Chapter 5
DISCUSSION
5. DISCUSSION

5.1. M₁ GENERATION

5.1.1. Germination

This parameter was used to assess the response of plant material to different concentrations of sodium azide, ethyl methane sulphonate and gamma radiation. In the present investigation it was observed that all the mutagens inhibited seed germination and caused a decrease in percent germination. The inhibitory effect was directly proportional to the concentration / dose of the mutagens, in both the varieties of mungbean. Maximum inhibition in germination was recorded at 0.04M concentration of both the chemical mutagens and 50 kR dose of gamma radiation.


Several views have been proposed to explain the inhibitory effect of mutagens on seed germination. According to Micke (1958 and 1961), Gottschalk and Schiebe (1960) and Sjodin (1962) the decrease in seed germination was mainly due to the interference of mutagens with metabolic activities of the seeds.
According to Aman (1968), endogenous growth regulators play an important role in seed germination and there exists a striking balance between the promoters and the inhibitors. He attributed the reduction in seed germination to the disturbance of this balance. According to Selin et al., (1974) the reduction in germination is due to the enhanced production of the active free radicals, which cause lethality. According to Gorden (1957) the inhibition in germination percentage is due to the inhibition of auxin synthesis. Woodstock and Justice (1967) reported that lowered respiratory quotient, may cause inhibition of seed germination in mutagen treated seeds. Favret (1963) stated that the depression in the rate of mitotic proliferation may cause delay in seed germination. Hutterman et al., (1978) proposed that the denatured DNA may be repaired after sometime resulting in the activation of biological process involved in germination and may cause delay in germination. Sinha and Godward (1972) opined that lower percentage of germination and survival may be due to the disturbance caused at the physiological level of cells and chromosomal damage.

EMS, SA and gamma radiations are potent mutagens well known for their action in inducing point mutations, enzyme inhibitions and chromosomal aberrations. The observed reduction in seed germination in mungbean as a result of treatment with these mutagens might be due to point mutations or the injuries caused to the genetic material resulting in production of altered or incomplete proteins. This may eventually lead to decrease in the rate of respiration. Reduction in rate of respiration and lack of required enzymes for carrying out normal metabolism eventually lead to decrease in the seed germination.

5.1.2. Seedling Survival and Maturity

Survival of seedlings is an important parameter for evaluating the harmful effects of any mutagen. In the present investigation all mutagens reduced the rate of survival and maturity of seedlings. The effect was directly proportional with the increased concentration or dose of the mutagens. The Vaibhav and Kopargaon-1 varieties differed from each other in their relative sensitivity to mutagenic treatments, regarding the rate of seedling survival. Kopargaon-1 showed maximum reduction in survival percentage than Vaibhav. Maximum reduction in rate of seedling survival was observed in Kopargaon-1 variety exposed to 50 kR.
dose of gamma radiation. Among the chemical mutagens, EMS was highly effective in decreasing seedling survival rate, than the sodium azide.

Similar reduction in seedling survival percentages as a result of mutagenic treatments has been reported by Heringa (1964) in pea, Zannone (1965) in Vicia, Sree Ramulu (1971) in Medicago sativa, Kharkwal (1978) in chickpea and Giriraj and Deshpande (1996) in sunflower. The effect of ionizing radiations on seedling survival was studied by several workers (Ehrenberg, 1955, Sudha Rani, 1990, Kiranmai, 1992, Padmavathi, 1993, Rayyan, 1995, Bale, 1999, Dixit and Dubey, 1986, Vandana and Dubey, 1990, and Solanki and Sharma, 1994). All these authors have observed a decrease in rate of seedling survival with increased dose of mutagens. Decrease in rate of seedling survival, as result of treatment with SA was reported by Rao and Reddi (1986) in rice.

Nilan et al., (1964), Blixt (1967) and Narayana and Konzak (1969) proposed that physiological imbalances or different types of chromosomal aberrations may be the prime cause of the lethality. According to Davis and Evans (1966), damage to genetic material is one of the causes. Gaul (1970) had reported that mutagenic effects on seedling survival might be due to chromosomal and extra-chromosomal injuries. These injuries affect the growth of seedlings and eventually lead to their death. All these factors and increase in lethality of cells, which is usually observed at higher doses/concentrations of the mutagens, might be responsible for the observed reduction in seedling survival rate in mungbean.

5.1.3. Pollen Sterility

Pollen sterility in M₁ generation is the prime criteria to determine genetical effectiveness of the mutagenic treatments (Kivi, 1962). Data on pollen sterility indicated that gamma radiation treatment induced higher pollen sterility followed by EMS and SA. Between the two varieties Vaibhav showed higher pollen sterility than the Kopargaon-1.

Induction of pollen sterility with ionizing radiation has been reported by Singh et al., (2000) in uredbean, Dnyansagar and Kothekar (1983) in Solanum nigrum and Kharkwal (1978) in chickpea. Induction of pollen sterility due to mutagenic effects of sodium azide, EMS and gamma radiations has been reported by

Different views have been expressed regarding the mechanism of mutagens on induction of pollen sterility. According to Konzak et al., (1961) and Sparrow and Woodwell (1962) induction of the pollen sterility is due to chromosomal irregularities caused by the mutagens. Gunkel (1957) proposed that gross injury at cellular level either due to gene controlled biochemical processes or due to acute chromosomal aberrations. Siddiq and Swaminathan (1969) reported that chromosomal aberrations especially high frequency of translocations was responsible for high sterility. According to Sato and Gaul (1967), the radiation induced sterility might be due to chromosomal aberrations and cryptic deficiencies, while the sterility induced by EMS might be due to the cryptic deficiencies and specific gene mutations. Sato and Gaul (1967) have proposed that, pollen sterility induced by EMS is of chromosomal, genic and purely physiological type. Similar mechanism may be responsible for the observed increase in pollen sterility with increased concentrations of the mutagenic agents in mungbean.

5.1.4. Seedling Height

The results indicated that all mutagens except 0.02M SA inhibited the growth of seedlings. Vaibhav variety was more sensitive to higher concentrations of EMS and gamma rays. Similar reduction in height of the seedlings with increasing concentrations of sodium azide was reported by Khan (1988) in Vigna mungo, Reddi and Mani (1985) in Sorghum and Rao et al., (1984) in rice. On the contrary, increase in plant height with increasing concentrations of sodium azide was reported by Venkatachalum and Jayabalan (1995) and Varshney and Siddiqui (1997). In the present study also we found that 0.02M concentration of SA in Vaibhav variety exerted stimulatory effect and increased the seedling height.
According to Sax (1963) mutagens might inactivate the meristem and cause hormonal disturbances leading to reduction in plant height. Sinha and Godward (1972) proposed that injury at cellular and chromosomal level is the prime cause of reduction of seedling height. According to Cladecott and North (1961) various environmental conditions under which seeds are irradiated e.g. physiological conditions of the seeds themselves and the type of ionizing radiation, may all contribute to the stimulation or retardation of growth in seedlings. According to Sree Ramulu (1970) the sensitivity of higher plants to mutagens vary, depending on various factors such as, the physiological status and structure of the tissues, capacity for reproduction and growth. Busey (1980) and Gaul (1970) have reported that inhibition of the growth was due to chromosomal and extrachromosomal damage of the cells. Sparrow (1966) has correlated the growth reduction with nuclear volume and DNA content. Bhamburkar (1981) found that the reduction in seedling growth was due to reduction in number of cells contributing to seedling growth. Conger and Stevenson (1969) reported that increased seedling injury at higher doses could be correlated with chromosomal damage. Gordon (1957) and Gowda (1977) believed that lower concentrations of chemical mutagens, stimulated growth due to the elevated auxin levels. According to Lakshmi et al., (1996) SA stimulated the synthesis of the protein at lower concentrations and may be employed to enhance the biochemical products. From the foregoing discussion it is evident that lower concentrations of mutagens stimulate growth either due to elevated auxin levels or by increasing protein synthesis and higher concentrations retard growth in mungbean.

5.2 STUDIES IN M2 GENERATION

5.2.1. Chlorophyll mutations

Chlorophyll mutations serve as an index for evaluating the efficiency and effectiveness of mutagens and its effective concentration so as to use them in mutation breeding. Chlorophyll mutations are used as markers in genetic studies and in physiological and biochemical research (Stadler, 1928 a and b; 1930). According to Gustafsson (1951) and Monti (1968), chlorophyll mutations are dependable index for evaluating genetic effects of mutagenic treatments. Robbelen (1968) indicated that the chlorophyll mutants could be used in studies...
involving the effect of specific gene products during differentiation, while according to Miller (1968), the chlorophyll mutants are potentially useful in the understanding of different functions, pathological invasion and various biochemical reactions. Chlorophyll mutations could be lethal, semi-lethal or viable type. According to Kothekar et al., (1994) the chlorophyll mutants are usually lethal but semi lethal and viable types are also known. The occurrence of chlorophyll deficient areas on the leaves has been used previously, as an indicator of the mutagenicity of different agents. According to Vig (1975) it is a very sensitive test system. According to Swaminathan (1965) chlorophyll development seems to be controlled by many genes located on different chromosomes. Stadler (1930) first observed that mutation induced by seed irradiation appeared in sectors in the M1 plants. Anderson et al., (1949) first demonstrated that the analysis of a mutated sector could greatly help in tracing the ontogeny of the organs in M1 plants. Chlorophyll formation in plant is the result of a long chain of steps in a biochemical pathway in which many loci are involved. The synthesis of chlorophyll is partly inherited by nucleus and cytoplasm. Chlorophyll mutations may appear due to the mutation in nuclear genes, which alter the stability of DNA. Chlorophyll deficiencies which occur due to mutations in genes are responsible for mutations in photosynthetic pigments. According to Gaul (1958) chimeras arise due to differential response of embryonic cells.

5.2.2. Chlorophyll frequency and spectrum

In the present investigation, five different types of chlorophyll mutations - striata, xantha, chlorina, albina and variegated, were observed in the mutagen administered mungbean populations. Vaibhav and Kopargaon-1 varieties differed noticeably in their chlorophyll mutation spectrum. All five types of chlorophyll mutations (chlorina, xantha, striata, variegated and albina) could be observed in Vaibhav variety while only three types (chlorina, xantha, and striata) could be found in the Kopargaon-1 variety.

It is surprising to note that the same mutagen induced different spectrum of chlorophyll mutations in different varieties. For e.g. EMS has induced all the five kinds of chlorophyll mutations in the variety Vaibhav, but could induce only three
types of mutations (chlorina, xantha and striata types) in Kopargaon-1. It is possible to conclude that different mutagens do differ in their effectiveness for inducing various kinds of chlorophyll mutations. Among the different types of chlorophyll mutations, the chlorina type of mutations was found in highest proportion in both the varieties. Sodium azide and gamma radiation could induce only three types of mutation e.g. chlorina, xantha and striata in both the varieties.


In the present investigation, EMS induced highest frequency of chlorophyll mutations than gamma radiation in both the varieties of mungbean. Natarajan and Upadhyay (1964) have attributed the high incidence of chlorophyll mutations, after EMS treatment, due to the specificity of the mutagen to certain regions of chromosomes. Reddy and Gupta (1989) suggested that EMS preferentially act on genes responsible for chlorophyll development.

From the spectrum of chlorophyll mutations, it is clear that certain mutants are exclusively found in some mutagenic treatments and absent in others. Albina and variegated mutants were found only in individual treatments of EMS in the

---

Chapter 5. Discussion
variety Vaibhav. The presence of certain chlorophyll mutants in some mutagenic treatments and absence in others indicate difference in the availability of mutagenic loci to the mutagen.

When the spectrum and frequency of chlorophyll mutations of the two varieties, are compared, the Vaibhav variety was found to be exhibiting more of them. Low frequency of albina and variegated mutants in the variety Vaibhav also suggests that the genotypic difference, early mutable nature and sensitivity of Vaibhav variety to chemical mutagen, specifically EMS. Such a varietal variation is generally attributed to differences in the mutagenic sensitivity or to a number of genes controlling the chlorophyll development.

Number of workers reported a significant difference between the relative proportions of different kinds of mutations induced by treatments with chemical mutagens and gamma radiation in chlorophyll deficient mutations (Ehrenberg et al., 1961, Nilan and Konzak, 1961, and Siddiq and Swaminathan, 1969). In all these investigations, chemical mutagens were found more effective than physical mutagens, a fact which was confirmed from the present investigation also.

5.2.3. Mutagenic Effectiveness and Efficiency

In the present investigation, lower concentrations of sodium azide, EMS and gamma radiation showed higher mutagenic effectiveness and efficiency values. In other words, values for mutagenic effectiveness and efficiency gradually decreased with an increase in concentration of the mutagens. High mutagenic effectiveness and efficiency at low concentrations of the mutagens has also been reported by Konzak et al., (1965) Solanki and Sharma (1994), Satpute (1994) and Harsulkar (1994) in different crop systems. According to Konzak et al., (1965) the higher efficiency at lower concentration of a mutagenic agent is due to the biological damages (like seedling injury, lethality and sterility) it causes at faster rate than the mutations. In the present work, sodium azide induced high percentage of chlorophyll mutations over gamma radiation. The response of cells of higher plants to physical and chemical mutagens is influenced by various biological, environmental and chemical factors. These factors modify the effectiveness and efficiency of mutagens in cells of higher
plants. The exact mechanism, by which many of these factors influence mutation frequencies, is not known.

5.2.4. Mutation Rate

In the present study both the chemical mutagens were found to be very effective than the physical mutagen in inducing highest rate of mutations as regards to lethality, sterility and effectiveness. EMS showed higher mutation rate for sterility and effectiveness in variety Vaibhav. Gamma radiation gave higher rate of mutations followed by EMS and SA for seedling injury in variety Kopargaon-1. Observations clearly indicated that high mutation rates can be obtained with moderate dose of mutagen and high mutagen doses are not suitable for achieving the mutation rates. From the observations, it is concluded that different mutagens can be used in recovery of a range of distinct mutant types and it is possible to increase the mutation frequency and mutagenic rate by employing specific mutagens in appropriate concentrations.

5.3 Viable Mutations

A wide range of viable mutants have been observed in the M$_2$ progeny of both the varieties of mungbean treated with various concentrations of sodium azide, EMS and different doses of gamma radiation. These mutants were characterized on the basis of specific mutant characters e. g. plant habit, Leaf structure, Flower type, pod type mutations, seed type, maturity and high yielding mutations.


In our studies, EMS has emerged out as the most effective mutagen than the sodium azide and gamma radiation, in terms of mutation spectrum. Comparison of the spectrum of viable mutation had shown that particular mutagens induced specific mutations in relatively large number, which was produced rarely by other
mutagens. According to Nilan and Konzak (1961) different mutagens and treatment procedure may responsible for the changes in the relative proportions of different types of mutations in higher plants. It was also observed that the spectrum of the mutations was dependent upon the nature of the mutagen employed. For example variegated leaf mutants and pentafoiliate leaf mutations were induced only by EMS. On the other hand, large flowers mutant, Hairy pod mutant, leathery leaf mutant and dissected leaf mutant was induced by gamma radiation only. Occurrence of tall mutations was due to SA and EMS treatment but not due to gamma radiation. Occurrence of dwarf mutations in both the varieties, with chemical (SA, EMS) and physical (gamma) mutagens showed the presence and similar mutability of this gene in both the varieties. These results strongly support the view that the spectrum of mutations produced by a mutagen is dependent upon the nature of the mutagen employed. The induction of certain mutation by a particular mutagen may be attributed to the fact that, the genes controlling these characters may be more responsive to particular chemical or physical mutagen. This could be due to differential mode of action of the mutagens on different base sequences in various genes.

Along with simple viable mutations, multiple mutagenic effects on two or more characters were also found in all the mutagenic treatments. However their frequencies differed with mutagen and variety. For example, 50 kR dose of gamma radiation induced a novel mutant that showed multiple morphological mutations like large flowers with dark yellow petals, dense thick hairy pods and black coloured seeds. It is named as Ihb mutant (large flower, hairy pod and black seed mutant). Such a mutant has not been reported earlier in mungbean. These Ihb mutants were also recovered in M₃ populations of mungbean. Lhb mutants also showed multiple mutagenic effects on various other traits. Important among them are semi-dwarf habit, late flowering, large-sized flowers, dark-yellow petals, late maturing, broad and short pods, thick dense hairs on pods.

The Ihb mutant raised through gamma radiation induced mutagenesis, in the present investigation, seems to be promising at least for two characters i.e., semi-dwarf habit and presence of thick dense hairs on the pods. The semi-dwarf habit of the plant enables it as lodging resistant. The thick dense hairs on the
pod helps in protecting them from insects and caterpillar predators and seems to be promising economical important character. It can be used or incorporated in breeding programmes that are aimed at genetic improvement of mungbean.

According to Patil (1966) multiple mutations are either due to mutation of pleotropic gene, mutation of the gene clusters or due to loss of chromosomal segments. Gaul (1961) and Mackey (1960) interpreted the occurrence of such mutants as due to chromosomal rearrangement or deletion. Occurrence of multiple mutations has also been reported in groundnut by Gregory (1961), Ashri and Golden (1965) and Patil (1966).

The second important conclusion in addition to the mutagen and its nature is the genetic makeup of the variety. The genetic makeup of the material has a significant role in determining its mutability. According to Gregory (1961), the genetic material or makeup of the experimental material is the prime factor that influences the induction and recovery of mutations and not the mutagen used. From the observations, it is clear that highly potent mutagen like EMS could not affect both the varieties equally. The mutation spectrum induced in the Vaibhav variety by EMS was quite broad as compared to Kopargaon variety. For example EMS could induce variegated mutants, albina mutants and various flower size mutations in Vaibhav variety but not in Kopargaon-1 variety. According to Gustaffson (1944), Konzak et al., (1961), Brojevic (1966) and Sparrow et al., (1965), even the small genetic difference of a single gene could bring about significant changes in the frequency and spectrum of recoverable mutations. Our experimental results fully support the view that the frequency and spectrum of induced mutations are affected by the genetic makeup of the variety.

The morphological mutants like early maturing, high yielding and early flowering mutants obtained in the present investigations are agronomically valuable and they may be utilized in future breeding programs. Other mutants like dense thick hairy pod mutants, dissected leaf mutants, black coat color seeds were exhibiting negative selection value and these might be useful only to plant breeders in hybridization programs. These mutants can be improved through selection by eliminating some of undesirable characters.
5.3.1. Habit mutants

Four different types of habit mutants were observed in the mutagen administered mungbean plants in M₂ and M₃ generations. They are Tall mutants, Dwarf mutants, Spreading mutants and Compact mutants.

Tall mutants have also been reported earlier in mungbean by Gajraj Singh et al., (2000), Yadav (1987), Khan and Tak (2000), Sharma and Haque (1983), Subramanian (1980), and Sharma and Singh (1992). Different workers have attributed increase in plant height to different factors. According to Webber and Gottschalk (1973), the increase in plant height is due to the changes in the internodal length. Blonstein and Gale (1984) opined that, the increase in plant height is due to increase in cell number, cell length or both. From the foregoing discussion it is clear that the tallness of the tall mutants is basically due to an increase in cell number and cell length which in turn may increase the internode length or internode number.

Large number of dwarf mutants were observed in the present investigation. Gamma radiations produced more frequency of dwarf mutants than EMS and SA. Dwarf mutants have also been reported by several workers (Down and Anderson, 1956, in Phaseolus vulgaris, Kundu, 1980 in Vigna mungo, Kothekar, 1989 a, b in mothbean and Coriandrum sativum, Hakande, 1992 in winged bean, More, 1992 in Medicago sativa, Kothekar and Kothekar, 1992 in mothbean, Satpute, 1994 in safflower and Kirtane, 2002 in onion). Different workers have attributed reduction in plant height to different factors like destruction or inhibition of auxin synthesis (Smith and Kerstein, 1942), genetic loss due to chromosomal aberration (Evans and Sparrow, 1961), interference with the synthesis of new DNA (Pele and Howard 1955), damage and deficiency of physiological pre-requisites to cell divisions (Stein and Sparrow, 1963), delay and loss of proliferation capacity and cell death (Evans, 1965) and inhibition of phytohormone responsible for normal growth (Tarar and Dnyansagar, 1974). Any one or all of these factors may be responsible for the observed reduction in plant height in the dwarf mutants. The induced dwarfness is definitely a desirable agronomic trait of importance, because more number of plants can be
accommodated per unit area and it will also facilitate easy translocation of metabolites from source to sink.

Compact and spreading type of mutants have been reported by Kharkwal (2000) in mungbean, chickpea, urdbean, cowpea, french bean and other pulse crops. Bhatia et al., (1999) obtained bushy and compact plant type mutants in several pulse crops. According to Malik (1988), mungbean mutant varieties NM 19-19 and NM121-25 have short stature, erect plants with determinate growth habit. Kharkwal et al., (1988) and Anonymous (1987) obtained high yielding, dwarf, determinate or semi-determinate mutants of cowpea through gamma radiation. It seems that the genetic material of cell is quite sensitive to radiation damage and both the primary and secondary physiological effects of the mutagens might be responsible for the observed habit mutations.

5.3.2. Leaf Mutations

Mutant plants with remarkable variation in leaf morphology have been observed in M₂ population of Vaibhav and Kopargaon-1 varieties of Mungbean. These variations appeared in shape, size, number and arrangement of the leaflets. Important among them are tiny leaves, broad leaves, leathery leaves, curly leaves, irregular leaves, dissected leaves, simple leaves, bifoliate leaves, tetrafoliate leaves, pentafoliate leaves and heptafoliate leaves.


According to Tarar and Dnyansagar (1979) changes in shape are due to chromosomal aberrations produced in the plant as a result of action of chemical mutagens and ionizing radiations. Joshua et al., (1972) have correlated the development of leaf abnormalities to the pleotropic action of mutated genes.
Prasad (1967) pointed that the splitting, rolling and constriction of organ in *Phalaris* are produced by irregularities in meristem after mutagenic treatment.

Among the three mutagens EMS was found to be very effective in inducing high frequency of leaf morphological mutations. All the mutagens have exerted clear cut effects on leaf morphology. Even though at this moment these leaf variations seems less important, they may be of immense value in understanding the genetic control of leaf formation and regulation of their size, shape and form.

### 5.3.3. Chlorophyll Mutants

In the present work, two viable chlorophyll mutants namely chlorina and variegated types were observed. Both the mutants grew well and developed pods. The growth of both the mutants was slow and yield was quite less than control. The viable chlorophyll mutants have been reported by Hakande (1992), Sonawane (2000) in winged bean. Variegated mutants may have ornamental and decorative value due to their attractive leaf colors.

### 5.3.4. Flower Mutation

Four different types of flower mutations (Small flower, large flower, abnormal flower and flower colour) were observed in the mutagen administered Vaibhav variety plants in $M_2$ and $M_3$ generations.


According to Datta and Banerji (1995), chromosomal aberrations, changes in chromosome number, gene mutations, rearrangement of different histogenic layers and changes in biochemical pathways leading to pigment formation, may be prime cause for flower colour mutations. Datta (1994) studied cytomorphological, anatomical and biochemical characters of mutant plants to understand the mechanism involved in the origin and evolution of somatic flower
color mutation at molecular level. According to Datta (1994) flower color mutation appeared or produced due to qualitative and quantitative changes in pigments during pigment biosynthetic pathways due to gamma radiation. We are of the opinion that the flower size and colour mutations are produced as a result of gene mutations causing changes in pigment biosynthetic pathways or genes that control ontogeny of the flower.

5.3.5. Pod Mutants

In the present investigation six types of pod mutations were observed. They are long, small, curved, hairy, flat pods and pod color mutants. Similar pod mutations were reported earlier by Singh and Agarwal (1986), Hakande (1992), Sonawane (2000), Boddu and Auti (2005), Singh and Chaturvedi (1982), Narashimha and Bhalla (1988) and Ganguli and Srivastava (1967).


5.3.6. Seed type mutation:

In the present investigation, large number of seed colour mutations (black colour, faint brown colour, dark green and reddish brown colour) and seed size mutations (small seeds, bold seeds, small seeds with rough seed coats, bold seeds with rough seed coats, small wrinkled seeds, large wrinkled seeds, dark green seeds, faint brown seeds, black coloured seeds, very small non-viable seeds) were isolated from M2 progeny of mutagen administered mungbean varieties. Among the two varieties, Vaibhav variety gave high frequency of seed
type mutations. Among the three mutagens EMS induced high frequency of seed type mutations than SA and gamma rays.


Dwivedi and Singh (1986) suggested that the factors P and R are responsible for purplish black and brown color seeds respectively. The concurrent presence of both factors produces purplish black color. In the absence of both P and R, the seed coat is white. The seed coat color seems to be under the control of different genetic factors like pigmentation factors, pigment complementary factors and modifying factors, which depend upon the presence of the dominant pigment factor in order to express their colors. Once the dominant pigment factor is present, the complementary factors either produce a definite color by themselves or interact to produce a wide range of color.

5.3.7. Early and late-flowering mutants

In the present investigation, mutants with alteration in days required for flowering and maturity were obtained in both the cultivars. The early flowering mutants isolated in both the cultivars in the present study exhibited rapid growth and normal seed production. Late flowering and maturing mutants have shown reduced seed production. Early flowering mutants are important from breeding point of view in all crop plants.


Chapter 5. Discussion
Late flowering mutants have been reported by Sonawane (2000) in winged bean, Kharakwal (1978) in Chickpea, Singh and Chaturvedi (1981 a) and Khalil et al., (1987) in mungbean.

According to Jana (1962), the early maturing mutants are produced as a result of physiological changes and increased production of flowering hormones, which are usually associated with mutagenesis. The earliness gets attained mainly due to early transition of vegetative meristem to a reproductive one. The transition from vegetative to reproductive growth is largely under genetic control. Flowering depends on a number of physiological changes that take place in the meristem during its transition from vegetative to reproductive phase.

The observed early maturity in the present investigation might be due to action of the mutagens in increasing the levels of auxins, which in turn stimulate the rate of vegetative growth of the mungbean plants. The increased hormone levels also help the plants in shifting from vegetative to reproductive phase. In the present investigation, along with early flowering mutants, late flowering mutants have also been recorded. Inadequate production of flowering hormone or inhibition of hormone production along with the physiological disturbances must be the cause of late flowering mutant.

5.3.8. Early and late Maturing Mutants

The early maturing mutants require less number of days and the late maturing mutants require more number of days for attaining maturity.

Early maturing mutants produced as a result of mutagenic action have been reported in Vigna mungo (Ramaswami and Sree Rangaswmy, 1974), dew bean (Kumar, 2000), Pisum sativum (Kaul, 1977), Cajanas cajan (Abrams and Velez Fortuno, 1962 and Pawar et al., 1979), pigeon pea (Pandy et al., 1996 and Rao et al., 1972) and Lathyrus sativus (Prasad and Das 1980).

According to Sparrow (1966) the change in phytohormones and reduction in photoperiodic cycle are the main causes for early as well as late maturity in the mutants.

We are of the opinion that late maturing mutants might be produced as result of genetic damage caused to the plants by the mutagens. The genetic damage, manipulated in the form of low levels of enzymes and hormones required for growth and reproductive development of plants ultimately result in delayed maturity.

5.3.9. High Yielding Mutants

The yield character in crop plants is genetically controlled. The genes concerned with yield follow quantitative inheritance and show additive interaction. They are influenced by various environmental factors.

High yielding mutants were reported in mungbean earlier by Dahiya (1973) and Shakoor et al., (1978) employing gamma radiation. Tikoo and Jain (1979) obtained high yielding and disease resistant mutants in mungbean. Pawar et al., (1986) developed a mungbean genotype with high yield potential and improved efficiency of nitrogen fixation. Pawar and Wanjari (1994) induced high yielding varieties of mungbean, blackgram and pigeonpea. Sharma and Singh (1992) reported similar mutants in mungbean. Mungbean lines with high yield and early maturity were obtained by Prasad (1976) and Bhal and Gupta (1983).

Singh et al., (2000) isolated a bold seeded mutant in urdbean following mutagenesis with gamma rays and EMS. This mutant showed vigorous growth and produced more leaves and pods per plant. Khan and Tak (2000) reported morphological and high yielding mutants in black gram. Bhatnagar et al., (1979) reported early and high yielding mutant in Cicer arietinum. Sonawane (2000) reported high yielding mutant in winged bean.

Mechanism of action of mutagens in induction of high yielding mutants is still unknown. The mutagens, through some unknown mechanism, must be inducing the expression of other yield controlling genes, which are otherwise remaining silent in the plant.
5.4. QUANTITATIVE CHARACTERS IN M₂ AND M₃ GENERATIONS

The experimental findings revealed that the chemical mutagen treatments showed positive as well as negative shift in mean values for various quantitative characters like plant height, number of primary branches, number of leaves per plant, pod number, pod length, seeds per pod, hundred seed weight, yield per plant, fresh weight, dry weight and number of nodules, in M₂ and M₃ generations. The gamma radiation showed only negative shift in mean values for quantitative characters.

Our experimental results on quantitative characters are in conformity with the observations of Dahiya, (1978), Singh and Chaturvedi, (1981a), Kundu and Singh, (1982), Khan, (1985), Verma and Singh, (1984), Yaqoob and Rashid, (2001), Singh and Yadav, (1991) and Chaudhary, (1988). All these workers have noted that the mean values for the quantitative characters reduced, enhanced or equal to that of control in all the mutagen treated populations.

In the present investigation, significant positive shift in the mean values of quantitative characters was found at low concentrations of the chemical mutagens. Similar results have been reported by Sharma and Haque (1983), Khan and Tak (2000), Yaqoob and Rashid (2001) in mungbean.

Gamma radiation and high concentration of chemical mutagens shifted means values in negative direction in both the varieties. For e.g. the number of pods decreased with the different doses of gamma radiation. Our results are consistent with the results of Gaul (1965), Bhatia and Swaminathan (1962), Scossiroli (1965) and Gaul and Aastveit (1966) who reported a negative shift in mean values of the quantitative characters due to treatment with gamma radiation and chemical mutagens. Sharma and Sharma (1982) and Dixit and Dubey (1986) in lentil, Wazir (1986) in wheat and Haq (1990) in Chickpea have reported significant reduction in plant height with gamma radiation.

According to the theory of quantitative genetics, the polygenic traits are controlled by a multiplicity of minor genes with small but cumulative effect and these "minor" genes can mutate with equal probability in both directions i.e. from the dominate state to the nonfunctional or recessive state and back. Sharma
and Sharma (2004) opined that since all genes, major or minor, are made up of same genetic material, their mutation pattern can not be different. Thus the possibility of shifting population means for a particular character in both directions through mutations in character specific genes with equal probability is ruled out. The genes controlling metabolism of a character frequently have dosage effect, but they all fall in the category of major genes. Therefore micromutations, which are considered to be induced in minor genes, could be consequence of minor changes in the functions of many other genes. Mutations with very small phenotypic effect would occur with high frequency and will have an equal probability of being positive or negative in their phenotypic effects (Gregory, 1968).

Yield per plant is an important trait as it measures the economic productivity in mungbean. But its inheritance is extremely complex. Its expression is inherited by many genes including those controlling production, transport and storage of assimilates, genes determining the plant growth and development and genes contributing to adaptation in stress environments. Studies for combining ability and types of gene action indicate that both additive and non-additive gene effects contribute to seed yield. According to Singh and Jain (1971), Singh and Singh (1971), Luthra et al., (1979), Reddy and Sree Ramulu (1982) and others, additive gene effects were predominant in the inheritance of seed yield, pods per plant, seeds per pod, seed weight, branches per plant, pod length, days to flowering and days to maturity. Ota et al., (1962) proposed that there exists a direct relationship between the dose of mutagen and variance. Hakande (1992) reported wider variability in yield parameters of mutagen treated winged bean in M₂ and M₃ generations. From the overall observations on the variability it was revealed that induced mutations made important contribution, to physiological efficiency of the plant resulting in yield improvement by creating new correlation. In the present study a positive correlation between the number of pods, length of pods, number of leaves and branches with increased yield was noted.

Comparative studies on macromutation data revealed that the chemical mutagens particularly alkylating agents are more effective than ionizing radiation. The above fact is reported by several workers (Brock, 1976; Sharma and
Sharma, 1979; Sharma, 1986; Sarkar and Sharma, 1988; Singh and Sharma, 1989; Vinod, 1994; Solanki and Sharma, 1999, Kharkwal, 2001 and Auti et al., 2005). Mutation induction through the application of radiation was most frequently (89% studies) for direct development of mutant varieties, where as use of chemical mutagens were relatively infrequent (Nichterlein et al., 2000). Brock (1976) attributed this disparity to later introduction of chemical mutagens. Now it is well established that there is a clear deficiency of information about the influence of chemical mutagens on quantitative characters compared to radiations. Chemical mutagens are better alternatives for inducing micromutations, as they induce mutations at a much higher rate and cause less chromosomal disturbances than radiation (Sharma, 2001). In the present investigation EMS and SA successfully induced variability in both the varieties of mungbean and broadened genetic diversity to an appreciable extent as compared to gamma radiation.

The morphological characters in M₁, M₂ and M₃ generations of mungbean in both Vaibhav and Kopargaon-1 showed consistency, ability to maintain the traits induced and stability in all the morpho-physiological character score. All the three generations studied in the present investigation has showed positive correlation in different aspects like mutagenic effectiveness and efficiency, frequency of viable mutations and quantitative characters.

5.5. BIOCHEMICAL STUDIES

5.5.1. Chlorophyll

Experimental results revealed that lower concentrations of chemical mutagens increased chlorophyll content in M₃ progeny, while the higher concentrations of chemical mutagens and all doses of gamma radiation decreased it.

We have also reported decrease in chlorophyll content in dissected leaf mutant, lhβ mutant, late maturing mutant and increased chlorophyll content in high yielding, early flowering and dwarf mutants of mungbean varieties.

The decrease in chlorophyll at higher concentrations of mutagens might due to lethal effects of mutagens on the chloroplast structure and integrity or point mutations, which disturb the metabolism of chlorophyll synthesis.

5.5.2. Carbohydrates

In the present study it is observed that lower concentrations of chemical mutagens increased the carbohydrate content of the M₃ progeny and the higher concentrations of the same and all doses of gamma radiation decreased it.

Laware and Dhumal (1998) and Laware et al., (1999) reported an increase in carbohydrate content in strawberry at low concentrations of SA, and decrease of the same at higher concentrations. Dhumal and Laware (2000) recorded a substantial increase in content of reducing sugars and starch in strawberry leaf mutants and decreased content of sugars in dwarf and early flowering mutants of strawberry induced through EMS and SA. Kirtane et al., (2000, 2001) observed an increase in reducing and non-reducing sugars and starch contents in Allium cepa due to treatment with gamma radiations and SA.

Different views were expressed by different workers on the mechanism of action of mutagens on carbohydrate metabolism. According to Gordon (1954), Halvey and Shoub, (1965) inhibition of auxin synthesis is one of the causes. Failure of assimilatory mechanism (Riley 1953), changes in the specific activites of some related enzymes (Huskins and Chapman 1956, Cherry et. al., 1961, 1962, Endo, 1967), rapid breakdown in protein and carbohydrate metabolism and a high rate of respiration (Joseph and Gaur, 1974) are the other factors effecting the levels of carbohydrates at higher concentrations of the mutagens.

The increase in carbohydrate levels observed in the present study with lower concentrations of the chemical mutagens might be due two factors. 1. Inability of the mutagen to cause mutations of the genes involved in carbohydrate metabolism, at the low concentrations. 2. Ability of the mutagens to act as
transcription factors and initiate transcription of genes involved in carbohydrate synthesis, at lower concentrations.

5.5.3. Proteins, Albumins, Globulins and Amino acids

It is observed that lower concentration of chemical mutagens increased the amount of total proteins, albumins, globulins and amino acids in the M₃ progeny while the higher concentrations of these mutagens and all doses of gamma radiation decreased them in both the varieties. Among the stable mutants only high yielding mutants showed high levels of proteins, aminoacids, albumins and globulins followed by Tall and dwarf mutants.


According to Venkatchalum and Jayabalan (1995) and Khanna (1991) increase in protein and amino acid contents is due to the saturation effect of mutagens. Number of workers attributed the increase in protein content at lower concentrations of mutagens to an increase in auxin levels.

Decrease in protein and amino acids contents due to high concentration of chemical mutagens was due to inhibition of auxin and DNA synthesis and degradation in protein and carbohydrate metabolism. Vardhini and Rao (1998) and Key (1969) suggested that the phytohormones regulate the growth of plants by regulating nucleic acid metabolism.

5.5.4. Protein profiles of mungbean mutants

In mutational studies, the morphological markers have been used to differentiate the individual plants or mutants. Such morphological markers could distinguish between all different genotypes but fail to distinguish between morphologically identical or near identical genotypes. Development of biochemical markers on the basis of protein banding patterns are of great importance, since they offer a great diversity (Tanksley and Orton, 1983). According to Ladizinsky and Hymowitz (1979) and Krishna and Mitra (1988), seed proteins provide a stable and convenient system for such analysis.

Chapter 5. Discussion

In the present study the SDS-PAGE analysis of seed proteins of viable mutants and their controls showed interesting results. The tall mutant, dwarf mutant, high yielding mutant, Vaibhav and kopargaon-1 controls resembled one another in banding pattern exhibited the presence of nine polypeptides in their seed proteins. No variation could be observed in the number, position and molecular weight of polypeptides of these five plants. But the early-flowering mutant and Lhb mutants did differ with each other as well as other mutants and controls in their protein-banding pattern.

The early maturing mutant exhibited the presence of 12 polypeptide bands while the lhb mutant showed the presence of 9 polypeptide bands. These bands differed from those of control and other mutants in position and molecular weight. This difference in banding pattern of these two mutants (early maturing and lhb mutants) can be used as molecular markers to identify them from other mutants and controls.

5.5.5. Albumin profiles of mungbean mutants

In the present investigation banding pattern of albumins in macromutants of Vaibhav (high yielding mutant, dwarf mutant and lhb mutant) and micromutants of Kopargaon-1 (seeds with rough seed coats, wrinkled seeds and dark green bold seeds) were analysed on SDS Polyacrylamide gels.

Electrophoretic analysis has revealed similarity in banding pattern of albumins in macromutants of Vaibhav and their control except high yielding mutant. Dwarf,
Ihb mutants and control exhibited 6 polypeptide bands. The high yielding mutants differed from other mutants and control in having three extra bands with molecular weights 58,884, 39,811 and 35,481 Daltons. It indicates that seed protein profile of high yielding mutant was superior to other mutants in their albumin profiles.

Similar was the case with the micromutants of Kopargaon-1. Here also all micromutants except the dark green bold seeded micromutant and their control resembled one another in albumin profiles and exhibited the presence of five light and narrow bands. But the dark green bold seeded micromutant, showed 8 polypeptide bands for albumins. The observed increase in the number of albumin bands in both high yielding macromutant and dark green bold seeded micromutant must be due to the mutagenic action in which the mutagen, through some unknown mechanism might be triggering other albumin producing genes.

5.5.6. Globulin profiles of mungbean mutants

Macromutants of Kopargaon-1 (dwarf mutant, high yielding mutant and early maturing mutant) resembled one another in their globulin banding pattern. All these macro mutants showed 11 bands while their control showed only 7 globulin bands. Thus all the macromutants of Kopergaon showed 4 extra bands for globulins as compared to control.

SDS-PAGE analysis of control and micromutants of Vaibhav (wrinkled bold seeds, black seeds, small seeds with rough coats, dark green bold seeds and large seeds with rough coats) exhibited differences in their globulin profiles. The control plants and wrinkled bold seeded, black seeded, and small seeds with rough coated micromutants showed 8 globulin bands. The large seeds with rough coated micromutants showed 9 and dark green bold seeded micromutants showed 11 globulin bands. Thus the two micromutants showed 1 and 3 extra globulin bands, as compared to their control.

The observed increase of 4 globulin bands in the macromutants of Kopargaon-1 and 1 & 3 bands in two micromutants of Vaibhav was certainly due to the effect of mutagens. The specific action however is not known.
5.5.7. Peroxidase isozyme profiles

Results indicate that all the mutants except the variegated mutant, and their controls resembled each other in peroxidase isozyme profiles. They showed only one band for peroxidase isozyme. The variegated mutant showed three distinct bands of peroxidase isozymes.

Peroxidase is an important oxygen-scavenging enzyme. It plays an important role in respiration and is an indicator of oxidative status of plants. According to Milan et al., (1973) and Warfield (1974) Sodium azide is a potent chemical mutagen responsible for inhibition of peroxidase activity. According to Malpathak and David (1991), peroxidase isozymes serve as very good markers for any mutational studies.

There are several reports on peroxidase isozyme profiles but on different plants (Macko et al., 1967 in Wheat; Bhatia and Nilson 1969 and Upadhyya and Yee 1968 in barely; Kadam et al., 1973 in maize; Cherry and Ory 1973 in pea; Johri et al., 1977 in Pearl millet; Dalave 2003 in mungbean and Apparao 2005 in Vigna unguiculata).

Several workers have observed changes in activity and profiles of peroxidase isozymes between the control and mutants. Subhash et al., (1981) recorded stimulation in peroxidase activity in the leafy mutants of tomato produced after the treatment of 0.1% hydroxylamine. Warfield et al., (1971) reported the stimulatory effect on peroxidase activity in Saintpaulia ionantha in response to ethylene gas and X-ray alone and in combination. Apparao (2005) studied the effect of sodium azide on peroxidase isozyme profiles in Vigna unguiculata (L.) Walp and reported disappearance of few peroxidase isozymes from the azide treated seedlings. From this he concluded that the sodium azide disrupts the genes of certain peroxidase isozymes, like any other known potent mutagen.

According to Scandalios (1974) isozymes have also been used as molecular markers in systematic, genetic and evolutionary studies. According to Kumar and Gupta (1985) and Mundges et al., (1990) isozymes have been used to identify the cultivars. In our study also the variegated mutant showed three distinct bands of peroxidase isozymes while all other mutants and their control exhibited
only one. Thus the variegated mutant can be identified from other mutants by the presence of three peroxidase isozyme bands.

5.5.8. Nitrate Reductase

Results on nitrate reductase (NR) activity indicated differences in their endogenous levels between the mutants and control and among the isolated mutants themselves. The control plants in general had higher levels of nitrate reductase than the mutants, in both the varieties. Tall mutant, dwarf mutant and high yielding mutant showed higher contents of NR among all the obtained mutants but less than control.

Similar results have been reported by Reddy and Vaidyanath (1989) in foxtail millet and Laware (2001) in strawberry. According to Reddy and Vaidyanath (1989) the altered levels of NR activity reflect difference in protein content. According to Laware (2001), the increased phytohormones and increased nitrogen levels enhance the activity of nitrate reductase in some mutants of strawberry. The regulation of NR activity is genetically controlled and stimulation or inhibition of NR activity in strawberry mutants is due to mutagenic action of EMS and SA.

Nitrate reductase is an importance enzyme that is considered to be vital for assimilation of inorganic nitrogen in plants. NR activity is correlated with nitrogen status of the plant (Misra and Srivatava, 1983; Eilrich and Hageman, 1973), dry matter accumulation (Goodman et al., 1974) and protein synthesis (Awasthi, 1988; Singh and Singh, 1985 and Nair and Abrol, 1982).

The increased NR activity may indirectly help in improving growth, number of pods and yield per plant in mungbean mutants.