetic improvement of such traits in turn depends upon the magnitude of genetic parameters and the breeding methodology adopted. Estimates of genetic parameters like genotypic coefficient of variation, heritability and genetic advance are therefore, needed to formulate suitable breeding procedures and to foresee the possibility upto which a particular trait could be improved.

In the present study, data on nine quantitative traits, namely, days to flowering, plant height, days to maturity, number of fertile branches, number of pods, pod length, number of seeds per pod, 100-seed weight and total plant yield were analyzed to assess the extent of induced variability in M₂ and M₃ generations of the two varieties of mungbean.

The extent of variability induced by three chemical mutagens differed from trait to trait. Study of the direction of shift in mean values of quantitative traits ascertainment whether mutation breeding can be restored for
the improvement of a trait under study. The mean values shifted to positive and negative directions for all quantitative traits in the present study. The positive shift was more pronounced at moderate concentrations, whereas negative shift was observed at the higher concentrations of the mutagens except for days to flowering, plant height and days to maturity where mean values shifted towards the negative side in almost all the mutagenic treatments. Although mean shifted on either side of the control mean, most of it went towards the positive side for yield and yield components. Enlargement in range of variability for yield and yield contributing traits such as number of fertile branches, number of pods, seeds per pod and 100-seed weight for the two varieties viz; PDM-11 and NM-1 of mungbean in M2 and M3 generations is indicative of the wider scope for selection. Opinions differ regarding the direction of the mutations. Gaul (1965) and Aastveit (1966) hold that the induced polygenic mutations do not follow any particular direction but occur at random. According to Bateman (1959), Brock (1965) and Goud (1967), the polygenic mutations always follow a particular direction opposite to the previous history of selection. Most quantitative traits have a complex genetic determination involving large number of genes interacting with one another, consequently, variation in both the directions is expected. From the results of the present study, it could be concluded that induced mutations are random, bi-directional and cause heritable changes in polygenic system. Besides, it is obvious from the results that the direction of the mutation depends upon the genotype/character under study and the dose applied.

The mean flowering time decreased significantly after the mutagenic treatments. Flowering was early by 4 days with 0.02% of HZ treatment in
both the varieties in $M_3$ generation. Reduction in flowering time accompanied by increase in variability indicated that variability has been induced in desired direction and would offer the possibility for selecting early flowering mutants in such treatment. Kaul (1980a) suggested that the mutations of two dominant genes to their recessive forms makes for an early flowering in peas.

The adverse effect of the mutagens on plant height was clear in both the varieties of mungbean. The treatments of SA at various concentrations gave the maximum reduction in plant height in both the varieties. The extent of reduction in growth is related to the mechanisms of action of a given mutagen. As a respiratory inhibitor, azide may inhibit an energy system resulting in the inhibition of mitosis which can be associated with seedling growth depression. Subba Rao (1988) has attributed the growth depression to slow rate of cell division, decreased amylase activity and increased peroxidase activity. The reduction in mean plant height was reported also by Bajaj et al. (1970) in Phaseolus vulgaris and by Rajput (1974) in Phaseolus aureus. On the other hand, Singh et al. (2000) reported an increase in plant height after treatments with gamma rays and EMS in Vigna mungo.

Use of mutations for obtaining early maturing varieties has been a frequent breeding objective (Micke, 1979). The data obtained on days to maturity resulted in a significant gain in reducing the maturity period by 3-4 days after mutagenic treatments in both the varieties in $M_3$ generation. The HZ treatments seems to be more effective in reducing the maturity period in mungbean. Early maturity would be ideal for a crop like mungbean where the drought approaches at pod filling stage in summer in this region. Wang et al. (2003) in soybean and Shamsuzzaman et al. (2005) in chickpea, also
reported a significant reduction in days to maturity after mutagenic treatments.

The mean number of fertile branches increased in most of the mutagenic treatments in both the varieties in M_2 generation. It is interesting to note that the treatments which showed increase in the number of fertile branches also showed the increase in the number of pods per plant, suggesting close correlation between these two traits. The mean values of these traits increased further in M_3 generation. It indicates that induced variability for these traits was in positive direction. The author is of the view that the increase in the number of pods per plant in the present study is obviously due to an increase in the number of flowers. Flower shedding was not noticed in the two varieties studied. It can also be said without any doubt that the number of pod sets were higher in the lines which produced large number of flowers. All these three traits namely, number of fertile branches, number of pods per plant and number of flowers seem to be highly correlated. Using chemical mutagens and gamma rays, Tickoo and Chandra (1999) have found that the mean values decreased significantly for pods per plant in M_2 generation of mungbean. There are also reports of increase in variance with the mean values remaining unchanged (Sharma, 1986).

The different treatments of the mutagens do not make much dent in the mean pod length in the var. PDM-11. It seems to be a very stable character. However, large poded mutants were obtained with certain mutagenic treatments in the var. NM-1. It was also observed that the increase in mean pod length was associated with a low genetic variability thus suggesting a limited scope of selection for improvement of this trait.
The mean number of seeds per pod increased significantly at lower and moderate mutagenic concentrations in M₂, which further increased in M₃ generation. However, a negative shift was noticed with the higher concentrations in M₂ generation. Khan (1985) assumed this depressive effect to be due to the high seed sterility induced by the higher doses of the mutagens. Similar results were reported by Singh and Chaturvedi (1990) in *Lathyrus sativus* and Singh *et al.* (2000) in *Vigna mungo*.

The character 100-seed weight is a reliable source of measuring yielding ability in pulses. Contrary to the findings of Tickoo and Chandra (1999) in mungbean and Waghmare and Mehra (2000) in Khesari, 100-seed weight, in the present study, has shown a significant increase from the controls with most of the mutagenic treatments in both the varieties of mungbean. The increase in mean values is due to the predominant incidence of favourable mutations in the treated population. This character has been reported to be governed by a relatively smaller number of genes, unlike other polygenic traits (Ghose *et al.*, 1960).

Seed yield in pulses is a complex trait and is influenced by many other quantitative traits like fertile branches per plant, pods per plant, seeds per pod and 100-seed weight. The mean plant yield increased in M₂ generation except at the higher concentrations of the mutagens, and in M₃ generation there was a complete positive trend in the mean values of seed yield per plant in all the selected mutagenic treatments in both the varieties of mungbean. EMS, HZ and SA treatments were effective in raising the seed yield by 3-5 g per plant in both the varieties. In *Vigna mungo*, reduction in mean seed yield per plant in M₂ and M₃ generations has been reported by Singh *et al.* (2000). Waghmare and Mehra (2000) achieved considerably
increased mean seed yield in M3 after gamma rays and EMS treatments in *Lathyrus sativus*. In the present study, increase in mean seed yield in M3 over M2 and the controls could be attributed to effective selection adopted for various yield contributing traits in M2 generation. Bhatia and Swaminathan (1962), working with wheat, concluded that the mean of the irradiated population where no selection has been applied with regard to the specific character under study tends to go down in comparison to the control.

There is a great discrepancy among the opinions of the researchers as to which generations are actually appropriate for the selection of quantitative traits. Gaul (1964), Kumar (1972), Jana and Roy (1973) and Tar’an et al. (2004) are of the opinion that selection for quantitative traits should be delayed until M3 or later generations following the mutagenic treatments. But many other workers have proposed that effective selection for polygenic traits can be done in early generations even in M2 itself (Sneep, 1977; Kharkwal, 1983; Kaul and Matta, 1985; Sarker and Sharma, 1988; Tickoo and Chandra, 1999; Singh et al., 2001; Solanki and Sharma, 2002 and Sheeba et al., 2003). The selection of progenies on the basis of superior mean and greater variance in M2 was found to be highly useful in the present study. Increase in the mean seed yield per plant may be due to the selection of normal-looking plants in M2 generation which could lead to the elimination of aberrant plants and also due to the genetic nature of the changes induced after the mutagenic treatments. It is clearly evident from the data that considerable amount of variability was induced in the mutagen treated population in M2 and M3 generations. The amount of induced variability however, varied not only among different treatments but also from trait to trait. The variability for days to flowering, plant height and days
to maturity was low in M3 than in M2 generation. However, there was an increase in the variability for yield and yield contributing traits in M3 than in M2 generation. These results clearly demonstrate that some characters have a tendency to stabilize sooner than others. Therefore, it is suggested that in mungbean, selection for some traits like days to flowering, plant height and days to maturity could be confined to M2 generation. The other traits, such as, yield and its contributing traits which have shown increased variability in M3 provide further chance to select more promising lines in M3 generation.

Both the varieties of mungbean viz; PDM-11 and NM-1 had not reached its peak in performance and still retained the capacity for effectively utilizing mutagenic changes in the positive way for yield and its components. This may be connected with the history of selection of these varieties which were developed through selection (var. PDM-11) and inter-varietal hybridization (var. NM-1).

The quantitative traits studied in the present study showed a wide range of phenotypic variation. The magnitude of the phenotypic variation, however, does not reveal the relative amounts of heritable (genetic) and non-heritable (non-genetic) components of variation. This was ascertained with the help of some genetic parameters, such as, genotypic coefficient of variation, heritability in broad sense and genetic advance in percent of mean. The estimates of genotypic coefficient of variation and heritability of various quantitative traits are essential (Kaul and Garg, 1979; Sakin and Yildirim, 2004; Khan and Wani, 2005b) since they indicate the degree of stability to the environmental fluctuations and the potential transmissibility of a trait from parent to offsprings and from generation to generation. It is clearly evident from the data that considerable amount of genotypic coefficient of
variation was induced by EMS, HZ and SA treatments. The genotypic coefficient of variation was recorded to be higher for yield and yield components, while it was comparatively lower for other traits like days to flowering, plant height, days to maturity and pod length in $M_3$ generation. Such differential behaviour of different traits has been reported earlier by Sharma (1986). The genotypic coefficient of variation of days to flowering, plant height, days to maturity and pod length was higher in $M_2$ as compared to $M_3$ generation. Shakoor and Haq (1980) reported negligible increase in variability due to generation advance for some quantitative traits in chickpea, suggesting that some polygenic traits are stabilized in early generation. In the present study, genotypic coefficient of variation did not increase with the concentration of the mutagen. Lack of a consistent dose response relationship may be due to an additional uncontrolled environmental variation (Conger et al., 1966).

Heritability is of interest to plant breeder as a measure of the value of selection for particular trait and also as an index of transmissibility. 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. The traits such as the number of fertile branches, number of pods, seeds per pod, 100-seed weight and total plant yield were found to have a high heritability. However, these estimates were low to moderate for days to flowering, plant height, days to maturity and pod length. In the present study, heritability estimates for yield were high. Lower heritability for yield has been reported by earlier workers in rice (Kaul and Kumar, 1983) and pigeonpea (Srivastava and Singh, 1993). 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 genotypes are subjected to. The heritability estimates for number of fertile branches, number of pods, seeds per pod, 100-seed weight and seed yield per plant in M₃ generation was greater than in M₂ generation of the two varieties of mungbean. High heritability in M₃ generation indicated that the induced variability in mutant population was fixed by selection. Ibrahim and Sharaan (1974) showed that the increase in heritability is an indication of effective selection. The high estimates of heritability in yield and yield components has been found to be useful from plant breeder’s view point as this would enable him to base his selection on phenotypic performance.

The heritability estimates for all the quantitative traits under study have increased over the controls except for a few instances, particularly for pod length, where heritability of the treated population was lower than the control. The decrease in heritability in some of the treatments indicates that, even though genetic variance has increased with the mutagenic treatment, the ratio of its increase was not at par with the total phenotypic variance which also increased.

A rational approach towards the improvement of any crop plant involves selection. Genetic advance gives the extent of stability and genetic progress for a particular trait under a suitable selection system and consequently carries much significance in self-pollinated crops like mungbean. Heritability in conjunction with genetic advance is more reliable in predicting the effect of selection than the heritability alone. This is because the heritability estimates are subjected to certain estimation errors (Lin et al., 1979) and genotype – environment interactions.

The estimated values of genetic advance, in percentage of mean, differed in different mutagenic treatments and also from one variety to
another. In both the varieties of mungbean, the traits viz., number of fertile branches, number of pods, seeds per pod, 100-seed weight and seed yield per plant possessed a high genetic advance than the other quantitative traits, for which such values were low. Although heritability in the present study has been calculated in the broad sense, its estimates can be reliably followed when the high genetic advance in the quantitative traits is accompanied by high heritability values. Results about yield and yield components are quite encouraging since they possess sufficiently high values of heritability and genetic advance.

5.6. High yielding mutants

A wide range of genetic variability was observed for the number of fertile branches, number of pods and seed yield per plant of the mutants isolated in M₃ generation. A glance at the data (Tables 46 & 47) indicates that the mutant progenies displayed a tremendous increase in mean values for these traits as compared to the control. Selection for number of fertile branches, number of pods and seed yield per plant in M₃ generation was found to be effective for all the isolated mutants, as is evident from the manifold increase in the values of the genotypic coefficient of variation, heritability and genetic advance as compared to the control and rest of the M₃ population. Therefore, these three traits have a high selection value and breeding significance.

The degree of association of plant characters has been helpful as a basis for selection. A comparison of mutated and control population of the two varieties of mungbean revealed that a significant increase in positive correlations between the number of fertile branches and pods, the number of fertile branches and the total plant yield and the number of pods and the total
plant yield were observed in the mutants isolated in $M_3$ generation. It was also observed that the negative correlation between the number of fertile branches and the total plant yield in the control population of the var. NM-1 was broken down in the mutants. This was highly desirable from the point of view of improvement of more than one trait. Such desirable changes in correlation with yield contributing traits have also been reported in *Cicer arietinum* by Kharkwal (2003). The correlation among yield contributing traits in a population is a composite of the effects of selection, gene linkage and pleiotropy (Sehrawat *et al.*, 1996). The usefulness of mutations in weakening, strengthening or altering character association has been reported earlier (Kaul and Garg, 1982; Reddy and Khan, 1984; Agarwal *et al.*, 2001; Yadav *et al.*, 2002; Khan and Wani, 2005b). If the nature of selection practiced in the control and treated population is the same, any difference in the correlation coefficient in the two populations will be due to the effect of mutagens or altered pleiotropic effects of newly mutated genes. However, according to Gottschalk (1987) climatic factors can also influence a pleiotropic pattern positively or negatively. Such alterations in correlation among various traits may be utilized to enhance the rate of selection response in quantitative traits. Since the number of fertile branches and the number of pods have shown a significant relationship with yield, it would be desirable to direct selection for these traits. The results show clearly that the mutagenic treatments have succeeded in generating more favourable associations between various components of yield.

5.7. **Seed protein content of the mutants**

Seed protein content is generally considered to be a complex character of a crop controlled by many genes located on several chromosomes (Frey,
1977; Konzak et al., 1978; Coffman and Juliano, 1979). Results on the estimates of total seed protein content of high yielding mutants isolated in M₃ generation showed that the mean protein content of the mutants did not differ significantly as compared to the controls. In different mutants, coefficient of variation for total seed protein content has not greatly altered over the controls indicating that further improvement is difficult to achieve. Seed protein showed a non-significant negative correlation with yield in different mutant lines. Hence simultaneous improvement of these traits is not possible in this crop. Similar negative correlation between yield and seed protein content has been reported earlier (Bliss et al., 1973; Kaul and Matta, 1976; Blixt, 1979; Imam, 1979; Gottschalk and Muller, 1982; Karjalainen and Kortet, 1987; Khan and Wani, 2005b). Protein content is influenced by the interactions of gene(s) and environmental factor(s) as has been reported in chickpea (Singh et al., 1990), mungbean (Ignacimuthu and Babu, 1989), rice (Kaul, 1980b) and pea (Gottschalk and Wolf, 1983; Santalla et al., 2001). Variation in total seed protein in the mutants and the control population of both the varieties may be due to change in environmental factors as the experiments were conducted in the field.

In brief, the results have revealed that moderate concentrations of chemical mutagens used in the present study proved to be efficient in increasing the genetic variability for yield-oriented selection in mungbean. Some isolated mutants possessed desirable plant architecture associated with high yield and slightly higher seed protein content than the controls. They can be evaluated in future generations and after multilocational trials released as 'new varieties. Thus the genetic variability induced by chemical mutagens can effectively be exploited for the improvement of mungbean.