Studies on chemical mutagenesis in varieties 'Ujwala' and 'Co – 1' of *Capsicum annum* L.

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on chemical mutagenesis in varieties 'Ujwala' and 'Co – 1' of *Capsicum annum* L.**" is an authentic record of work carried out by Mr. ABDUL SALAM C.M. in the Department of Botany, University of Calicut during 1999 – 2007 under my supervision and guidance and that no part thereof has been presented earlier for any other degree or diploma.

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DECLARATION

I hereby declare that the thesis entitled "**Studies on chemical mutagenesis in varieties 'Ujwala' and 'Co – 1' of *Capsicum annum* L.**" submitted for the Ph. D Degree of the University of Calicut has not been submitted earlier for the award of any other degree or diploma and that it represents the original work carried out by me.

Date: 19-01-2007

ABDUL SALAM C.M.

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CHAPTER - 1

INTRODUCTION

Spices have long been known throughout the world as an inevitable ingredient to add flavour and piquancy in food items. Spices have played an important role in the history of civilization, exploration and commerce. So great was the value of spices in ancient and medieval times that they were often equated with gold and precious stones. Spices are also used as an essential ingredient in the manufacture of high quality cosmetics and medicines. Condiments are also spices, products of which are used as food adjuncts to add taste only. Both spices and condiments contain essential oils, which provide the flavour and aroma.

Chilli (*Capsicum annum* L.) has a unique place in human diet as a condiment and as a vegetable. It is grown both for home market and for export to other countries. In recent years, chilli has assumed greater importance as a commercial crop on account of its export value. Chilli production in the country in 2004-05 is around 12 lakh tones as against 10.6 lakh tones of dry chilli in 2003-04. The average productivity is around 1,112 kg/ha. Andhra Pradesh leads with the maximum productivity of 1,948 kg/ha followed by Punjab (1,607 kg/ha). Despite the Sudan Red-1 scare, the demand for Indian chillies has been rising in the international market. By April 2005 chilli exports touched an all time high of an estimated 1.3 lakh tones valued at over Rs.480 crore in 2004-05. At present about 13 per cent of the total chilli production in the country is exported. The estimated current

world import of chilli is around 1.5 lakh tones i.e., about 23 per cent of total world import of spices (Anonymous, 2005).

Capsicum, belongs to the family Solanaceae and is widely grown for their fruits, which may be eaten fresh, cooked or used as dried powder or processed into oleoresin. Red pepper products, both pungent and non-pungent types represent one of the most important spice commodities in the world. They add spice flavour and colour to foods while providing essential vitamins and minerals.

Five species of *Capsicum* are commonly recognized as domesticated; they are *C. annum*, *C. baccatum*, *C. chinense*, *C. fruitescens* and *C. pubescens*. Out of these, most products used commercially for food processing are obtained from C*apsicum annum*.

The dried ripe fruits of the *Capsicum* are also called chillies or red peppers. Chilli is actually reported to be native of South America and the natives of Peru knew its cultivation since pre - historic times. Wide spread geographic distribution of *Capsicum annum* and *Capsicum fruitescens* from the New World to other continents occurred in the sixteenth century via Spanish and Portuguese traders, whereas the other spices were little distributed outside South America (Andrews, 1995). Chilli was not known to Indians about 400 years ago, since this crop was first introduced into India by the Portuguese towards the end of the15th Century (Varmudi, 2001).

The varieties of chillies are broadly divided into two groups namely (i) the long pungent type, including pickling type, used as spice and (ii) the bell-shaped, non-pungent or mild, and thick-fleshed type, popularly known as 'Simla mirch' or Paprika, which is commonly used as a vegetable.

Capsicum annum is the most widely grown and economically important of the domesticated chilli peppers. This suffrutescent annual shrub is with many angular branches. The leaves are simple, entire and acuminate usually wrinkled. Flower is solitary, extra axillary, some times occur in pairs or clusters, ebracteate, actinomorphic, pedicellate, bisexual and hypogynous. Calyx is companulate, sepals usually five, gamosepalous and is shorter than fruit. Corolla is bell shaped rotate, 5 to 6 lobed and twisted in bud. Lobes are thin veined and incurred in tips. Androecium consists of five introse anthers dehisce longitudinally by lateral sutures. Carpels two, syncarpous and obliquely placed. Ovary superior, 2 or 4 celled with numerous ovules in each locule on swollen axile placenta. The style is slender, terminal and linear. Stigma is subcapitate and faintly bifid. In the fruit of chilli, unlike the usual berries, the seeds are not embedded in the fleshy pericarp. The pericarp in chilli fruit is leathery or succulent which turns from green to purple or red, orange or orange red. The placenta carries numerous seeds.

The main source of pungency in peppers is the group of alkaloid compounds called capsaicinoides. Capsaicin (C_{18} H_{27} NO_3, trans-8-methyl-N-vanillyl-6-nonenamide) is the most abundant capsaicinoid, followed by

dihydro capsaicin, with minor amounts of nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin *etc.* Capsaicin is a white crystalline, fat-soluble compound formed from homovanillic acid that is insoluble in water, odourless and tasteless (Andrews, 1995).

The red colour of mature pepper fruits is due to several related carotenoid pigments, including capsanthin, capsorubin, cryptoxanthin and zeaxanthin, which are present as fatty acid esters. The most important pigments are capsanthin and its isomer capsorubin, which makes up 30-60% and 6-18% respectively, of the total carotenoids in the fruit of *Capsicum* (Govindarajan, 1985).

Chilli peppers are widely used for food processing, as a food colourant, to add pungency to food, to impart flavour and as a source of pain relief for pharmaceutical use. Paprika, paprika oleoresin, red pepper oleoresin, and dried chilli are used as a source of red colour in various processed products. *Capsicum* oleoresin has considerable advantages over dried chilli including more stable colour retention, easier to handle compared to the rather bulky dried chilli. Dried chilli is valued for its contribution to flavour in chilli sauces and chilli powders. The flavouring principle is associated with volatile aromatic compounds and colour.

The antioxidant vitamins A, C and E are present in higher concentrations in various pepper types. Peppers are good sources of many essential nutrients. Pepper produces high amounts of vitamin C, provitamins A, E, P (Citrin), B_i (thiamine), B_2 (ribo flavin) and B_3 (niacin).

Chilli peppers stimulate the flow of saliva and gastric juices that serve in digestion. It has been said that pepper raises body temperature, relieves cramps, stimulates digestion, improves the complexion, reverses inebriation, and soothes gout.

Capsaicin has significant physiological action, which is used in many pharmaceutical preparations like liniments and ointments for cold, sore throat, chest congestion *etc.* It is also used in cosmetics like prickly heat powders and skin ointments. It is also used as carminative, tonic and stimulant (Pruthi, 1979). Capsaicin is being used to alleviate pain and is the most recommended topical medication for arthritis. At nerve endings a neuro transmitter called 'substance P' informs the brain that something painful is occurring. Capsaicin causes an increase in the amount of 'substance P' released. Eventually, the 'substance P' is depleted and further releases from the nerve endings are reduced. A decrease in 'substance P' also helps to reduce long-term inflammation. Prolonged use of the cream containing capsaicin has been found to help reduce the itching in dialysis patients, the pain from shingles (Herpes zoster) and cluster headaches (Carmichael, 1991). Capsaicin and dihydrocapsaicin have the capacity to inhibit the formation of mutagenic and carcinogenic heterocyclic amines (Millic *et al.*, 1998).

Improvement of crop plants through breeding programme depends on the creation of genetic variability and availability of new gene recombinations. But with the increase in human population and his

indiscriminate interference into the natural gene pool lead to the destruction of valuable and desirable wild gene resources. At this juncture, mutation breeding plays a significant role in creating new genetic variability in existing species. Mutation induction and selection methodology has now progressed to the point where, it provides a viable additional option to plant breeders for creating useful genetic variability. In most situations, mutation induction should be looked upon as a tool that, in combination with other tools such as crossbreeding, can be used to achieve desired plant breeding goals. Genetic variability available in germplasm collection can be supplemented by induced mutations, when the germplasm sources may be inadequate or in a particularly poor genetic background. Induced mutations have their most prominent place when an otherwise good cultivar is to be improved in only one, easily recognizable character, leaving the rest of the genotype more or less untouched.

Gene technology can modify only the individually recognizable 'Major genes' present in the economically important plants. Crop production, however, relies upon many genes especially those, which control quantitative characteristics polygenically. Therefore, the chemical induction of mutations can be regarded as a useful tool in modifying the polygenically controlled traits in plants (Micke, 1995).

The idea of inducing mutations artificially and using them for crop improvement was first introduced by De Vries in 1901. However, it received due attention only after 1927 when Muller demonstrated artificial induction of

mutations by ionizing radiations in *Drosophila melanogaster*. Later, Stadler (1928) produced mutations in barley and maize by X - rays and gamma rays. The discovery of chemical mutagens in 1940 by Auerbach opened new vistas for improving crop plants through the induction of mutations. After that, several chemical compounds, which possess powerful mutagenic properties than ionizing radiations in terms of mutation rates, have been extensively used for inducing genic changes in various crop plants. Extensive work with chemical mutagens has, however, begun only since 1960 following the introduction of the alkylating agent, ethyl methanesulphonate (Heslot, 1964). Chemical mutagens especially alkylating agents, in contrast to radiations, produce wider spectrum of mutations (Swaminathan *et al.*, 1962). Mutagens such as ethyl methanesulphonate, diethyl sulphate, sodium azide and certain base substitution and nitroso compounds appear to induce higher proportions of point mutations than chromosomal aberrations (Konzak *et al.*, 1977).

The alkylating agents have a reaction pattern more suited than ionizing radiations for breaking down the buffering characters of polyploid germplasm and for creating maximum of genic diversification and allelic interactions between homologous loci (Mac Key, 1967). According to Gaul (1967), ethyl methanesulphonate exhibits relatively more positive micro-mutation inducing potential.

Sodium azide (SA) mutagenecity was first observed by Wyss *et al.* (1948) in their studies on the role of peroxides in radiation-induced

mutagenesis. Later, sodium azide was found to be a very effective mutagen under certain treatment conditions (Kleinhofs *et al.*, 1974). Sideris *et al.* (1974) postulated a base transition mechanism to explain the mutagenic effect of sodium azide. The mutagenic effect of sodium azide was reported by various workers in a number of crop plants of economic importance (Sander *et al.,* 1978).

In a series of experiments carried out in various crops, it has been established that chemical mutagens, when applied to plants, induce mutations that are polygenic in nature (Abrams and Frey, 1964; Gottschalk, 1973; Brock, 1977; Khan, 1982; Siddiqui, 1982; Khan *et al.,* 1999). The isolated desirable mutants showed increased yield, high protein content, resistance to certain specific diseases and many other desirable traits beneficial to mankind, which opened a new era in the history of crop breeding.

Therefore a comprehensive study was undertaken to evaluate the extent of variability induced by the two potent chemical mutagens, **Ethyl methanesulphonate (EMS)**, an alkylating agent and **Sodium azide (SA)**, a respiratory inhibitor in two varieties of *Capsicum annum* namely **Ujwala** and **Co-1**.

The main objectives of the present investigation are:

- to study the biological damage induced by mutagens like inhibition in seed germination, seedling injury, cotyledonary abnormalities and pollen sterility in M_1 generation.
- to analyze the frequency and spectrum of morphological and chlorophyll mutants in M_2 generation.
- to select the mutants having desirable characters in M_2 generation.
- to isolate mutants in M_3 generation having superior characters and to estimate capsaicin content of some selected mutants.
- to calculate mean, standard deviation, phenotypic and genotypic coefficient of variability, heritability and genetic advance of both control and treated populations in M_2 and M_3 generations in order to assess the extent of genetic variability induced by the mutagens.

CHAPTER - 2

REVIEW OF LITERATURE

2.1. Induction of Mutations

2. 1. 1. Mutation studies in other crops:

The induction of mutations has been accepted as a useful tool in plant breeding programmes. Mutation breeding has helped much in evolving improved varieties with better agronomic characters leading to higher productivity. However, the efficacy of mutation breeding programmes depends on the amount of genetic variability available in the crop species and the efficiency of the selection techniques employed.

The excellent works of Muller (1927) and Stadler (1928) opened new vistas in plant breeding by introducing artificial induction of mutation. Later, the usefulness of micro mutations in plant breeding was established by Gregory (1956, 1961).

Most of the varieties developed by mutation breeding so far have arisen from material treated with ionizing radiations. Extensive work with chemical mutagens has begun only since 1960 following the introduction of ethyl methanesulphonate (Heslot, 1964). However the alkylating agents have a reaction pattern more suited than ionizing radiations for breaking down the buffering characteristics of polyploid germplasm and for creating a maximum of genic diversification and allelic interaction between homologous loci (Mac Key, 1967). Chemical mutagens, especially alkylating agents, in

contrast to radiations, produce wider spectrum of mutations (Swaminathan *et al.*,1962).

Konzak *et al.* (1961) and Arnason *et al.* (1962) reported that EMS gives higher frequencies of gene mutations than ionizing radiations. Swaminathan *et al.* (1962) observed chromosome and chromatid aberrations and isochromosomes in cells when different wheat cultivars of various ploidy levels were treated with different concentration of ethyl methanesulphonate. Gaul (1963) found an increase in M_2 mutation frequency with an increase in M_1 sterility. Jana (1963) obtained different chlorophyll mutants after the X-ray irradiation of seed of variety T-9 of black gram. The chlorophyll deficient mutants included albina, xanthoalba, xantha, chlorina, variegata and virescent type. Swaminathan (1965) further reported that EMS is supposed to be specific to certain chromosome regions containing genes for chlorophyll development located near centromere. Goud (1967a) reported that the EMS is more effective in producing biological damage than gamma rays in bread wheat. Sato and Gaul (1967) reported that sterility induced by EMS and other chemical mutagens might be due to cryptic deficiencies and specific gene mutations. Prasad (1968) identified a yellow rust resistant mutant in the M_2 generation of EMS treated *Triticum durum* variety NP 404. Izvorska (1969) irradiated the seeds of the eggplant variety Delicacy with different doses of X-rays and reported that the germination was earlier in various treatments and M_1 plants flowered earlier and produced more fruits than controls.

Sharma (1970) observed a synergistic action of gamma rays and EMS on pollen sterility. Siddiqui (1972) worked out extensively on chemical mutagenesis and obtained high yielding mutants of *Solanum melongena*. Gustafsson (1972) obtained erectoid mutants in barley, which are of direct importance in barley breeding. Kaul and Bhan (1973) studied the effect of gamma rays, EMS and DES singly and in combination upon seedlings of three rice varieties. Patel and Shah (1974) irradiated brinjal seeds and observed morphological and structural changes in shoot apex. Reddy and Reddy (1974) recovered various grain shape mutants after treatment with EMS and DES in rice.

Chowdhury (1978) reported that chemical mutagens are more efficient in inducing wide range of mutations with a higher frequency in bread wheat. Singh *et al.* (1978) studied the biological damage and morphological mutations induced by gamma rays and EMS in pearl millet. Khan and Hashim (1979) studied the mutagenic effectiveness and efficiency of gamma rays, EMS and hydrazine in *Phaseolus aureus*. Raina *et al.* (1979) carried out investigations in a number of wheat varieties and revealed that both additive and non-additive genetic variances were considerably enhanced in EMS treated populations. Sharma and Sharma (1979) compared the effectiveness and efficiency of NMU in lentil and found that NMU was about three times more effective than gamma rays. Farook (1979) reported the increase in mean values of quantitative characters and variability of protein characters in treated material as compared to control in chickpea.

Haq and Shakoor (1980) irradiated the seeds of *Cicer* with gamma rays and found a mutant, which was resistant to blight disease. Singh *et al.* (1980) treated the seeds of pigeon pea with gamma rays and NEU and selected the plants with good plant type and uniform maturity. Singh and Chaturvedi (1980) treated two inbreds of mung bean with different mutagens i.e. EMS, NMU and gamma-rays and reported that irrespective of the varieties involved, EMS was found to be most efficient for germination, NMU for pollen fertility and seedling height in M_1 generation and NMU for chlorophyll mutations in M_2 generations. Tiwari and Singh (1981) treated the seeds of T_2 variety of pigeon pea with gamma rays and observed that the M_1 plants showed more than 20% pollen sterility. Singh and Chaturvedi (1981) treated two inbreds of mung bean [*Vigna radiata* (L.) Wilczek] *viz.* Pusa Baisaki and S-8 with EMS, NMU and gamma rays to find out their relative mutagenic susceptibility and specificity. Sharma and Sharma (1982) induced polygenic variability for yield and its components by different mutagenic treatments in Lentil. Bhamburker and Balla (1983) conducted experiments on the seeds of black gram with single and combined treatments of gamma rays and hydrazine. An increase in genetic advance values was observed in treated population. Bandyopadhyaya and Bose (1983) isolated tall vigorous mutants with variation in leaf shape, early flowering and high pod production in M_2 generation of black gram treated with EMS and X-rays. Khan (1983) observed a considerable increase in genetic variance, heritability and expected genetic advance in mung bean after treatment with EMS and gamma rays. Rao (1984) treated varieties of pigeon pea with various doses

of gamma rays and isolated wide range of mutants with different forms of habit and profuse branching with increase in yield. Verma and Singh (1984) reported the gamma-ray induced variability in green gram. The M_3 population showed higher variance than the control for pods per plant, seeds per plant and yield per plant. Bahl and Gupta (1984) treated two inbreds of mung bean ML 5 and K 851, with gamma rays and EMS separately and in combination and found that albina and chlorina chlorophyll mutations occurred in the M_2 segregation population. Khan (1984) treated the seeds of mung bean with gamma rays, EMS and hydrazine. The mean values and variances of number of days of flowering, number of fertile branches per plant and number of pods per plant was higher than that in the control. Sharma and Kaul (1984) reported that the EMS induced mutant of IR8 rice exhibits higher salt tolerance than the parent variety. Grover and Virk (1984) reported various viable mutants after treatment with MNNG (N-methyl-N-nitro-N-nitrosoguanidine), EMS and HA (Hydroxyl amine) in mung bean. Khamankar (1984) studied the spectrum of induced mutations and found marked differences for rates at specific loci by hydrazine and hydroxylamine while, gamma-rays gave highest frequency of lethals, EMS showed highest chlorophyll mutations.

Meiotic anomalies induced by EMS in three hexaploid wheat varieties were observed by Al-Saheel (1985). He further reported that the differences in chiasma frequency and varietal treatment interaction were significant. Nadarajan *et al.* (1985) treated the seeds of two varieties of *Cajanus cajan*

with gamma-rays and diethyl sulphate and found that low doses of the mutagen slightly increased M_1 plant height but higher doses reduced the height. Kumar and Sinha (1985) observed the most common occurrence of chromosome bridges from the mitotic root and shoot tip cells of the seedling in the gamma irradiated population of *Cajanus cajan*. Khan (1987) evaluated the various agronomic characters of eight mutants obtained after treatment with EMS and HZ in mung bean. Yadav (1987) treated the seeds of mung bean with different doses of EMS, DES and gamma rays separately and in combination. In general the mitotic index and seedling vigour decreased with increasing mutagenic doses. However, frequency and spectrum of chlorophyll mutation increased in the M_2 generation. Rosaiah *et al.* (1987) found an increase in the nitrogen fixing ability of green gram by the gamma-irradiation of the seeds. Singh and Raghuvanshi (1987) treated the seeds of *Vigna mungo* with gamma rays alone or followed by treatment with EMS. Two mutants with pentafoliate leaves, high yield and greater number and dry weight of nodules were isolated. A depression in seed germination, seedling height and pollen fertility was noted by Khan and Siddiqui (1988) after mutagenic treatments in *Vigna radiata* (L). Wilczek. Narasimhachary and Bhalla (1988) isolated a male sterile mutant in the M_2 generation in EMS treated population of pigeon pea. Venkateswarulu *et al.* (1988) isolated various types of chlorophyll mutants after treatment with gamma rays and EMS singly and in combination in *Carthamus roseus* L. Rajput *et al.* (1988) treated the seeds of *Vigna radiata* with gamma-rays and a high yielding mutant line was obtained from the treated population having 17- 49%

increase in the yield over the control. Pande and Raghuvanshi (1988) irradiated the seeds of *Vigna radiata* variety K581 with gamma rays and EMS. A dwarf mutant isolated in M_2 and found true breeding in M_3 generation showed increased number of pods per plant and seeds per pod. Jayabalan and Rao (1988) obtained different chlorophyll mutants in two cultivars of tomato after the treatment with gamma-rays, ethyl methanesulphonate and n-nitroso-n-methylurea. The incidence of various types of chlorophyll mutants did not follow dose related trend. Bahl (1988) has reviewed the heritability, genetic advances and correlation studies in chickpea and has observed that most of the studies were based on broad sense heritability and breaking of negative correlation by the mutagenic treatment. Chary and Bhalla (1988) reported a male sterile mutant from the M_2 generation of pigeon pea after treatment with EMS. Reddy (1989) identified various types of chlorophyll mutants in *Triticale* after treatment with gamma rays and EMS. Reddy and Gupta (1989) observed that EMS produced high frequency of mutation in comparison to gamma rays in *Triticale*. Bhalla (1989) studied the effect of gamma rays and EMS in pigeon pea. They observed several mutations affecting the plant height, seed and pod characters and yield. Ignacimuthu and Babu (1989b) isolated several high protein mutants with high level of lysine after the treatment of seeds with gamma rays and EMS in *Vigna mungo* and *Vigna radiata*. Ignacimuthu and Sakthivel (1989) treated the seeds of *Vigna radiata* variety PS-16 with EMS and gamma rays and found a positive significant correlation between chromosomal abnormalities and pollen sterility induced. Srivastava *et al.*

(1989) induced genetic variations in sugarcane by treating the single bud sets with gamma-rays and ethyl methanesulphonate.

Natarajan and Palaniswamy (1990) treated the seeds of two varieties of mung bean with EMS and in combination with gamma rays. In M_2 generation, it was found that the 30 KR gamma ray treatment produced the highest frequency of micro mutations. Combination treatment of low doses of EMS and gamma rays were recommended for inducing mutation in yield components. Singh and Raghuvanshi (1991) isolated a bold seeded mutant from gamma-irradiated M_2 population of black gram. Srivastava and Singh (1991) isolated a number of agronomically important mutants from M_2 population of two varieties of pigeon pea by gamma-ray treatment. Sharma and Talukdar (1991) induced genetic diversity in mung bean by gamma-rays, EMS and their combination treatments and found that primary branches, pods per cluster and clusters per plant showed high heritability with high genetic advance. Sharma and Singh (1992a) gave post irradiated heat treatment after 30 KR gamma-ray dose and observed that treatment administered with higher temperature (60^0C) caused maximum reduction in M_1 parameters and induced higher frequency of chlorophyll and viable mutations in M_2 generation of mung bean. Sharma and Singh (1992b) obtained two male sterile mutants from 30 KR gamma ray treated M_2 population of mung bean. Sharma and Singh (1992c) reported induction of a number of important mutants *i.e.* bushy type, tall type, long pod, high yielding clustered pod, synchronous maturing and early maturing plants from EMS

and gamma ray treated population of mung bean. Srivastava *et al.* (1992) obtained a few male sterile mutants from gamma ray and EMS treatments in M_2 generation of pigeon pea. Srivastava and Singh (1993 a) obtained higher estimates of variability and heritability in M_2 population of pigeon pea after mutagenesis. Srivastava and Singh (1993b) reported synchronous maturing mutants in pigeon pea by treating the seeds with gamma rays, EMS and NMU. Siddiqui (1993) observed an increase in phenotypic variability of polygenic traits in eggplant after NMU mutagenesis. Micke (1995) evaluated the role of induced mutations in crop improvement and concluded that radiation and other mutagens are helpful in producing genetic variation useful for plant breeders. Khan *et al.* (1999) isolated high yielding mutants from ethyl methanesulphonate and methyl methanesulphonate treated M_3 population of mung bean. The mean values of characters under study were increased significantly over control.

Khan *et al.* (2000) studied the extent of biological damages and polygenic variability induced by methyl methanesulphonate in green gram. A dose dependent biological damage in M_1 and a higher coefficient of variability and heritability values for quantitative traits was observed in M_2 generation of treated population. Rizwana Banu *et al.* (2002) found that when compared to ethyl methanesulphonate, gamma ray treatments induced more variation in the quantitative characters in cowpea. Kumar *et al.* (2003) reported that the ethyl methanesulphonate and gamma irradiated plants of *Lens culinaris* showed varying degrees of meiotic irregularities almost at all

treatment doses. The frequency of meiotic irregularities was found to be more in the sets of combined treatments as compared to the individual ones.

The mutagenic action of sodium azide in bacteria has been known. However, the mutagenic property of azide in plants has now been an established fact. The mutagenic action of sodium azide as well as its metabolite in barley and bacteria appears to be unique among the present day known mutagens (Kleinhofs *et al.*, 1978).

Sideris *et al.* (1969,1973) reported that the mutation frequency obtained with sodium azide treatments was increased to about 20 percent by using treatment solutions at pH values below the pKa of azide. Angstrom *et al.* (1972) used azide in conjunction with N - nitroso - N - methylurea in *Arabidopsis*. Nilan *et al.* (1973) reported mutation frequencies on M_1 spike of up to 46 percent with 4 h germination with azide treatment in barley. Conger (1973) studied the effect of ascorbic acid on azide – radiation synergism on seedling growth in barley.

Levy and Ashri (1973) observed varietal differences in response to azide sensitivity in peanut. Vig (1973) reported that azide increases frequencies of non-disjunction in soybean. Ashri and Levy (1974) studied the response of successive developmental stages of peanut embryos to azide and reported that earlier stages of embryo development were more sensitive than later stages. Sideris and Aggrakis (1974) postulated a base transition mechanism to explain the mutagenic effect of sodium azide. Konzak *et al.*

(1975) observed a positive azide concentration effect on mutation frequencies on barley.

Warfield (1974) and Chaudhary and Kaul (1976) reported that azide induces few chromosome aberrations above control levels in barley. Similar results were also obtained in *Vicia faba* (Kihlman and Sturelid, 1975) and in soybean (Vig, 1973). Pearson *et al.* (1975) studied the effect of sodium azide on the barley cell cycle and found that the principal effect was a delay in the inhibition of metabolism following germination, which resulted in a uniform delay in mitotic activity, seedling growth and ATP and DNA synthesis. Choudhary and Kaul (1976), Niknejad (1976) and Walther (1976) reported that azide induces reduction in seed germination in M_1 seedling growth and an increase in sterility and recessive embryo lethals as scored by the reduced number of M_2 seedling per spike in barley. The proportion of the different chlorophyll deficient mutants in barley induced by azide, gamma rays and diethyl sulphate have been reported by Nilan *et al.* (1976). Walther (1976) reported no change in certain biological end-points in barley after 9 months storage of azide treated seeds at 5^0C. Sander and Muehlbaner (1977) suggested that the lack of leaf variants from azide treatment of peas might be due to the lack of chromosome breaks. Veleminsky *et al.* (1977) reported the induction of dose dependent frequency of single strand breaks of DNA of non-germinating embryos in barley. Owais *et al.* (1978, 1981) established that sodium azide has been shown to be metabolically activated into a mutagen in barley. Activation mechanism of azide is related to the L-

cysteine biosynthetic pathway. Sharma *et al.* (1979) studied the effect of concentration and soaking time on induced chlorophyll mutation frequency after azide treatment in rice. Hasegawa and Inoue (1980) reported that sodium azide produces high frequency of mutations with negligible amount of chromosomal aberrations in rice. Cheralu and Reddy (1985) studied the mutagenic effect of hydrazine and sodium azide on four different cultivars of *Sorghum*.

Khan and Siddiqui (1995) isolated various types of morphological mutants by using chemical mutagens viz., ethyl methanesulphonate, and methyl methanesulphonate and sodium azide. These mutants differ from control and also among themselves in height, growth and flowering habit. Gaikwad and Kothekar (2003) recorded various morphological mutants in ethyl methanesulphonate and sodium azide treated M_2 and M_3 generation of *Lens culinaris*. Out of nine morphological mutants isolated, the early maturing, high yielding and bold seed type mutants have the potential to be incorporated into breeding programmes. Khan and Wani (2004) observed higher genotypic coefficient of variation, heritability and genetic advance for three quantitative characters in ethyl methanesulphonate, methyl methanesulphonate and sodium azide treated M_2 and M_3 populations in mung bean.

2. 1. 2. Mutation studies in *Capsicum*

Various workers adopted the technique of induction of mutations by using physical and chemical mutagens as an effective tool for the genetic improvement of *Capsicum.*

Subhash and Nizam (1973) observed chromosome aberrations in root tips of *Capsicum* seedlings grown from X-ray irradiated seeds. Aberrations included deletions, parallel bridges and single and paired fragments of acentric chromosomes. Zubrzycki and Vonder (1973) treated the seeds of *C. annum* cv. California Wonder with ethyl methanesulphonate (EMS) and X-rays. Shifriss (1973) identified a recessive single – gene mutation for stable male sterility in the variety Gambo of *Capsicum annum.* Bansal (1973) treated the seeds of variety NP 46A with ethyl methanesulphonate and N-nitroso – N – methylurea. Sterile mutants were isolated in the M_1 and M_2 generations, in which reproductive parts were transformed into vegetative ones. Deskaloff and Daskalov (1974) found that the male sterile mutants isolated after irradiation showed shrunken anthers and lack pollen grains.

Brecils and Pochard (1975) transferred the recessive gene ms 509, obtained by chemical mutagenesis, to the parents of the commercial pepper hybrid Lumuyo – INRA. Subhash and Nizam (1975) exposed *Capsicum annum* seeds to X-rays. In M_2 generation 90% of the PMCs had an extra chromosome and the variant plants were taller, bore larger flowers, matured earlier and gave higher yields than normal plants. Solomatin (1976) studied the effect of chemical mutagens on mutation frequency in different varieties

of pepper and the highest mutation frequency was obtained using ethyleneimine. A large percentage of mutation was obtained using N - nitroso – N - ethylurea and dimethyl sulphate. Daskaloff *et al.* (1977) treated the seeds of Pazardzhik pepper 794, Kalinkov 800/7, Zlaten meda (gold medal) and Albina with gamma rays, X-rays and ethyl methanesulphonate. A total of six male sterile mutants were obtained, of which the most important are those with the genes ms 3 and ms 8 and a cytoplasmic male sterility system derived from Kalinkov. Nine marker genes wre also reported, which include marbled white zones on the leaves (m) and absence of anthocyanin on leaves and stem (al). Saccardo and Ramulu (1977) treated the seeds of *Capsicum* var. Qurdratod' Asti (Astisquare) with fast neutrons and var. Corno di. Toro (Bull's Horn) with ethyl methanesulphonate. The frequency of M_2 plants that showed no symptoms of cucumber mosaic virus after inoculation was 0.54% for fast neutrons and 0.05% for 0.2% ethyl methanesulphonate. Saccardo *et al.* (1977) reported that *C. pendulam, C. fruitescens* and *C. chinense* were good source of resistance and are being used for hybridization with *C. annum*. Irradiation with gamma rays and fast neutrons was used and a screening technique was set up to select resistant mutant types. Ramalingam (1977a) introduced MDU1, a new mutant chilli variety derived from the M_2 of K1 following gamma irradiation. The mutant has a compact, determinate habit, clusters of flowers and an increased capsaicin content. Ramalingam (1977b) reported that the treatment of the variety K1 with X-rays in two successive generations or with X-rays in the first and diethyl sulphate in the next did not appreciably increase the frequency of

chlorophyll mutation above that was expected on an additive basis. Daskaloff and Daskalova (1978) obtained six male-sterile mutants following gamma and X-irradiation of seeds of *Capsicum annum*. Ilieva *et al.* (1978) observed meiotic aberrations in *Capsicum annum* and *Capsicum pendulum* after gamma irradiation. Chromatin was unevenly distributed between the poles and disturbance of chromosome segregation led to a reduction in the number of nuclei formed in PMC's. Katiyar (1978) observed meiotic abnormalities after irradiation in *Capsicum*. Indira and Abraham (1979) isolated a tricarpellate mutant in *Capsicum* through gamma irradiation.

Ramalingam and Sethupathi Ramalingam (1980) found that the spectrum of mutations differed according to variety and mutagen and interactions between variety and dose affected both the spectrum of mutations and their frequency. Subhash *et al.* (1981) isolated twelve multilocular mutants after the seed treatment with mitomycin C. The flowers of the mutants were larger than those of the original variety. Raghuvanshi and Singh (1982) treated the seeds of cultivar NP 46A with dimethyl sulphate, ethyl methanesulphonate, methyl methanesulphonate, hydrazine sulphate, hydroxylamine hydrochloride and gamma rays. Five viable mutants were obtained in the M_2, characterized by erect fruit, small pointed fruit, long orange fruit, round orange fruit and purple fruit. Saccardo and Vitale (1982) irradiated the male and female gametes of *Capsicum annum* with gamma rays and seeds with fast neutrons. Selection for useful agronomic characters was carried out and in the M_7, eight lines from seed irradiation and 35 from

gamete irradiation were grown. Zatyko and Moor (1982) obtained a new type of spontaneous mutant, Feherozon with outstanding features that include continuous fruiting, determinate growth habit, low light requirements, slow pod growth, resistance to red spider mite, suitability for both forcing and outdoor culture from transplants or direct sowing and suitability for manual or mechanical harvesting and all marketing purposes. Rostaino (1983) obtained two recessive brachytic mutants in M_4 generation after the treatment of *Capsicum annum* with ethyl methanesulphonate and gamma rays. Saccardo and Monti (1983) selected a number of dwarf mutant lines and mutant lines with improved fruit colour and size after gamete irradiation. Saccardo and Vitale (1983) isolated short forms of *Capsicum annum* with synchronous ripening and thick-fleshed fruits of good processing quality in the M_5 and M_7 generations from gamma-irradiation of seeds and gametes of the variety pimento. Rajam and Subhash (1984) induced mutations in *Capsicum* by treating seeds with various concentrations of mitomycin C. Almost all the treatments induced variability for nine quantitative characters, several morphological mutants such as clustered bud, tall, multilocular spindle fruit, erect fruit and orange fruit were isolated in M_2 generation.

Asha and Nayar (1986) tested fifteen genotypes of *Capsicum annum* to study the effect of gamma rays and ethyl methanesulphonate on pollen sterility. Gamma ray induced higher percentage of pollen sterility when compared to the action of ethyl methanesulphonate and a genic status influenced variation was noted in the effect of mutagens. Devadas *et al.*

(1986) studied the mutagenicity of four organophosphorus insecticides in the meiotic system of red pepper. Germination and seedling survival were significantly reduced by the treatments. All the treatments induced meiotic abnormalities in PMC's, which resulted in dose – dependent pollen sterility. Kumar *et al.* (1986) studied the mode of bivalent formation in desynaptic mutant of chilli pepper and found that the frequency distribution of PMCs with different numbers of bivalents deviated significantly from the expected binomial distribution in two groups of desynaptic mutants, the mutation in each case being controlled by a single recessive gene. Nushikyan *et al.* (1987) treated the seeds of *Capsicum annum* cv. Nork and cv. Lastochka with N-methyl-N-nitrosourea, N, N-dimethyl-N-nitrosourea and ethyleneimine. The mutants selected in M_5 and M_6 generations showed high resistance to diseases and exceeded the initial cultivars in fruit yield and vitamin C content. Joshi *et al.* (1989) treated dry diploid seeds of *Capsicum annum* with NMG (N –methyl-N-nitrosoguanidine) at various concentrations and found that NMG was effective in inducing mutants with dark red, yellow and orange pericarps. They also found that frequency of colour mutations increased with mutagen concentration. Rostaino (1989) isolated a new dwarf pepper from ethyl methanesulphonate and gamma ray treated population of local Italian variety Friariello. The new dwarf Friari KS 80, with compact habit was found suitable for protected cultivation under conditions of high plant density.

Raghuvanshi and Kumar (1991) isolated nine mutants of *Capsicum annum* cv. Pusa-jwala after chemical and physical mutagenic treatments. Capsaicin content was significantly increased in the fruits of four of them. Daskalov and Baralieva (1992) identified a new cultivar of *Capsicum annum* with 2 to 2.5 times more β - carotene (Provitamin A) content in gamma irradiated M_2 population. Rajam and Subhash (1995) produced a wider frequency and spectrum of mutation in combination treatment of X-rays and ethyl methanesulphonate in *Capsicum annum.*

Alcantara *et al.* (1996) treated the seeds of *Capsicum annum* cv. 'Key stone resistant Giant - 3' with various concentrations of ethyl methanesulphonate. The mutants observed included glossy – spinach (gs), vein chlorosis (vc), dwarf virescent (dwv), dwarf yellow (dwy), dwarf chlorotic (dwc), white-spotted (ws), yellow green mottle (mot - yg), light green mottle (mot - lg) and golden yellow mottle (mot - gy). Subhash *et al.* (1996) treated the shoots regenerated from cotyledon explants of *C. annum* cv. G4 and obtained streptomycin resistant shoots developed from the green sections of the cotyledons. Patil *et al.* (1997) induced quantitative variation by treating the seeds of cv. Jwala with ethyl methanesulphonate and dimethyl sulphonate. The mean values were significantly shifted in a negative direction for plant height, primary branches and girth, and in a positive direction for secondary branches, number of fruits, length of fruit and yield/plant. Siddiqui and Azad (1998) treated the seeds of *Capsicum annum* var. California wonder with ethyl methanesulphonate and the resulting plants

exhibited a number of variations in several of the sixteen quantitative characters studied. Mehta *et al.* (1999) studied the effect of chemical mutagensis in *C. annum* varieties S-49 and Jwala. A shift of mean in the positive direction was found for fruit yield and some other characters, and differential varietal and character responses through mutagenic treatments were observed.

Rangaiah and Manjunath (2000) irradiated two varieties of chilli viz., Ceylon and Byadagi, seeds with different doses of gamma rays. The comparison of mean in different populations indicated shifts in both the directions of control F_2 mean, due to mutagenesis of hybrid and parental lines depending on the trait. The direction of shifts varied with genotypes, mutagen and character. Kumar *et al.* (2001) induced chlorophyll mutations in chilli pepper by gamma irradiation. Rangaiah *et al.* (2001) conducted correlation and path coefficient analysis for 10 traits using M_2 and F_2M_2 populations obtained by gamma irradiation of *C. annum*. At the genotypic level, a positive association was observed between days to maturity and plant height, number of primary branches and number of fruits per plant and fruit width; and number of secondary branches and number of fruits per plant and fruit width. Boseland (2002) generated a mutant that causes a novel flaccidity phenotype in *Capsicum annum* by treating seeds with ethyl methanesulphonate. Inheritance studies indicated that the mutant was controlled by a single recessive gene.

CHAPTER - 3

MATERIALS AND METHODS

3.1. Plant Materials

Two south Indian varieties of chilli (*Capsicum annum* L.) namely, Ujwala and Co -1 were selected as the plant material in the present investigation. Seeds of the varieties Ujwala and Co -1 were procured from the information and sales centre, Kerala Agricultural University, Mannuthy and Department of Horticulture, Tamilnadu Agricultural University, Coimbatore respectively.

3. 1. 1. Variety Ujwala

This variety was released at the Horticulture College, Kerala Agricultural University, Vellanikkara, Thrissur. It is highly resistant to bacterial wilt disease and possesses a compact plant type, upright fruit forms and extensive axillary shoots terminating into cluster of fruits. Fruits are bright red in colour and possess high pungency. It yields an average of 625.0 kg of dry fruits per hectare.

3. 1. 2. Variety Co-1

It was released at the Horticulture College, Tamilnadu Agricultural University, Coimbatore. It possesses dichotomous growth pattern and result in the production of a solitary fruit at each branching node. Fruits are pendent, thick and bright red in colour and possess high pungency. It yields an average of 928.7 kg of dry fruit per hectare.

3. 2. Mutagens used

Two chemical mutagens were used in the present study, *viz.* ethyl methanesulphonate (EMS) and sodium azide (SA).

3. 2. 1. Ethyl methanesulphonate (EMS) – $CH_3SO_2OC_2H_5$

(i) Manufactured by Sigma chemical company, St. Louis, Missouri 63178, U S A.

(ii) EMS is a monofunctional alkylating agent, causes depurination, transition and formation of triesters in the backbone of DNA molecule.

3. 2. 2. Sodium azide (SA) – NaN_3

(i) Manufactured by Sigma chemical company, St. Louis, Missouri 63178, U S A.

(ii) It is used as a respiratory inhibitor. During duplication of DNA by base transition mechanism, it causes point mutation.

3. 3. Experimental Procedures

3. 3. 1. Preparation of mutagenic solutions

The solution of chemical mutagen EMS was prepared in phosphate buffer at pH = 7, and solution of SA was prepared in phosphate buffer at pH = 3. Only freshly prepared solutions were used for all the treatments.

3. 3. 2. Pre – Treatment

Healthy, disease free and uniform sized seeds of C. annum var. Ujwala and var. Co - 1 were used in the present investigation. The seeds were soaked in distilled water for 12 hours (9pm to 9 am). prior to the treatment with the mutagens of different concentrations.

3. 3. 3. Mutagen administration

Concentrations: - The following concentrations of the two mutagens were used for treating the pre – soaked seeds.

EMS: 0.1%, 0.2 % and 0.3%

SA: 0.01%, 0.02% and 0.03%.

Sample size: 200 seeds were used for each treatment.

Treatment time: The treatments were given at room temperature ($27\pm1^{0}C$) for 9 hours duration (9am to 6pm).

Controls: For each variety 200 pre-soaked seeds were again soaked in distilled water for 9 hours to serve as controls.

To facilitate uniform absorption, higher quantity of solutions of mutagens, approximately three times than the volume of the seeds were used. During the treatment, the test tubes containing the solution and seeds were frequently shaken to ensure sufficient aeration. Immediately after

treatment the seeds were thoroughly washed in running tap water so as to remove even traces of mutagen.

3. 4. Selection in different generations

3. 4. 1. M_1 generation

Fifty seeds each of every treatment and control of both the variety were sown in poly bags (20 x 30 cms) in order to raise the seedlings. 45 days old seedlings were transplanted to experimental field adjacent to the Unity Women's College, Manjeri campus in three replicates. The distance between seedlings in a row and between the rows was kept at 45 x 50 cms respectively.

Recommended agronomic practices were employed for preparation of field, sowing and subsequent management of the population. The remaining lot of 150 seeds were used for determining basic characters such as seed germination and seedling height *i.e.* root and shoot length.

Seeds of each treatment with their respective controls of both varieties were spread over moist cotton in petriplates. The petriplates were kept in room temperature at 27 ± 1^0C in order to study the effect of mutagens on seed germination and seedling growth.

3. 4. 1. 1. Observations

A detailed study of the effect of different mutagenic treatments in the two varieties was undertaken using the following biological parameters.

3. 4. 1. 1. 1. Seed germination

The percentage of seed germination was calculated on the basis of total number of seeds sown in petriplates. Seeds, which gave rise to both radicle and plumule, were considered as the criterion for germination of seed.

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

3. 4. 1. 1. 2. Seedling height

On the 20th day the seedling height was estimated by measuring the root and shoot lengths for each treatment along with control. Seedling injury was measured by the reduction in the root or shoot lengths or no development of both the organs or any one organ.

3. 4. 1. 1. 3. Pollen fertility

The pollen fertility was determined by staining the pollen grains with 1:1 acetocarmine - glycerin solution. For this purpose ten plants at random were selected from each treatment including their controls for both the varieties and five young flower buds from each plant were used for microscopic analysis. Pollen grains, which took the deep stain and had a regular outline were considered as fertile, while the shrunken, empty and unstained ones as sterile.

The following formula was used to calculate the percentage of pollen fertility inhibition or injury or reduction.

Percentage of inhibition

or

$$\text{Percentage of injury} = \frac{\text{Control} - \text{Treatment} \times 100}{\text{Control}}$$

or

Percentage of reduction

3. 4. 1. 1. 4. Cytological Studies

For meiotic analysis young flower buds from individual plants for each treatment and control were fixed in Carnoy's fluid (1 glacial acetic acid: 3 chloroform: 6 ethyl alcohol) for at least 30 minutes. The flower buds were washed and preserved in 70% ethyl alcohol. Anthers were smeared in acetoorcein and pollen mother cells (PMC's) were examined for detecting various meiotic stages during microsporogenesis.

3. 4. 1. 1. 5. Cotyledonary abnormalities

The plants showing abnormalities in cotyledons were recorded in the field. The frequency of cotyledonary abnormalities in various treatments was calculated by the formula given below: -

$$\text{Cotyledonary abnormality (\%)} = \frac{\text{No. of seedling showing cotyledonary abnormalities} \times 100}{\text{Total no. of } M_1 \text{ seedlings.}}$$

3. 4. 2. M_2 Generation

For raising M_2 generation, 50 healthy seeds of both the varieties from each normal looking M_1 plants of all the different treatments with their respective controls were selected and sown in poly bags to raise M_2 seedlings. Forty five days old seedlings were transplanted in the field in plant progeny rows. The spacing between plants in a row and between rows was 45 cms and 50 cms respectively in each treatment as well as in controls. The experiments were conducted in Completely Randomized Block Designs (CRBD) with three replicates (Plate 6).

3. 4. 2.1. Observations

3. 4. 2. 1. 1. Chlorophyll mutations

Chlorophyll mutations were scored when seedlings were 15-30 days old. They were identified and classified according to Gustafsson (1974). The frequency of chlorophyll mutations was calculated by the following formula.

$$\text{Mutations frequency (\%)} = \frac{\text{No. of mutant seedlings}}{\text{Total no. of } M_2 \text{ seedlings.}} \times 100$$

3. 4. 2. 1. 2. Quantitative characters

Observations were also made on normal looking plants of each progeny, for each treatment with their controls. The progenies segregating for macro mutations were not used for such analysis. The following ten quantitative characters were thoroughly studied in two different generations.

1. Days to flowering: - Number of days taken from sowing to anthesis of the first flower was recorded.

2. Days to maturity: - Days to maturity were noted as the number of days taken from the date of sowing to the date of ripening of the first fruit.

3. Plant height (cm): - The height of the plant was measured at the time of final harvest from the base up to the apex of the plant.

4. Number of primary branches / plant: - The branches that arise from the main stem were recorded as primary branches and counted at the time of final harvest.

5. Number of secondary branches / plant: - The branches that arise from the primary branches were recorded as secondary branches and counted at the time of final harvest.

6. Number of fruits / plant: - The red ripened fruits picked in each harvest were counted and the total of all the harvests was taken as the number of fruits per plant.

7. Weight of fresh fruit (g): - The ripe fruits were weighed immediately after the harvest. Weight of twenty fresh ripe fruits per plant was taken.

8. Length of the fruit (cm): - Five fruits from each plant were selected at random from the second and third harvest and the length of individual fruit was measured from the calyx end to the tip of the fruit and the mean was taken and expressed in centimeters.

9. Width of the fruit (cm): - The red ripe fruits, which were used for measuring the length of the fruits, were taken for measuring the width of the fruits. The broadest part of the fruit was measured in centimeters.

10. Total plant yield (g): - Plant yield was measured from the weight of total number of fruits harvested per plant and the yield of each plant was recorded in grams.

3. 4. 3. M_3 generation

For raising the M_3 generation of the mutagen treated population, the treatments of each chemical mutagen for each variety were selected, which gave the maximum total plant yield in M_2 generation. The selected treatments were 0.1 and 0.2 per cent EMS and 0.01 and 0.02 per cent SA in the variety Ujwala and 0.2 and 0.3 per cent EMS and 0.02 and 0.03 per cent SA in the variety Co-1. All the selected M_2 progenies of each treatment together with controls were sown in plant progeny rows. The spacing was the same as followed in M_2 generation. The experiments were conducted in Completely Randomized Block Designs (CRBD) with three replicates. The plants showing morphological variations were discarded from each progeny. Observations were recoded for the quantitative characters from the normal looking plants and these plants were harvested individually at maturity.

3. 5. Capsaicin estimation

The estimation of capsaicin content in some selected mutants was carried out at the Division of Biochemistry, Indian Institute of Spices Research (IISR), Marikunnu, Calicut. The dried chilli was powdered and then extracted with ethyl alcohol. The extract was concentrated and the resulting concentrate was subjected to High Performance Liquid Chromatography (H P L C) using Shimadzu H P L C system, L C - 6A pump and SPD 6A UV detector. The C 18 chromatographic column; 25 cm x 4.6 mm id., 5 µm particle size, 40% acetonitrile and 60% DI - H_2O with 1% acetic acid (v/v) as mobile phase and a variable wavelength UV spectrophotometric detector set at 280nm. The data was compiled using Winchrom software.

3. 6. Statistical analysis

Data collected for ten quantitative characters in M_2 and M_3 generations were subjected to statistical analysis in order to assess the extent of induced variation, as indicated below.

3. 6. 1. Mean ($\overline{X}$)

The mean was computed by taking the sum of the number of values ($X_1 + X_2 + \ldots\ldots X_n$) and dividing the total number of values (N) involved, thus:

$$X = \frac{(X_1 + X_2 + \ldots\ldots X_n)}{N}$$

Or, $$\overline{X} = \frac{\Sigma X_n}{N}$$

Where, X_1, X_2.....X_n = observations

and N = total number of observations involved.

3. 6. 2. Standard error (S.E.)

$$S.E. = \frac{S.D. \text{ of Sample}}{\sqrt{N}}$$

Where, S.D = Standard deviation.

N = Number of observations.

3. 6. 3. Genetic Parameters

Analysis of variance was done according to Singh and Chaudhary (1985) to find out the variance between the families and within the families of plant progenies.

The Components of variance considered were:

Within – family variation in the control and in the treated material which was an estimate of environmental variation.

Between – family variation which was an estimate of the between family genetic variation.

3. 6. 3. 1. Genotypic Variance ($\sigma^2 g$)

The estimation of genotypic variance ($\sigma^2 g$) was done by the following way:

$$\sigma^2 g = \frac{(MsBf) - (MSe)}{N}$$

Where, (MsBf) = Mean sum of squares for between families.

MSe = Mean sum of squares for within families or error.

N = Number of replication.

3. 6. 3. 2. Genotypic coefficient of variation (CVg %)

$$CVg\ (\%) = \frac{\sqrt{(\sigma^2 g)}\ x\ 100}{\overline{X}}$$

$\sigma^2 g$ = Genotypic variance

$\overline{X}$ = Mean

3. 6. 3. 3. Phenotypic Variance (σ^2 p)

Phenotypic variance was estimated by summing the estimated genotypic variance ($\sigma^2 g$) to the environmental variance (MSe or $\sigma^2 e$).

$$\sigma^2 p = \sigma^2 g + \sigma^2 e.$$

3. 6. 3. 4. Phenotypic coefficient of variation (CVp %)

$$CVp\ (\%) = \frac{\sqrt{(\sigma^2 p)}\ x\ 100}{\overline{X}}$$

$\sigma^2 p$ = Phenotypic variance

$\overline{X}$ = Mean

3. 6. 3. 5. Heritability (h^2)

It is the ratio of the genotypic variance to the total phenotypic variance. The broad sense heritability (h^2) was estimated by the formula suggested by Johnson *et al* (1955).

$$h^2(\%) = \frac{\sigma^2 g \times 100}{\sigma^2 t}$$

Where, $\sigma^2 g$ = induced genotypic variance.

$\sigma^2 t$ = total phenotypic variance.

($\sigma^2 t = \sigma^2 g + \sigma^2 e$) calculated from the treated populations.

3. 6. 3. 6. Genetic advance (Gs)

The estimates of genetic advance (Gs) with 1% selection intensity were based on the formula derived by Allard (1960) and modified by Khan (1979).

$$Gs = K \sigma^2 p \, h^2$$

h^2 = broad sense heritability

$\sigma^2 p$ = phenotypic standard deviation of the mean performance of treated population.

K= 2.64 constant for 1% selection intensity.

$$Gs\ (\%\ of\ \overline{X}) = \frac{Gs \times 100}{\overline{X}}$$

3. 5. 3. 7. Test of significance

The comparison of the means of various treatments and control was carried out by the least significance difference (L. S. D) method suggested by Khan and Khanum (1994).

$$LSD = t\alpha \frac{\sqrt{2S^2}}{n}$$

$t\alpha$ = 't' value at 0.05 or 0.01 level of probability

S^2 = residual mean square.

n = number of replicates.

If the difference between the control mean and the treatments is greater than the calculated L. S. D. value obtained at 5% or 1% level, the difference between the two means is taken to be significant at 5% and 1% level.

CHAPTER – 4

EXPERIMENTAL RESULTS

Seeds of Chilli (*Capsicum annum* L.) varieties Ujwala and Co -1 were treated with different concentrations of potent chemical mutagens *viz.* ethyl methanesulphonate and sodium azide. Various types of biological damages in M_1 generation and induced variability of ten quantitative characters in M_2 and M_3 generation were observed. Capsaicin content of some selected mutants was also estimated by HPLC method.

4.1. M_1 Generation

The effect of chemical mutagens on biological activities *viz.* inhibition of seed germination, seedling injury, cotyledonary abnormalities, pollen sterility and meiotic chromosomal abnormalities were studied in M_1 generation.

4. 1.1. Seed germination

The data recorded on seed germination are presented in Table 1. A gradual decrease was observed in seed germination with the increasing concentrations of mutagens in both the varieties. Both the varieties responded differently to various mutagenic treatments. In variety Ujwala, the control seeds showed 83.33% germination. The different concentrations of EMS caused a gradual decrease in seed germination, it ranged from 78.89% with the lowest concentration (0.1%) and 66.67% with the highest concentration (0.3%) of EMS. The other variety (Co -1) also behaved more

Table 1. Effect of mutagens on seed germination in M_1 generation

Treatment	Var. Ujwala		Var. Co - 1	
	Percentage of seed germination	Percentage of inhibition	Percentage of seed germination	Percentage of inhibition
Control	83.33	--	82.50	--
0.1% EMS	78.89	5.33	75.00	9.09
0.2% EMS	73.33	12.00	65.00	21.21
0.3% EMS	66.67	19.99	55.50	32.72
0.01% SA	76.66	8.00	70.00	15.15
0.02% SA	71.11	14.66	60.00	27.27
0.03% SA	66.66	20.00	48.50	41.12

or less in the similar way, with germination ranging from 75% to 55.5% after EMS treatment. In the variety Ujwala, the percentage of seed germination ranged from 76.66% to 66.66% with SA treatments. Similarly, in the variety Co -1, it declined from 70.00% to 48.5% after treatment with SA.

The percentage of inhibition also increased from lower to higher concentrations of mutagenic treatments in both the varieties. It was highest (41.12%) at 0.03% SA treatment in Variety Co -1. Both the varieties showed the highest percentage of inhibition in seed germination in SA treatments. The percentage of inhibition in variety Ujwala with EMS treatments ranges from 5.33% to 19.99% whereas, in variety Co -1 it was 9.09% to 32.72%. In the variety Ujwala, the SA treatments caused the percentage inhibition from 8.00% to 20.00%, however, in Co -1, it was 15.15% to 41.12%.

A dose dependent inhibition in seed germination was noticed in both the varieties. Compared to EMS treatments, SA treatments exhibited more severe effect. The variety Co -1 showed greater sensitivity to the mutagenic treatments.

4. 1. 2. Seedling Growth

The results of growth of seedlings presented in Tables 2 and 3, show that all the mutagenic treatments caused a reduction in seedling growth. Sodium azide treatment exhibited more pronounced reduction in seedling growth than the EMS treatment. The variety Ujwala was relatively more sensitive with respect to the effect of seedling growth.

Table 2. Growth of seedlings raised from EMS treated seeds in M_1 generation

Variety	Treatment	Length in cm			Percentage of injury
		Root Mean ± S. E.	Shoot Mean ± S. E.	Total Mean ± S. E.	
Ujwala	Control	3.14 ± 0.22	2.59 ± 0.08	5.73 ± 0.30	--
"	0.1%	2.60 ± 0.19	2.12 ± 0.07	4.72 ± 0.26	17.63
"	0.2%	2.30 ± 0.23	1.92 ± 0.07	4.22 ± 0.30	26.35
"	0.3%	1.86 ± 0.17	1.61 ± 0.09	3.47 ± 0.26	39.44
Co -1	Control	3.79 ± 0.07	2.81 ± 0.05	6.60 ± 0.12	--
"	0.1%	3.63 ± 0.07	2.30 ± 0.10	5.93 ± 0.17	10.15
"	0.2%	3.01 ± 0.14	2.02 ± 0.07	5.03 ± 0.21	23.79
"	0.3%	2.80 ± 0.17	1.89 ± 0.09	4.69 ± 0.26	28.94

Table 3. Growth of seedlings raised from SA treated seeds in M_1 generation

Variety	Treatment	Length in cm			Percentage of injury
		Root Mean ± S. E.	Shoot Mean ± S. E.	Total Mean ± S. E.	
Ujwala	Control	3.14 ± 0.21	2.59 ± 0.08	5.73 ± 0.29	--
"	0.01%	2.00 ± 0.09	2.46 ± 0.06	4.46 ± 0.15	22.16
"	0.02%	1.80 ± 0.07	2.21 ± 0.07	4.01 ± 0.14	30.01
"	0.03%	1.60 ± 0.14	1.63 ± 0.09	3.23 ± 0.23	43.63
Co -1	Control	3.79 ± 0.07	2.81 ± 0.05	6.60 ± 0.12	--
"	0.01%	3.21 ± 0.07	2.20 ± 0.07	5.41 ± 0.14	18.03
"	0.02%	2.97 ± 0.07	1.89 ± 0.14	4.86 ± 0.21	26.36
"	0.03%	2.68 ± 0.16	1.60 ± 0.12	4.28 ± 0.28	35.15

The percentage of injury in seedling growth after EMS treatments ranges from 17.63 to 39.44 in the variety Ujwala and in the variety Co -1, it was 10.15 to 28.94. In SA treatments the percentage injury ranges from 22.16 to 43.63 and 18.03 to 35.15 in variety Ujwala and Co -1 respectively.

As in the case of seed germination, the seedling growth also showed a dose dependent injury in both the varieties. However, in contrast to the seed germination, the variety Ujwala showed more sensitivity to seedling injury compared to the other variety Co -1.

4. 1. 3. Cotyledonary abnormalities

The frequency of cotyledonary abnormalities is shown in Table 4. Cotyledonary abnormalities such as seedlings with splitted cotyledonary leaves, three cotyledonary leaves (Plate 1) and cotyledonary leaves with white spots and cotyledonary leaves with yellow streaks were recorded in M_1 generation.

These abnormalities were recorded in EMS treated populations of both the varieties and SA treated populations of variety Co -1. The frequency of cotyledonary abnormalities was not dependent on the dose of mutagen. However, the higher concentration of EMS (0.3%) induced such abnormalities in both the varieties. The higher concentration of SA (0.03%) caused cotyledonary abnormality (2.33%) only in the variety Co -1.

In the variety Co -1, all the concentrations of EMS i.e., 0.1%, 0.2% and 0.3% induced cotyledonary abnormalities at the rate of 2.22%, 2.75%

Table 4. Frequency (%) of cotyledonary abnormalities in M_1 generation

Treatment	Var. Ujwala	Var. Co -1
Control	--	--
0.1% EMS	--	2.22 (CLWS)
0.2% EMS	--	2.75 (CLWS, CLYS)
0.3% EMS	2.75 (CLYS)	2.41(TCL, SCL)
0.01% SA	--	--
0.02% SA	--	--
0.03% SA	--	2.33 (TCL)

CLWS – Cotyledonary leaves with white spots, CLYS – Cotyledonary leaves with yellow streaks, TCL – Three cotyledonary leaves, SCL – Splitted cotyledonary leaves.

PLATE - 1

Fig 1. Splitted cotyledonary leaf

Fig 2. Tricotyledonary leaf

and 2.41% respectively whereas, in the variety Ujwala only the 0.3% EMS treatment caused the abnormalities in the cotyledons (2.75%). No such abnormalities were recorded in SA treatments, except in the variety Co -1 at 0.03% SA. The cotyledons of the seedlings in other treatments were as normal as those of control.

4. 1. 4. Pollen fertility

Pollen fertility reduced with increasing concentrations of both the mutagens showing a linear decrease of fertility on dose. About 90.65% and 93.06% pollen fertility was observed in control plants of Ujwala and Co -1 respectively (Table 5).

The highest percentage of reduction was recorded in EMS treatments. For variety Ujwala the percentage reduction ranged from 5.17% to 17.66% with EMS treatments whereas, in the variety Co -1, it ranged from 4.27% to 14.46%. In SA treatments it ranged from 2.17% to 11.90% in Ujwala and 1.91% to 8.97% in Co -1. The variety Ujwala was found to be more sensitive than Co -1.

4. 1. 5. Meiotic chromosomal abnormalities

Various types of meiotic chromosomal abnormalities with different frequencies were observed in M_1 generation in both the varieties after treatment with different concentrations of EMS. The frequency of meiotic chromosomal abnormalities is presented in tables 6 and 7 and plate 3 and 4. Meiosis was normal in control treatments. Multivalents, stickiness, clumping

Table 5. Effect of mutagens on pollen fertility in M_1 generation

Treatment	Var. Ujwala		Var. Co -1	
	Pollen fertility	Percentage reduction	Pollen fertility	Percentage reduction
Control	90.65	--	93.06	--
0.1% EMS	85.96	5.17	89.09	4.27
0.2% EMS	82.81	8.65	85.84	7.76
0.3% EMS	74.64	17.66	79.44	14.46
0.01% SA	88.68	2.17	91.28	1.91
0.02% SA	85.43	5.76	89.98	3.31
0.03% SA	79.86	11.90	84.71	8.97

Table 6. Frequency (%) of various induced meiotic abnormalities in M_1 generation of the var. Ujwala.

Treatment	Total PMC's scored	Diakinesis & Metaphase			Anaphase & Telophase				Total
		Mv	St	Clp	Br	Lg	Mnu	Nsyn	
Control	608	--	--	--	--	--	--	--	--
0.1% EMS	606	--	--	0.50	0.99	0.66	0.50	0.50	3.15
0.2% EMS	578	0.69	0.35	1.04	0.52	1.04	0.69	0.87	5.20
0.3% EMS	614	0.81	114	0.65	1.47	1.63	1.63	0.98	8.31
0.01% SA	589	--	--	--	--	--	--	--	--
0.02% SA	573	--	--	--	--	--	--	--	--
0.03% SA	557	--	--	--	--	--	--	--	--

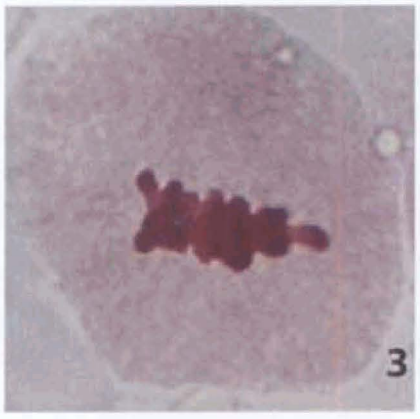
PMC – Pollen Mother Cell, Mv – Multivalent, St – Stickiness, Clp – Clumping, Br – Bridges, Lg – Laggards, Mnu – Micronuclei, Nsyn – Non synchronous separation.

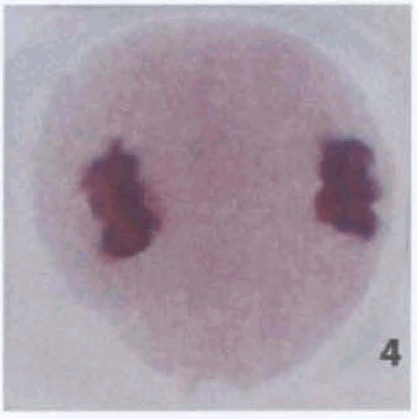
Table 7. Frequency (%) of various induced meiotic abnormalities in M_1 generation of the var. Co -1.

Treatment	Total PMC's scored	Diakinesis & Metaphase			Anaphase & Telophase						Total
		Mv	St	Clp	Br	Lg	Mnu	Tro	Ptd	Nsyn	
Control	610	--	--	--	--	--	--	--	--	--	--
0.1% EMS	594	--	0.34	0.51	0.51	0.17	0.17	--	--	0.34	2.04
0.2% EMS	630	0.32	--	0.32	0.78	0.63	0.48	0.32	--	0.63	3.48
0.3% EMS	586	0.68	0.68	0.51	1.02	1.37	0.34	0.68	0.68	0.34	6.30
0.01% SA	621	--	--	--	--	--	--	--	--	--	--
0.02% SA	578	--	--	--	--	--	--	--	--	--	--
0.03% SA	542	--	--	--	--	--	--	--	--	--	--

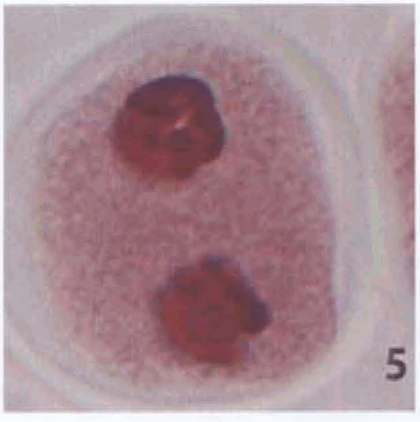
PMC – Pollen Mother Cell, Mv – Multivalent, St – Stickiness, Clp – Clumping, Br – Bridges, Lg – Laggards, Mnu – Micronuclei, Tro – Tripolar orientation, Ptd – Pentad, Nsyn – Non synchronous separation.

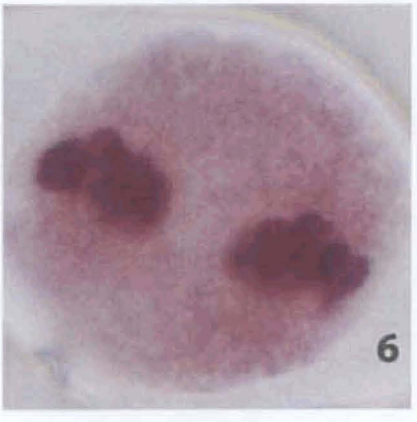
PLATE - 2



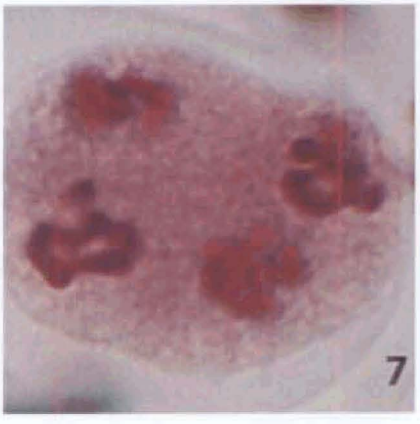


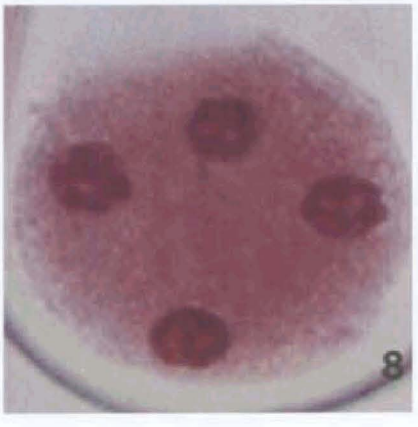
Figs 3 - 4. Normal metaphase I and anphase I





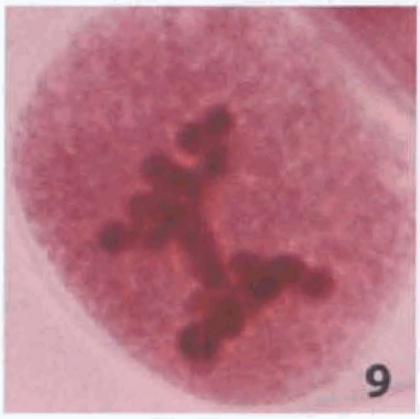
Figs 5 - 6. Normal telophase I and metaphase II

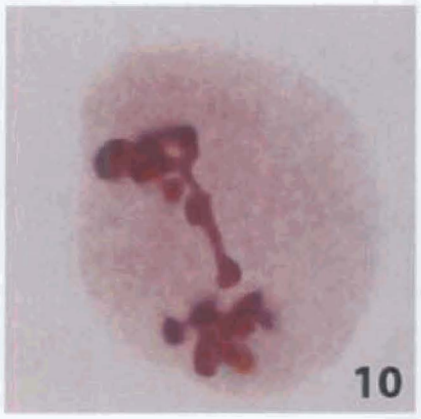


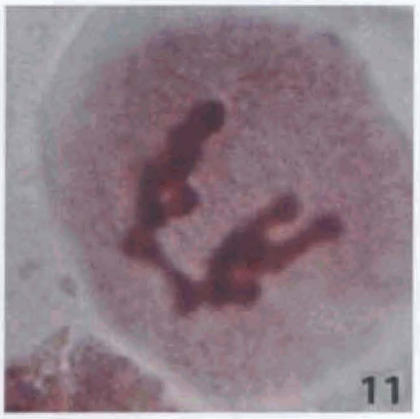


Figs 7 - 8. Normal anaphase II and telophase II

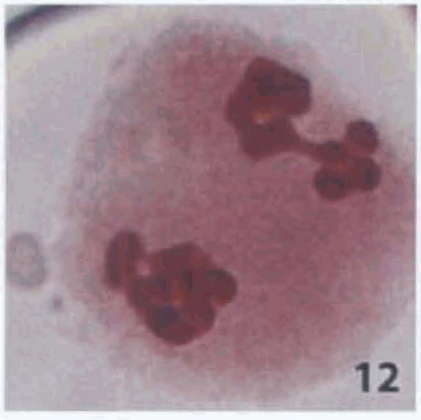
PLATE - 3

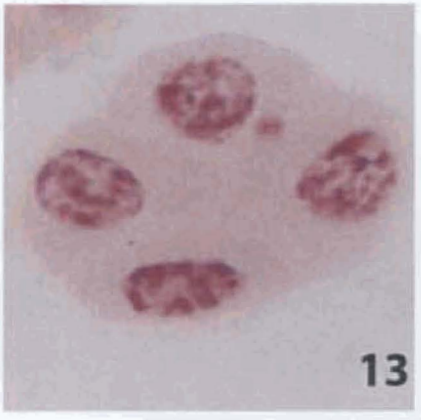






Figs 9 -11. Chromosome bridges at anaphase I





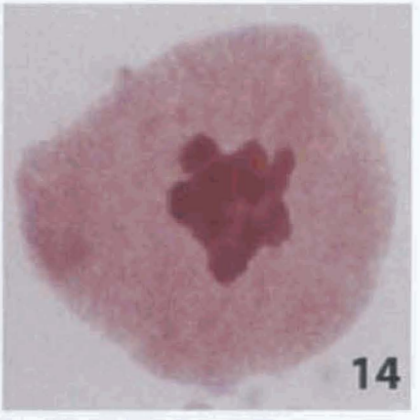
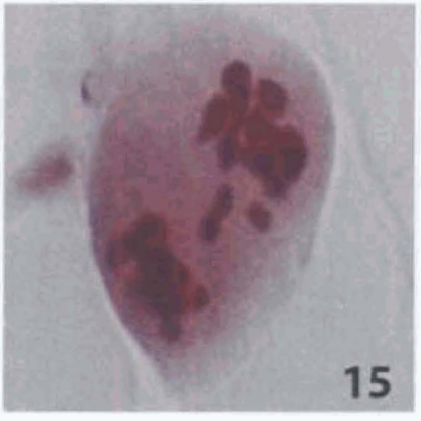
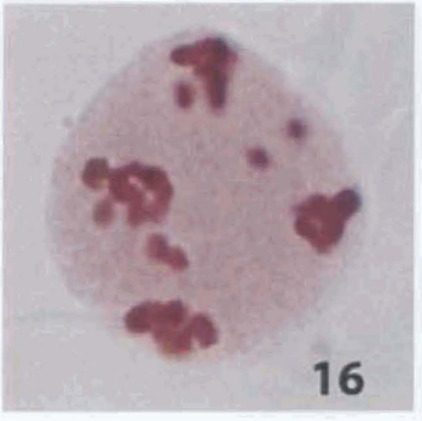
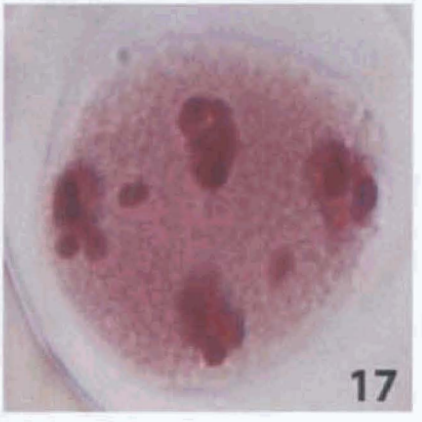


Fig 12.Bridge at anaphase II **Fig 13. Micronucleus** **Fig 14. Clumping**

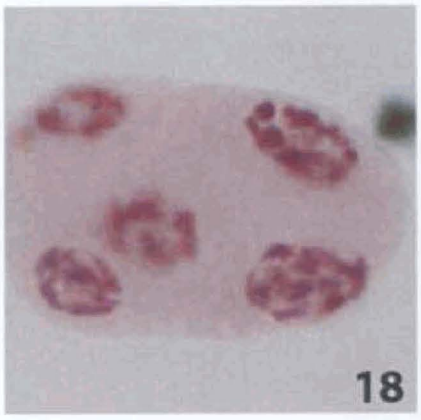


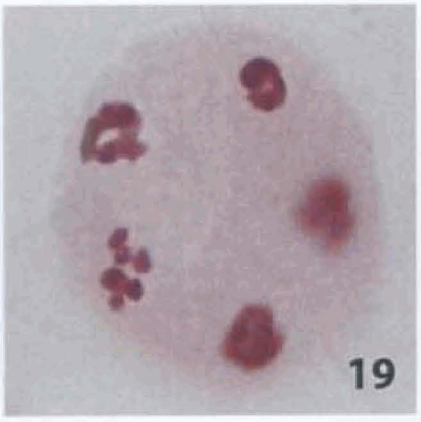




Figs 15 - 17. Laggards at anaphase I and anaphase II

PLATE - 4





Figs 18 - 19. Pentads

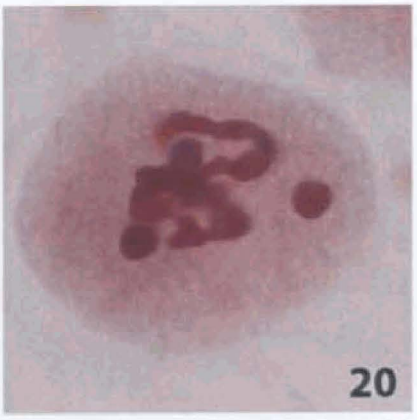
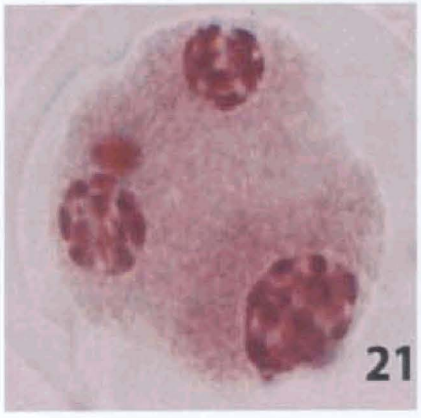
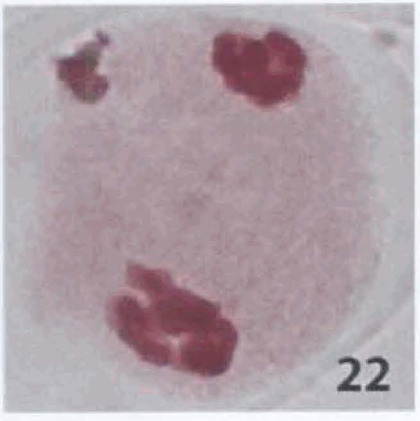


Fig 20. Stickiness





Figs 21 - 22. Tripolar orientation

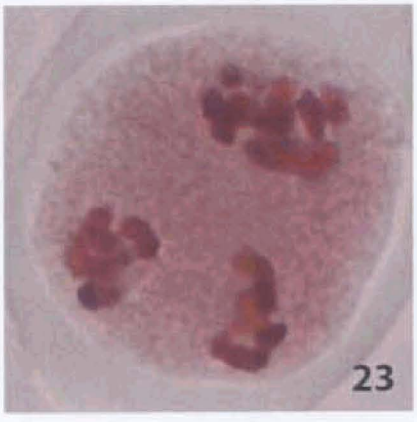
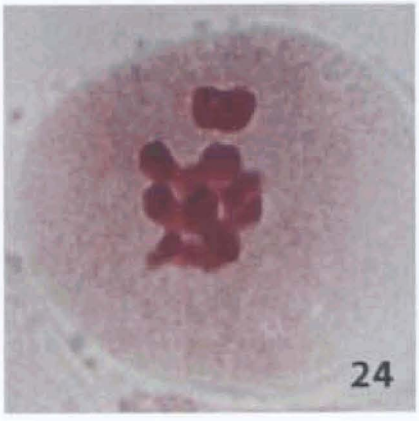
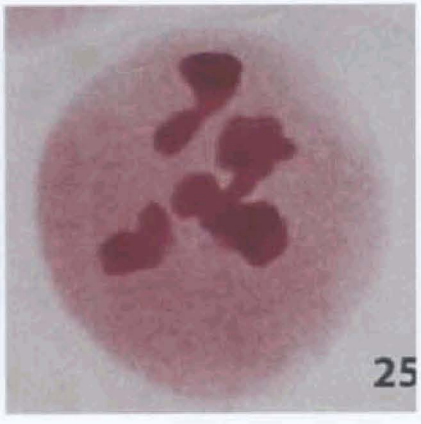
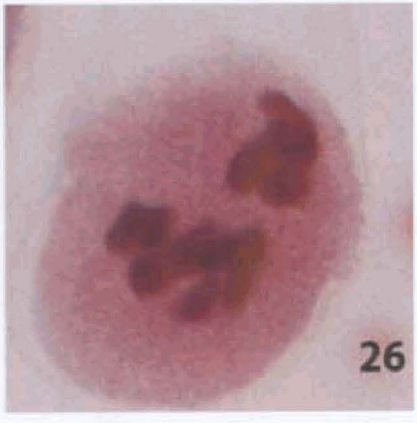


Fig 23. Non - synchronous separation







Figs 24-26. Multivalents

bridges, laggards, micronuclei and non – synchronous separation were recorded in EMS treatments of the variety Ujwala. In variety Co - 1, tripolar orientation of chromosomes and pentads were also observed in addition to the above mentioned aberrations. The frequencies of chromosomal abnormalities increased with the increase in mutagenic concentrations. The total frequencies of abnormalities were 3.15%, 5.2% and 8.31% in the variety Ujwala and 2.04%. 3.48% and 6.30% in the variety Co – 1 in 0.1%, 0.2% and 0.3% EMS treatments respectively. Moreover, varietal response to the chromosomal aberrations was very pronounced, *i.e.* the variety Ujwala was more sensitive and the frequency of aberrations was comparatively high at all the mutagen doses. The frequency of such aberrations was comparatively low in the variety Co – 1. Stickiness, clumping of chromosomes, bridges, laggards and micronuclei were observed in higher frequencies in almost all the treatment doses in both the varieties. However, all the SA treatments failed to induce chromosomal aberrations in PMC's in both the varieties , when compared to the control (plate 2).

4. 2. M_2 Generation

4. 2. 1. Chlorophyll mutations

Chlorophyll mutations were recorded in M_2 generation when seedlings were 10 to 20 days old. A total of four types of chlorophyll mutants (Albina, Xantha, Viridis and Striata) were noticed at seedling stage from the M_2 segregating progenies of both the genotypes viz., Ujwala and Co -1. The fifth

type of chlorophyll mutant (Maculata) was obtained only at higher concentration of EMS treatment. It was observed that in many cases the seedling, which initially looked to be normal, started showing different types of chlorophyll deficiencies at the later stage of growth. At the initial stage, the growth of the seedling was normal but later all the chlorophyll deficient mutants were found to be lethal except viridis, which showed stunted growth and produced few fruits (Plate 5). A brief description of these chlorophyll mutants is given below:

Albina: The seedlings with white leaves and lack chlorophyll. The mutation is lethal and survived only for about 13 to 22 days after germination or two-leaf stage.

Xantha: It is also a lethal mutation and survived for 17 to 25 days. Leaves have little or no chlorophyll and yellow in colour.

Viridis: The leaf size was reduced but leaf shape remains unaltered. The plants were slow growing and had a reduced size and produced few small fruits. The mutant was distinguished because of its reduced height and viridine green colour of leaves.

Striata: Seedling with yellow or white longitudinal bands and spots. They showed slow growth and survived 20 to 31 days.

Maculata: Seedlings showed yellow or whitish patches on the surface of leaves. These mutants survived only for about 20 – 26 days.

Table 8. Frequency (%) of chlorophyll mutation in M_2 generation after mutagenic treatment

Treatment	Var. Ujwala				Var. Co -1				
	Albina	Xantha	Viridis	Striata	Albina	Xantha	Viridis	Striata	Maculata
Control	--	--	--	--	--	--	--	--	--
0.1% EMS	--	--	--	--	1.01	--	--	--	--
0.2% EMS	--	--	--	--	1.09	--	--	0.55	--
0.3% EMS	1.09	--	1.01	6.59	--	2.41	--	1.81	5.42
0.01% SA	--	--	--	--	--	--	--	--	--
0.02% SA	--	--	--	--	--	--	--	--	--
0.03% SA	--	2.56	1.92	--	--	1.16	2.33	--	--

PLATE - 5

Fig 27. Albina seedling

Fig 28. Viridis and Normal seedlings

4. 2. 2. Frequency and spectrum of chlorophyll mutations

Mutations for chlorophyll defects are largely of theoretical interest in crop plants. Five different types of chlorophyll mutants were observed and their frequency was summarized in table 8. The chlorophyll mutation frequency was calculated on M_2 plant basis. The variety Co -1 has shown the greater frequency of chlorophyll mutation as compared to the variety Ujwala. The frequency of chlorophyll mutations was found to be higher at higher concentrations of both the mutagens. The different chlorophyll mutant types observed were albina, xantha, viridis, striata and maculata. In the variety Ujwala only the higher concentration of mutagens caused the chlorophyll mutations, however, in variety Co -1, all the EMS treatments induced such mutations. It indicated that the EMS is very effective in inducing chlorophyll mutations than SA.

4. 2. 3. Morphological mutations in M_2 and M_3 generations

The phenotypically visible, easily perceivable genetic alterations were observed to recover desirable mutations in M_2 and M_3 generation. The controls as well as the mutagenized populations were screened for all phenotypically detectable mutations at different stages of growth in both the varieties. Twelve different types of morphological mutants were isolated from M_2 and M_3 populations. The frequency of different mutants observed is summarized in Table 9. These mutants were categorized on the basis of the trait affected. The characteristic features of such mutants are described below: -

Tall mutants (high yielding): These mutants were isolated from 0.3% EMS treated M_3 population of the variety Co –1 (Fig 43). These mutants were considerably taller than the control plants, their mean height was 82.7 cm where as, it was 51.49 cm for the control plants. They produced normal sized fruits and appeared at a frequency of 13.63% of the total morphological mutations.

Tall with short fruit mutants: These mutants were isolated from the 0.2% EMS treated M_3 population of the variety Co –1 (Fig 42). These plants were characterized by the production of large number of short fruits with an average weight of 2 gm and size of 6.8 cm long and 0.9 cm width. The mean height of these mutants was 70.45 cm. Their frequency was found be 9.09%.

Tall with crinkled fruit mutants: These mutants were appeared in 0.03% SA treated M_2 population of the variety Co – 1 (Fig 47). The mean height of this mutant was 68.73 cm whereas, it was 49.59 cm in the control plants. These mutants produced normal fruits but showed crinkled pericarp. These were low yielding when compared to the control plants. Their frequency was found to be 9.09%.

Dwarf mutants: These mutants appeared in 0.3% EMS treated M_2 population of the variety Ujwala (Fig 33). These mutants were conspicuous by the short stature and reduced yield components. The mean height of the control was 72.83 cm whereas, it was 48.12 cm in the dwarf mutants. Their frequency was 13.63% of the total morphological mutations.

Short fruit mutants: These mutants were observed in 0.3% EMS treated M_2 population of the variety Co –1 (Fig 45). The average weight of the fruit was 2.75 gm. The mean fruit length and fruit width were 6.0 cm and 1.0 cm respectively. The frequency of occurrence of such mutants was 11.36% of the total morphological mutants.

Erect fruit mutants: These mutants were recovered from the 0.2% EMS treated M_3 population of the variety Co - 1 (Fig 40). The fruits of these mutants were erectly oriented. The number of branches and yield were considerably reduced. The frequency of this mutation was 13.63% of the total morphological mutations.

Yellow slender fruit mutants: These mutants were screened from the 0.01% SA treated M_3 population of the variety Co – 1 (plate 10). They produced greenish yellow slender fruits with viable seeds. The average fruit weight was 1.75 gm and fruit length and width, 8.00 cm and 0.78 cm respectively. The frequency of such mutants was 4.55% of the total morphological mutants.

Long thick fruit mutants: The fruits of this type of mutants are comparatively longer and thicker (Fig 44). They appeared in the 0.02% SA treated M_2 population of the variety Co -1. The average fruit length was 11.52 cm and fruit width, 1.7 cm. The mean fruit weight was 7.00 gm whereas, 4.3 gm in control plants. The frequency of such mutants was 11.36% of the total morphological mutants.

Long slender fruit mutants: These mutants were recovered from 0.02% SA treated M_2 population of the variety Co –1 (Fig 48). They produced longer fruits with an average fruit length of 10.98 cm and width of 1.12 cm. These mutants were low yielding when compared to the control plants. They appeared at a frequency of 4.55% of the total morphological mutants.

Seedless mutants: Two seedless mutants were isolated from the 0.03% SA treated M_2 population of the variety Ujwala (plate 8). The growth of these mutants was normal but produced small sized fruits with aborted seeds. The average fruit weight was only 1.5 gm and fruit length and width, 4.34 cm and 0.84 cm respectively. The frequency of these mutations was 4.55% of the total morphological mutants.

Non-viable mutant: One mutant, which failed to produce fruits was noticed in the 0.03% SA treated M_2 population of the variety Co –1 (Fig 46). The mutant showed normal growth habit but produced less number of branches and flowers. The flower development was very late, but the anthers produced 58% viable pollen grains. The frequency of this mutant was 2.27%.

Viable chlorophyll deficient mutant: One viridis chlorophyll deficient mutant, which was isolated from the 0.2% EMS treated M_2 population of the variety Co – 1 was grown to maturity, flowered and produced viable seeds (Fig 41). This mutant showed slow growth rate with viridine green colour of leaves. The fruit size was comparatively smaller than the control. The mean fruit weight was only 2.31 gms and the fruit length and width, 3.48 cm and

Table 9. Frequency (%) of morphological mutants following chemical mutagenesis

Sl. No.	Mutant type	Mutagen	Variety	Generation	Frequency
1	Tall mutant (high yielding)	0.3% EMS	Co -1	M_3	13.63
2	Tall with short fruit mutant	0.2% EMS	Co -1	M_3	9.09
3	Tall with crinkled fruit mutant	0.03% SA	Co -1	M_2	9.09
4	Dwarf mutant	0.3% EMS	Ujwala	M_2	13.63
5	Short fruit mutant (high yielding)	0.3% EMS	Co -1	M_2	11.36
6	Erect fruit mutant	0.2% EMS	Co -1	M_3	13.63
7	Yellow slender fruit mutant	0.01% SA	Co -1	M_3	4.55
8	Long thick fruit mutant	0.02% SA	Co -1	M_2	11.36
9	Long slender fruit mutant	0.02% SA	Co -1	M_2	4.55
10	Seedless mutant	0.03% SA	Ujwala	M_2	4.55
11	Non viable mutant	0.03% SA	Co -1	M_2	2.27
12	Viable chlorophyll deficient mutant	0.2% EMS	Co -1	M_2	2.27

PLATE - 6

Figs 29 - 31 : Sections of the experimental field

PLATE - 7

Fig 32. Var. Ujwala - High yielding mutant

Fig 33. Var. Ujwala - Dwarf mutant

PLATE - 8

Fig 34. Var. Ujwala - Seedless mutant

Fig 35. Var. Ujwala-L.S.of Seedless fruits

PLATE - 9

Fig 36. Var. Co - 1 - Control plant

Fig 37. Var. Ujwala - Control plant

PLATE - 10

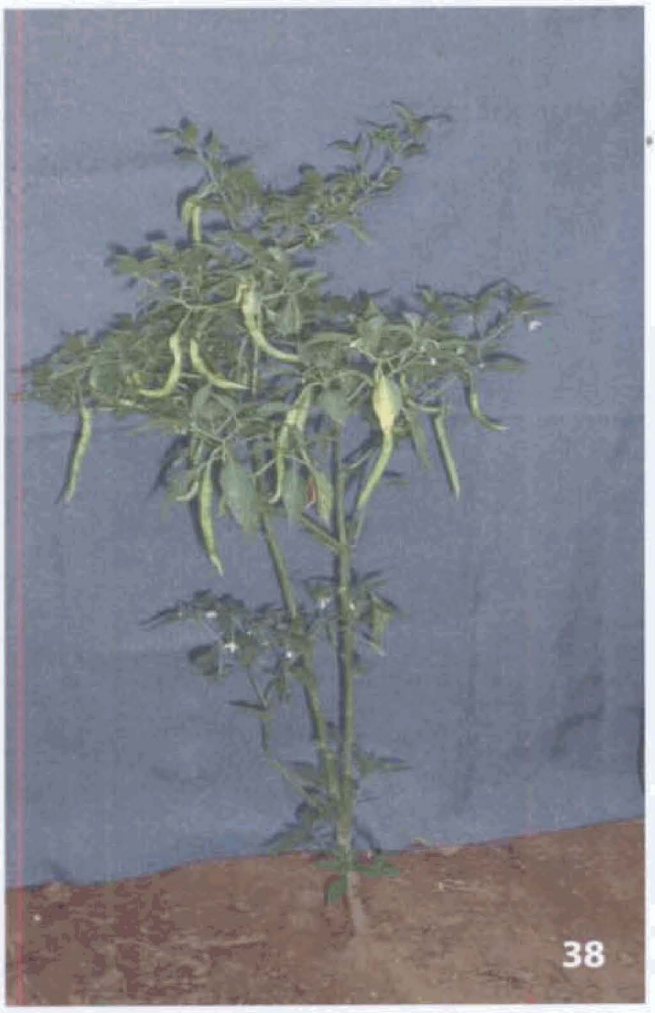


Fig 38. Var. Co - 1 - Yellow slender fruit mutant

39

Fig 39. Normal and yellow slender mutant fruits

Fig 40. Var. Co - 1 - Erect fruit mutant

Fig 41. Var. Co - 1 - Viable chlorophyll deficient mutant

Fig 42. Var. Co - 1 - Tall with short fruit mutant

Fig 43. Var. Co - 1 - Tall mutant

PLATE - 13

Fig 44. Var. Co - 1 - Long thick fruit mutant

Fig 45. Var. Co - 1- Short fruit mutant

PLATE - 14

Fig 46. Var. Co - 1 - Non - viable mutant

Fig 47. Var. Co - 1 - Crinkled fruit mutant

PLATE-15

Fig 48. Var. Co - 1 - Long slender fruit mutant

Fig 49 . Var. Co - 1 - High yielding mutant

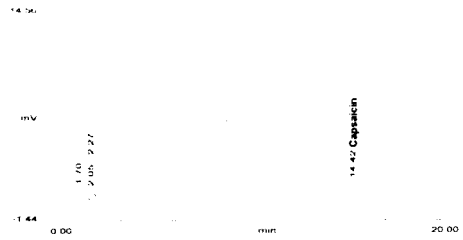


Fig 50. HPLC chromatogram of standared capsaicin

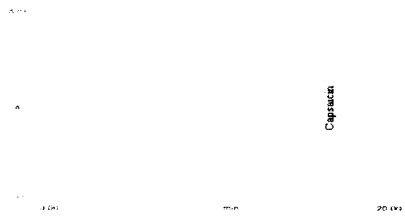


Fig 51. Var. Co - 1 - Control plant

Fig 52. Long thick fruit mutant

Fig 53. Tall with short fruit mutant

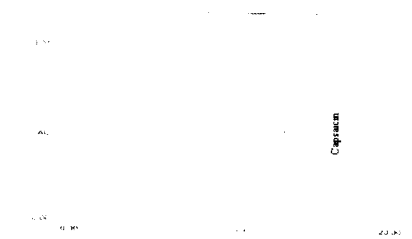


Fig 54. Short fruit mutant - High yielding

Fig 55. Viable chlorophyll deficient mutant

Fig 56. Tall high yielding mutant

Table 10. Capsaicin content of some selected mutants

Sl. No.	Variety/Mutant	SHU Total	% of capsaicin
1	Co -1	40667.50	0.25
2	Tall with short fruit mutant	46818.06	0.30
3	Long thick fruit mutant	7236.12	0.05
4	Short fruit (high yielding) mutant	33797.80	0.21
5	Viable chlorophyll deficient mutant	1517.00	0.01
6	Tall high yielding mutant	33266.20	0.20

SHU = Scoville Heat Unit

0.99 cm respectively. The frequency of this mutant was found to be 2.27% of the total morphological mutations.

4.3. Capsaicin content

Table 10 shows the capsaicin content and pungency in SHU (Scoville Heat Unit) of variety Co -1 and it's five selected mutants. No effective morphological mutants could be isolated from the variety 'Ujwala' (Table 9). The tall with short fruit mutant contains maximum capsaicin (0.30%) and pungency at the rate of 46818.06 SHU. There was no much increase in capsaicin content in other mutants. The long thick fruit mutant and viable chlorophyll deficient mutant contain very low percentage of capsaicin. The long thick fruit mutant contains only 0.05% capsaicin and pungency of 7236.12 SHU. The viable chlorophyll deficient mutant has 0.01% capsaicin and pungency of 1517.00 SHU. Short fruit mutant (high yielding) and tall high yielding mutant contain 0.21% capsaicin with 33797.80 SHU and 0.20% capsaicin with 33266.20 SHU respectively (plate 16).

4. 4.Quantitative characters

Genetic variability induced by the chemical mutagens in two varieties *viz*, Ujwala and Co -1 of *Capsicum annum* L. in M_2 generation was estimated. Attempts were made to isolate micro mutations using statistical methods for ten polygenic traits. In each treatment, statistical analysis was made to determine mean, shift in mean, phenotypic and genotypic

coefficients of variation, heritability and genetic advance (as percentage of mean). The comparison of the mean values of various treatments and control was carried out by the least significant difference method (L. S. D) method. If the difference between the control and the treatment mean values is greater than the calculated L. S. D value, it is taken to be significant and noted in tables 11 – 30.

(I) Days to flowering

Estimates of mean values, shift in mean, phenotypic and genotypic coefficient of variation, heritability and genetic advance for days to flowering for treated and control populations are given in tables 11 and 12. Significant early flowering was observed in both the varieties except at 0.01% SA treatment in the variety Ujwala. The mean shifted in negative direction in all the treatments except at 0.01% SA treatment in the variety Ujwala. The mean days to flowering reduced approximately 3 to 8 days in treated population.

The phenotypic and genotypic coefficients of variation increased in the treated population. The increase in these two values was not in linear order with respect to the concentration of both the mutagens. The highest value of phenotypic and genotypic coefficients of variability (6.69% and 6.11%) was observed in the variety Ujwala with 0.1% EMS treatment. The EMS treatments were most effective in inducing high value of genotypic and phenotypic variability than SA treatments in both the varieties.

Table 11. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to flowering in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	102.50 ± 0.44	--	2.92	1.34	21.20	1.63	--
0.1% EMS	94.63 ± 1.21	-7.87	6.69	6.11	83.60	14.76	1% = 2.910
0.2% EMS	93.67 ± 0.80	-8.83	4.43	3.89	77.20	9.03	5% = 1.921
0.3% EMS	96.17 ± 1.09	-6.33	5.76	5.58	88.00	13.39	--
0.01% SA	104.21 ± 1.09	+1.71	5.40	4.11	58.00	8.26	1% = 2.835
0.02% SA	98.70 ± 0.82	-3.80	4.30	2.23	27.00	3.07	5% = 1.872
0.03% SA	97.96 ± 0.10	-4.54	5.13	2.32	20.40	2.76	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 12. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to flowering in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	90.41 ± 0.33	--	0.47	1.35	12.07	0.41	--
0.1% EMS	85.63 ± 0.72	-4.78	4.14	2.99	52.43	5.73	1% = 3.712
0.2% EMS	86.65 ± 0.40	-3.76	2.35	2.07	77.59	4.81	5% = 2.450
0.3% EMS	85.29 ± 0.50	-5.12	3.11	2.43	61.10	5.01	--
0.01% SA	86.46 ± 0.48	-3.95	2.87	1.81	39.90	3.02	1% = 2.763
0.02% SA	86.22 ± 0.31	-4.19	1.85	1.26	46.38	2.26	5% = 1.824
0.03% SA	85.39 ± 0.36	-5.02	2.17	1.65	57.43	3.29	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

The heritability also increased in the treated population except at 0.03% SA treatment in the variety Ujwala. The highest heritability estimates, 83.60%, 77.20%, and 88.00% were observed at 0.1%, 0.2% and 0.3% EMS treatments respectively, in the variety Ujwala. In variety Co -1, the highest values *i.e.* 77.59% and 61.10% were recorded with 0.2% and 0.3% EMS treatments.

The genetic advance also increased after the mutagenic treatments in both the varieties. The highest value of genetic advance was estimated with 0.1% and 0.3% EMS treatments. The genetic advance value of the variety Ujwala with 0.1% and 0.3% EMS treatments was 14.76% and 13.39% respectively. Where as, in the variety Co -1, the genetic advance was found to be 5.73% and 5.01% respectively.

(2) Days to maturity

The data recorded on days to maturity are summarized in Tables 13 and 14. The analysis pertaining to the effects of the different mutagens upon days to maturity indicate that the mean values shifted towards the negative direction with 0.1% and 0.2% EMS treatments in the variety Ujwala. However, in all other treatments, except at 0.03% SA in the variety Ujwala, the mean values shifted towards the positive direction. In the variety Ujwala a significant earliness in maturity for about 4 days was observed with 0.2% EMS treatment whereas, in the variety Co -1 delayed maturity for about 2 to 3 days was noticed with 0.1% and 0.2% EMS treatments. Significant delay in

Table 13. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to maturity in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	136.29 ± 0.55	--	2.03	1.06	27.30	1.40	--
0.1% EMS	134.46 ± 1.07	-1.83	4.14	3.84	86.20	9.41	1% = 3.355
0.2% EMS	132.25 ± 0.87	-4.04	4.89	4.31	77.60	10.02	5% = 2.215
0.3% EMS	136.96 ± 0.86	+0.67	2.97	2.31	60.00	4.71	--
0.01% SA	142.25 ± 0.71	+5.96	2.57	2.01	61.20	4.17	--
0.02% SA	138.67 ± 0.91	+2.38	3.39	1.25	13.96	1.23	1% = 3.983
0.03% SA	136.29 ± 0.78	0.00	2.78	1.79	41.30	3.03	5% = 2.629

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 14. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to maturity in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	122.81 ± 0.37	--	1.52	1.16	58.96	2.36	--
0.1% EMS	124.89 ± 0.53	+2.08	2.22	2.03	84.14	4.92	1% = 1.377
0.2% EMS	126.61 ± 0.42	+3.80	1.79	1.44	64.00	3.03	5% = 0.909
0.3% EMS	123.57 ± 0.52	+0.76	2.21	1.96	78.80	4.59	--
0.01% SA	125.50 ± 0.43	+2.69	1.79	1.61	80.09	3.80	1% = 1.7941
0.02% SA	124.31 ± 0.35	+1.50	1.42	1.01	50.43	1.89	5% = 1.1843
0.03% SA	124.10 ± 0.37	+1.29	1.53	0.93	36.77	1.89	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

fruit maturity was observed with 0.01% SA treatment in the variety Ujwala and with all the SA treatments in the variety Co -1.

The phenotypic and genotypic coefficient of variation increased after the mutagen treatments except at 0.02% SA treatment in the variety Co -1. The highest phenotypic (4.14% and 4.89%) and genotypic (3.84% and 4.31%) was estimated at the 0.1% and 0.2% EMS treatments in the variety Ujwala.

Similarly, heritability values increased for all the mutagen treatments except for 0.02% SA treatment in the variety Ujwala and 0.02% and 0.03% SA treatments in the variety Co -1. The highest values of heritability were recorded at 0.1% EMS treatment in both the varieties. In the variety Ujwala 86.20% heritability was estimated at 0.1% EMS treatment while, it was 84.14% at the same mutagen concentration in the variety Co -1.

The genetic advance also increased in the treated population except at 0.02% SA treatment in the variety Ujwala and 0.02% and 0.03% SA in the variety Co -1. The highest values of genetic advance (9.41% and 10.02%) in the variety Ujwala was noticed at 0.1% and 0.2% EMS treatment and in the variety Co-1 (4.92% and 4.59%) at 0.1% and 0.3% EMS treatments.

(3) Plant height

The tables 15 and 16 present the data recorded on plant height. It clearly reveals that the mutagen treatments induced considerable variation on plant height. The mean values shifted to the negative direction in the

variety Ujwala whereas, in the variety Co -1, it shifted to the positive direction in all the mutagen treatments. Significant reduction in plant height was observed in the population treated with 0.2% and 0.3% EMS and 0.02% SA in the variety Ujwala. On contrary, in the variety Co -1, the above concentrations of the mutagen caused an increase in plant height.

The phenotypic coefficient of variation was increased in all the mutagen concentrations in the variety Ujwala. The genotypic coefficient of variation also increased over the control population, except at 0.01% SA treatment. In the variety Co -1, both the phenotypic and genotypic coefficient of variation increased in majority of the mutagen treated population. The highest phenotypic coefficient of variation 14.25% and genotypic coefficient of variation 11.82% was estimated at 0.03% SA treatment in the variety Ujwala. The variety Co -1 also exhibited the similar trend i.e. 13.01% phenotypic and 12.39% genotypic coefficient of variation at 0.03% SA treatment.

The highest heritability value was estimated in all the EMS treatments and at higher concentration of SA i.e. 0.03% in the variety Ujwala. In the variety Co -1, all the mutagen concentrations, except 0.02% SA exhibited higher heritability value over the control. In the variety Co -1, the highest heritability value (90.68%) was observed at 0.03% SA whereas, the variety Ujwala exhibited 82.20% and 82.50% heritability at 0.1% and 0.2% EMS treatments respectively.

Table 15. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for plant height (cm) in M_2 generation

Var. Ujwala

Treatment	Mean ± S .E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	72.83 ± 0.98	--	6.99	5.09	53.00	9.78	--
0.1% EMS	71.33 ± 1.16	-1.50	8.22	7.46	82.20	17.84	1% = 4.689
0.2% EMS	68.04 ± 1.37	-4.79	10.44	9.48	82.50	22.75	5% = 3.095
0.3% EMS	67.88 ± 1.05	-4.95	7.83	6.96	79.00	16.33	--
0.01% SA	71.04 ± 1.40	-1.79	9.29	4.03	18.80	4.61	--
0.02% SA	67.71 ± 1.81	-5.12	13.78	10.07	53.40	19.42	1% = 7.221
0.03% SA	70.71 ± 1.93	-2.12	14.25	11.82	68.80	25.88	5% = 4.767

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 16. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for plant height (cm) in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	49.57 ± 0.58	--	5.77	4.83	69.94	10.66	--
0.1% EMS	49.87 ± 0.63	+0.30	5.34	4.61	74.39	10.48	1% = 2.148
0.2% EMS	51.80 ± 1.01	+2.23	9.87	8.81	79.65	20.75	5% = 1.418
0.3% EMS	54.88 ± 1.12	+5.31	9.53	8.74	84.08	21.15	--
0.01% SA	50.49 ± 1.06	+0.92	8.84	7.90	79.84	18.62	1% = 1.807
0.02% SA	55.25 ± 0.96	+5.68	8.87	6.65	56.25	13.17	5% = 1.193
0.03% SA	54.60 ± 1.39	+5.03	13.01	12.39	90.68	31.14	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Genetic advance also increased in almost all the mutagen treated populations, except at 0.1% EMS treatment in the variety Co - 1 and 0.01% SA in the variety Ujwala. The increase in values of genetic advance was not dose dependent. The highest genetic advance was recorded with 0.03% SA i.e. 31.14% in the variety Co -1. The variety Ujwala also showed the similar trend *i.e.* 25.88% at 0.03% SA treatment.

(4) Primary branches/plant

The mean values for number of primary branches induced by the mutagen treated population shifted mainly to the positive direction (Tables 17 and 18). However, the induced variation of the character was significant only at 0.1% and 0.2% EMS in the variety Ujwala. The data clearly indicates that all other treatments failed to induce significant variation in both the varieties.

The phenotypic and genotypic coefficient of variation was highest in almost all the treatments. In the variety Ujwala, the highest phenotypic coefficient of variation 23.46% was noticed at 0.3% EMS treatment and 20.84% at 0.03% SA treatment. However, the highest genotypic coefficient of variation was, 17.07% at 0.2% EMS and 12.10% at 0.03% SA treatments in the same variety. In the variety Co -1, the highest phenotypic and genotypic variability, 19.62% and 10.34% was found at 0.2% EMS treatment.

Heritability values also increased over the control population except at 0.1% EMS treatment in the variety Co -1. It varied from 20.90% to 59.40% in

Table 17. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for primary branches/plant in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	4.88 ± 0.11	--	12.36	4.77	14.90	4.86	--
0.1% EMS	5.25 ± 1.39	+0.37	16.76	12.89	59.20	26.19	1% = 0.559
0.2% EMS	5.33 ± 0.23	+0.45	22.14	17.07	59.40	34.72	5% = 0.369
0.3% EMS	4.71 ± 0.22	-0.17	23.46	15.80	45.40	28.12	--
0.01% SA	5.17 ± 0.20	+0.29	19.92	11.07	30.90	16.25	1% = 0.744
0.02% SA	5.00 ± 0.19	+0.12	19.39	8.86	20.90	10.70	5% = 0.491
0.03% SA	4.92 ± 0.02	+0.04	20.84	12.10	33.70	18.54	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

the variety Ujwala and 2.34% to 41.67% in the variety Co -1. The highest heritability values (59.20% and 59.40%) were recorded in the variety Ujwala with 0.1% and 0.2% EMS treatments. Whereas, in the variety Co -1, the highest heritability value was 41.67% with 0.3% EMS treatment.

The genetic advance values increased after mutagenic treatments except at 0.1% EMS treatment in the variety Co -1. In the treated population it varied from 10.70% to 34.72% in the variey Ujwala and 0.95% to 16.96% in the variety Co -1.

(5) Secondary branches/plant

The data recorded on number of secondary branches per plant are given in tables 19 and 20. In all the treatments, the mean shifted to the positive direction. In general, there was an increasing trend of mean values of the secondary branches in almost all the concentrations of mutagen in both the varieties.

A glance at the data shows that the phenotypic and genotypic coefficient of variation increased with all the treatments in both the varieties. In the variety Ujwala the highest phenotypic and genotypic coefficient of variation, 26.75% and 21.21% was noticed at 0.03% SA treatment. Whereas, in Co -1 it was 27.84% at 0.01% SA and 26.35% at 0.2% EMS treatments.

The heritability values increased in all the mutagen treatments in both the varieties. The highest heritability value in the variety Ujwala was 87.40%

Table 19. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for secondary branches/plant in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	11.04 ± 0.25	--	9.02	2.88	10.20	2.43	--
0.1% EMS	13.75 ± 0.33	+2.71	12.14	8.17	45.30	14.52	1% = 1.905
0.2% EMS	13.13 ± 0.54	+2.09	21.01	14.12	45.20	25.07	2% = 1.257
0.3% EMS	12.88 ± 0.56	+1.84	22.16	17.74	64.00	37.43	--
0.01% SA	13.33 ± 0.73	+2.29	15.37	14.37	87.40	35.46	1% = 2.268
0.02% SA	13.25 ± 0.65	+2.21	21.55	17.55	69.60	39.60	5% = 1.497
0.03% SA	12.50 ± 0.55	+1.46	26.75	21.21	62.90	44.42	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 20. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for secondary branches/plant in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	11.29 ± 0.18	--	6.44	3.94	37.40	6.36	--
0.1% EMS	15.55 ± 0.76	+4.26	25.41	23.87	88.22	59.18	1% = 2.124
0.2% EMS	16.45 ± 0.85	+5.16	26.60	26.35	98.20	68.93	5% = 1.402
0.3% EMS	20.85 ± 0.64	+9.56	16.19	14.24	77.40	33.09	--
0.01% SA	17.12 ± 0.88	+5.83	27.84	25.89	86.50	63.58	--
0.02% SA	17.42 ± 0.51	+6.13	14.94	12.51	70.03	27.63	1% = 1.427
0.03% SA	17.44 ± 0.68	+6.15	20.55	16.48	64.29	34.89	5% = 0.942

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

at 0.01% SA treatment and it varied from 45.20% to 87.40%. In the variety Co -1, it was highest at 0.2% EMS treatment and varied from 64.29% to 98.20% in treatments and 37.40% in control.

The values of genetic advance also increased over control in the treated population. It increased with increasing doses of both the mutagens in the variety Ujwala. It varied from 14.52% to 44.42% in treatments and 2.43% in the control population. There was no dose related response in the variety Co -1 and varied from 27.63% to 68.93% in treatments and 6.36% in the control population.

(6) Number of fruits/plant

A significant increase in number of fruits /plant was noticed after the mutagen treatments (Table 21 and 22). In all the treatments a positive significant shift in mean was obtained in both the varieties. It was more pronounced in the variety Ujwala than Co -1.

Similarly, the values of phenotypic and genotypic coefficient of variation also increased in the treated population. The highest values of phenotypic (17.45%, 17.66%) and genotypic (15.61%, 15.80%) coefficient of variation in the variety Ujwala were observed at 0.2% and 0.3% EMS treatments. In the variety Co -1, 0.3% EMS treated population exhibited highest value of phenotypic (34.83%) and genotypic (33.99%) coefficient of variation.

Table 21. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for number of fruits /plant in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	67.02 ± 0.97	--	6.99	5.04	52.00	9.59	--
0.1% EMS	86.58 ± 2.36	+19.56	14.17	12.18	73.37	27.55	1% = 6.993
0.2% EMS	80.50 ± 2.71	+13.48	17.45	15.61	80.00	36.85	5% = 4.616
0.3% EMS	79.17 ± 2.70	+12.15	17.66	15.80	80.00	37.29	--
0.01% SA	82.96 ± 2.48	+15.94	15.37	14.37	87.40	35.46	1% = 7.033
0.02% SA	77.46 ± 2.63	+10.44	11.15	8.18	53.90	15.86	5% = 4.643
0.03% SA	80.46 ± 2.56	+13.44	16.66	14.73	78.20	34.39	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 22. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for number of fruits /plant in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	22.42 ± 0.33	--	7.59	6.54	73.95	14.84	--
0.1% EMS	25.30 ± 0.88	+02.88	17.94	16.82	87.95	41.64	1% = 2.155
0.2% EMS	32.72 ± 1.53	+10.30	24.89	24.26	95.00	62.42	5% = 1.423
0.3% EMS	36.76 ± 2.42	+14.34	34.83	33.99	95.30	87.62	--
0.01% SA	23.30 ± 1.01	+00.88	23.39	21.34	83.30	51.43	1% = 2.142
0.02% SA	28.11 ± 1.37	+05.69	25.25	23.81	88.90	56.26	5% = 1.414
0.03% SA	26.48 ± 1.17	+04.06	22.79	18.82	68.34	41.11	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Heritability values of the treated population also increased over control. In EMS treatments the heritability values showed somewhat an increasing trend along with the increasing doses of mutagen but the SA treatments did not exhibit such a dose dependent increase in heritability values. In the variety Ujwala, it varied 53.90% to 87.40% in treatments and 52.00% in control. In the variety Co -1, it varied from 68.34% to 95.30% with a control value of 73.95%.

Genetic advance also increased in the treated population in both the varieties. The highest value of genetic advance was recorded at 0.3% EMS treatment, i.e. 37.29% and 87.62% in Ujwala and Co -1 respectively. In control population it was 9.59% and 14.84% in the variety Ujwala and Co -1 respectively.

(7) Fruit weight

The data recorded on fruit weight is presented in tables 23 and 24. It clearly shows that significant increase in fruit weight was obtained after mutagenesis at 0.1% EMS and 0.02% SA concentrations in the variety Ujwala. In the variety Co -1, all the treatments of both the mutagens failed to induce significant increase in this trait. The mean shifted to positive direction in the variety Ujwala whereas, in Co -1, a negative shift in mean values with EMS treatments and a positive shift with SA treatments were noticed.

The phenotypic coefficient of variation was found to increase in most of the mutagen doses in the variety Ujwala. The highest values were

Table 23. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for weight of twenty fruits (gms) in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	41.50 ± 0.48	--	5.97	2.66	19.90	3.13	--
0.1% EMS	45.42 ± 1.18	+3.92	13.23	11.98	82.10	28.66	1% = 3.525
0.2% EMS	43.29 ± 0.94	+1.79	10.94	4.96	20.60	5.95	5% = 2.327
0.3% EMS	41.96 ± 0.82	+0.46	10.10	1.30	1.66	0.44	--
0.01% SA	42.67 ± 0.89	+1.17	10.81	6.07	31.30	8.93	1% = 2.349
0.02% SA	44.88 ± 0.96	+3.38	11.15	8.18	53.90	15.86	5% = 1.550
0.03% SA	42.96 ± 0.57	+1.46	6.43	2.70	17.60	2.99	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 24. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for weight of twenty fruits (gms) in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	86.24 ± 0.64	--	3.58	1.51	17.85	1.69	--
0.1% EMS	86.15 ± 2.75	-0.09	17.18	16.82	95.85	43.47	1% = 3.837
0.2% EMS	84.91 ± 2.18	-1.33	11.52	10.61	84.81	25.79	5% = 2.533
0.3% EMS	81.45 ± 1.28	-4.79	6.12	3.24	28.10	4.53	--
0.01% SA	90.53 ± 1.63	+4.29	7.18	5.52	59.15	11.21	--
0.02% SA	89.65 ± 1.63	+3.41	9.80	8.63	77.50	20.06	1% = 4.859
0.03% SA	88.72 ± 1.72	+2.48	10.49	8.49	65.48	18.13	5% = 3.208

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

obtained in the EMS treatments and also in the lower concentrations of SA *i.e.* 0.01% and 0.02%. In the variety Ujwala, the genotypic coefficient of variation differed in the treatments, at 0.3% EMS, it was very low (1.30%) and at 0.1% EMS, it was very high (11.98%) when compared to other concentrations. In the variety Co -1, the highest values of phenotypic and genotypic coefficient of variation were recorded at 0.1% and 0.2% EMS treatments. All other doses also showed increased values over the control.

Heritability values increased at lower doses (0.1% and 0.2% EMS and 0.01% and 0.02% SA) in the variety Ujwala. The highest value of heritability, 82.10% was recorded at 0.1% EMS treatment. In variety Co -1, all the mutagenic treatments showed high heritability value than the control. The highest value, 95.85% was recorded at 0.1% EMS treated population.

Genetic advance also increased in the lower mutagen concentrations in the variety Ujwala, The highest value (28.66%) was recorded at 0.1% EMS treatment. In the variety Co -1, all the concentrations of both the mutagens caused an increase in genetic advance over the control. The highest value, 43.47% was recorded at 0.1% EMS treatment.

(8) Fruit length

The data recorded on fruit length reveals that the mean values shifted to both positive and negative direction after the mutagen treatments (Tables 25 and 26). Significant positive shift in mean value was noticed only at 0.1%

EMS treatment in the variety Ujwala and negative shift at 0.3% EMS and 0.01% SA treatments in the variety Co -1.

Phenotypic coefficient of variation showed an increase in values in all the treatments of both the varieties. The highest phenotypic coefficient of variation was estimated at 0.01% SA treatment in the variety Ujwala i.e. 8.72%. In the variety Co -1, the highest value, 17.63% was estimated at 0.1% EMS treatment. Genotypic coefficient of variation also increased in the treated population except at 0.02% SA treatment in the variety Ujwala. The highest values of genotypic coefficient of variation, 6.26% and 17.31% were estimated at 0.01% SA and 0.1% EMS in the variety Ujwala and Co -1 respectively.

Heritability values increased in the entire EMS treated population and 0.01% SA treatment in the variety Ujwala. The highest value, in the variety Ujwala, was estimated at 0.2% EMS treatment, *i.e.* 81.00 %. In the variety Co -1, highly increased values of heritability were estimated in all the concentrations of both the mutagens. It varied from 24.03% to 96.44% whereas; in control population the heritability value was 4.93%.

Genetic advance in the variety Ujwala increased over control in all the EMS treatments and at 0.01% SA treatment. The highest values were, 12.78% and 11.86%, observed at 0.2% EMS and 0.01% SA respectively. In the variety Co -1, it varied from 7.19% to 44.89%.

Table 25. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit length (cm) in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	5.38 ± 0.07	--	4.69	3.31	49.70	6.15	--
0.1% EMS	5.53 ± 0.05	+0.15	4.85	3.57	54.20	6.94	1% = 0.171
0.2% EMS	5.41 ± 0.06	+0.03	5.98	5.37	81.00	12.78	5% = 0.113
0.3% EMS	5.47 ± 0.07	+0.09	6.36	4.84	57.90	9.73	--
0.01% SA	5.35 ± 0.09	-0.03	8.72	6.26	51.50	11.86	1% = 0.335
0.02% SA	5.54 ± 0.07	+0.16	6.34	1.78	7.86	1.36	5% = 0.221
0.03% SA	5.37 ± 0.08	-0.01	6.43	2.69	17.60	2.99	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 26. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit length (cm) in M_2 generation

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	9.01 ± 0.04	--	2.16	0.48	4.93	0.28	--
0.1% EMS	8.82 ± 0.30	-0.19	17.63	17.31	96.44	44.89	1% = 0.329
0.2% EMS	8.92 ± 0.16	-0.09	9.84	9.41	91.40	23.76	5% = 0.217
0.3% EMS	8.36 ± 0.19	-0.65	11.70	11.16	91.44	28.25	--
0.01% SA	8.25 ± 0.10	-0.76	11.34	5.56	24.03	7.19	--
0.02% SA	9.05 ± 0.21	+0.04	12.21	11.53	89.00	28.68	1% = 0.242
0.03% SA	9.04 ± 0.16	+0.03	9.35	8.69	86.31	21.31	5% = 0.160

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

(9) Fruit width

The data on fruit width is presented in tables 27 and 28. All the concentrations of both the mutagens did not induce any significant variation in mean values in the variety Ujwala. However, in SA treatments a positive insignificant shift in mean values was observed. In the variety Co -1, 0.1% and 0.3% EMS treatments and 0.02% and 0.03% SA treatments induced slight increase in fruit width over control whereas, a slight decrease in fruit width was recorded at 0.2% EMS treatment.

An increase in phenotypic coefficient of variation was noticed in almost all the mutagen concentrations except at 0.2% and 0.3% EMS treatments in the variety Co -1. It varied from 4.15% to 16.03% in the treated populations, while it was 5.32% in the control population. In the variety Ujwala, all the mutagen treated population showed an increase in values of phenotypic coefficient of variation. It varied from 4.79% to 6.27% in the treated population, while it was 3.73% in the control population. The genotypic coefficient of variation was found to increase in the treated population except at 0.01% and 0.03% SA treatments in the variety Ujwala. It ranged between 2.71% to 11.09% with a control value of 2.23% in Co -1 and 1.47% to 5.38% with a control value of 2.97% in Ujwala.

An increase in heritability value, in the variety Ujwala, was observed only at 0.3% treatment *i.e.* 73.50% while; it was 63.00% in the control population. However, in variety Co -1 except at 0.1% EMS, the heritability

Table 27. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit width (cm) in M_2 generation

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	0.93 ± 0.006	--	3.73	2.97	63.00	6.20	--
0.1% EMS	0.93 ± 0.010	0.00	5.77	4.54	62.00	9.45	1% = 0.023
0.2% EMS	0.92 ± 0.010	-0.01	5.89	3.02	26.30	4.09	5% = 0.015
0.3% EMS	0.93 ± 0.012	0.00	6.27	5.38	73.50	12.17	--
0.01% SA	0.94 ± 0.008	+0.01	4.79	2.87	35.96	4.55	1% = 0.045
0.02% SA	0.95 ± 0.010	+0.02	5.50	4.00	52.90	7.69	5% = 0.030
0.03% SA	0.94 ± 0.010	+0.01	5.08	1.47	8.40	1.13	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 28. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit width (cm) in M_2 generation.

Var. Co - 1

Treatment	Mean± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	1.18 ± 0.01	--	5.32	2.23	17.50	2.46	--
0.1% EMS	1.20 ± 0.02	+0.02	16.03	5.20	10.51	4.45	1% = 0.053
0.2% EMS	1.15 ± 0.01	-0.03	5.32	4.01	56.32	7.94	5% = 0.035
0.3% EMS	1.23 ± 0.01	+0.05	4.15	2.71	42.69	4.67	--
0.01% SA	1.17 ± 0.02	-0.01	8.50	4.90	22.90	5.14	--
0.02% SA	1.21 ± 0.02	+0.03	6.81	4.27	39.41	7.08	1% = 0.032
0.03% SA	1.22 ± 0.03	+0.04	11.76	11.09	89.01	27.63	5% = 0.021

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

values increased over control. In control population it was 17.50% while, in other treatments it varied from 22.90% to 89.01%.

Genetic advance increased at 0.1% and 0.3% EMS and 0.02% SA treatments in the variety Ujwala. The highest value, 12.17% was recorded at 0.3% EMS treatment. In the variety Co -1, the values of genetic advance were increased over control in all the doses of mutagens. In control, it was 2.46% whereas; in treatments it varied from 4.45% to 27.63%.

(10) Fruit yield/plant

The data recorded on fruit yield/plant is given in tables 29 and 30. It is clear from the data that a significant increase in fruit yield was occurred in the lower doses of the mutagens in the variety ujwala. The highest increase in fruit yield was noted in 0.1% EMS treatment. The mean values shifted towards the positive direction in all the mutagen doses. In contrary, in variety Co -1, the increase in fruit yield was significant at higher doses of the mutagens.

In both the varieties the phenotypic coefficient of variation increased in the population treated with the mutagens. It varied from 14.16% to 24.50% in the variety Ujwala and 18.76% to 30.24% in the variety Co -1. The genotypic coefficient of variation also increased after the treatments and varied from 10.08% to 20.29% in the variety Ujwala and 17.22% to 28.44% in the variety Co -1.

Table 29. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit yield/plant (gms) in M_2 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	139.22±2.73	-	10.30	7.15	48.18	13.09	--
0.1% EMS	195.79±8.09	+56.57	21.54	19.12	78.68	44.75	1% = 21.518
0.2% EMS	175.80±8.15	+36.58	24.50	20.29	68.55	44.34	5% = 14.204
0.3% EMS	145.43±5.44	+06.21	17.60	13.29	57.06	26.51	--
0.01% SA	175.73±4.91	+36.51	14.16	10.08	50.73	18.96	1% = 18.165
0.02% SA	172.35±6.80	+33.13	21.01	16.14	63.53	35.24	5% = 11.991
0.03% SA	146.69±4.53	+07.47	14.44	10.64	54.27	20.69	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation

Table 30. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit yield/plant (gms) in M_2 generation.

Var. Co - 1

Treatment	Mean± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	96.71±1.63	--	8.57	6.06	49.96	11.30	--
0.1% EMS	106.54±3.79	+09.83	18.76	17.22	84.27	41.93	1% = 14.086
0.2% EMS	138.63±6.58	+41.92	25.78	24.09	87.30	59.94	5% = 9.298
0.3% EMS	145.80±8.07	+49.09	29.44	28.44	93.33	72.53	--
0.01% SA	105.79±4.91	+09.08	25.58	22.44	75.63	51.06	1% = 22.186
0.02% SA	126.47±7.23	+29.76	30.24	28.01	85.79	68.48	5% =14.645
0.03% SA	119.16±6.68	+22.45	29.64	25.03	71.30	55.80	--

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

The heritability values also increased in the treated population over the control. In the variety Ujwala, it varied from 50.73% to 78.68% and the highest value was noticed at 0.1% EMS treatment. The highest value in the variety Co -1, 93.33% was recorded at 0.3% EMS treated population and varied from 71.30% to 93.33%.

The genetic advance values increased in the treated population. In the variety Ujwala maximum values were recorded at the lower doses of the EMS treatments, *i.e.* 44.75% and 44.35% at 0.1% and 0.2% EMS respectively. However, there was no such dose related trend in SA treatments and it varied from 18.96% to 35.24%. In the variety Co -1, it increased with the increasing doses in most of the treatments and varied from 41.93% to 72.53% and 51.06% to 68.48% with EMS and SA respectively.

4. 5. Quantitative characters in M_3 generation

The M_3 population was raised from the M_2 plants of the selected treatments *i.e.* 0.1% and 0.2% EMS and 0.01% and 0.02% SA of the variety Ujwala and 0.2% and 0.3% EMS and 0.02% and 0.03% SA of the variety Co -1, which gave the maximum total plant yield. The seeds from the selected M_2 plants were sown in plant progeny rows and seedlings were transplanted in the field in Completely Randomized Block Designs (CRBD). The data on ten quantitative characters were recorded and statistically analyzed.

(1) Days to flowering

The data recorded in tables 31 and 32 shows that the days to flowering were reduced significantly in almost all the treatments except in 0.02% SA in the variety Ujwala and 0.2% EMS in the variety Co -1. The highest reduction was observed at 0.2% EMS treatment in the variety Ujwala and 0.03% SA treatment in the variety Co -1.

The phenotypic coefficient of variation increased in the variety Ujwala in all the mutagenic treatments and highest values were noticed at 0.01% and 0.02% SA treatments, i.e. 5.87% and 4.30% respectively. In the variety Co -1, except at 0.2% EMS, the values of phenotypic coefficient of variation increased over control. Somewhat similar trend was observed in the case of the genotypic coefficient of variation, in both the varieties.

Heritability values also increased in all the treated populations of variety Ujwala, it varied from 46.09% to 86.83%. In Co -1, 0.2% EMS treatment showed lower value of heritability than the control. In other treatments it varied from 41.30% to 70.31%, with a highest value at 0.3% EMS treatment.

Genetic advance also increased in all the treated populations of the variety Ujwala, with a highest value of 13.47% at 0.01% SA treatment. In Co1, genetic advance was low in 0.2% EMS treatment than the control, but all other treatments showed high values.

Table 31. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to flowering in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D
Control	100.38 ± 0.39	--	1.89	0.80	17.79	0.89	--
0.1% EMS	92.54 ± 0.69	-7.84	3.91	3.14	64.57	6.66	1% = 2.774
0.2% EMS	92.13 ± 0.54	-8.25	2.92	1.98	46.09	3.55	5% = 1.672
0.01% SA	96.71 ± 1.12	-4.21	5.87	5.47	86.83	13.47	1% = 3.319
0.02% SA	98.78 ± 0.83	-1.60	4.30	3.80	78.39	8.89	5% = 2.001

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 32. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to flowering in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S .D.
Control	89.76 ± 0.31	--	1.68	0.82	23.96	1.06	--
0.2% EMS	87.03 ± 0.28	-2.73	1.55	0.59	14.50	0.59	1% = 5.188
0.3% EMS	84.28 ± 1.51	-5.48	3.40	2.85	70.31	6.32	5% = 3.565
0.02% SA	85.21 ± 0.38	-4.55	2.15	1.38	41.30	2.34	1% = 2.509
0.03% SA	83.26 ± 0.37	-6.50	2.26	1.60	50.43	3.01	5% = 1.513

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

(2) Days to maturity

The data presented in tables 33 and 34 clearly shows that the mean values shifted to negative direction in the variety Ujwala while, in variety Co -1, it shifted mainly to the positive direction. A significant decrease in days to maturity was observed in the variety Ujwala except at 0.01% SA treatment. However, in Co -1, the days to maturity was increased to about 4 days in the population treatment with 0.2% EMS.

The phenotypic coefficient of variation showed a slight increase in treated population, except at 0.01% SA in the variety Ujwala. While in Co -1, it increased only at 0.3% EMS treatment. The values of genotypic coefficient of variation were increased after mutagenic treatments in both the varieties. However, when compared to the M_2 treated population, the increase in both the values was very low in M_3 generation.

Heritability values exhibited increasing trend after the treatments in both the varieties. It varied from 49.25% to 68.44%, with a highest value at 0.2% EMS treatment, in the variety Ujwala. In Co -1, the range of heritability value was 53.40% to 79.12%, with highest value at 0.2% EMS treatment.

Genetic advance also increased in all the treated population in both the varieties. In the variety Ujwala, it varied from 2.81% to 4.48%, with 1.29% in the untreated population while, in variety Co -1, it was 2.40% to 4.51%, with 1.38% in the untreated population.

Table 33. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to maturity in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	140.67 ± 0.61	--	2.17	1.03	22.62	1.29	--
0.1% EMS	132.21 ± 0.71	-8.46	2.66	1.88	49.25	3.46	1% = 4.328
0.2% EMS	129.80 ± 0.62	-10.87	2.48	2.05	68.44	4.48	5% = 2.610
0.01% SA	140.00 ± 0.52	-0.67	2.03	1.47	52.39	2.81	1% = 2.460
0.02% SA	135.38 ± 0.71	-5.29	2.68	1.94	52.19	3.69	5% = 1.483

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 34. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for days to maturity in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	122.64 ± 0.46	_	1.89	0.99	27.72	1.38	
0.2% EMS	126.69 ± 0.44	+4.05	1.77	1.57	79.12	3.70	1% = 1.895
0.3% EMS	121.99 ± 0.55	-0.65	2.29	1.98	74.59	4.51	5% = 1.143
0.02% SA	123.86 ± 0.43	+1.22	1.74	1.27	53.40	2.45	1% = 2.085
0.03% SA	123.39 ± 0.39	+0.75	1.61	1.21	56.25	2.40	5% = 1.257

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

(3) Plant height

The data recorded on plant height in M_3 generation is presented in tables 35 and 36. The mean shifted towards both positive and negative direction after mutagenic treatments in both the varieties. In the variety Ujwala, the shift in mean was not significant, whereas, in Co -1, except at 0.2% EMS treatment, the mean values showed significant shift towards positive direction. In 0.3% EMS, 0.02% SA and 0.03% SA treatments the plant height increased by 58.61 cm, 56.74 cm and 57.81 cm respectively over control *i.e.* 51.49 cm.

Phenotypic coefficient of variation increased in the treatments in both the varieties, except at 0.2% EMS and 0.01% SA in the variety Ujwala. The highest value of phenotypic coefficient of variation (10.81%) was recorded at 0.02% SA treatment while; in Co -1 it was 13.88% at 0.3% EMS treatment. Genotypic coefficient of variation also exhibited the similar trend, with the highest value 10.04% at 0.02% SA treatment in Ujwala and 13.00% at 0.3% EMS treatment in Co -1.

Except at 0.01% SA treatment in the variety Ujwala, the values of heritability also showed an increase in the treated population. It was very high in the variety Co -1, when compared to the other variety Ujwala. It varied from 85.25% to 87.71% in the treated population of the variety Co -1.

Genetic advance values increased at 0.1% EMS and 0.02% SA treatments in the variety Ujwala. In the variety Co -1, all the treatments

Table 35. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for plant height (cm) in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	73.04 ± 1.19	--	7.63	6.33	68.81	13.87	--
0.1% EMS	74.63 ±1.21	+1.59	8.05	7.08	77.47	16.46	1% = 7.766
0.2% EMS	71.70 ± 0.85	-1.34	6.99	5.64	65.13	12.01	5% = 4.869
0.01% SA	71.04 ± 1.07	-2.04	7.71	4.65	36.44	7.42	1% = 7.493
0.02% SA	71.69 ± 1.53	-1.35	10.81	10.04	86.29	24.62	5% = 4.518

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 36. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for plant height (cm) in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	51.49 ± 0.55	--	5.16	3.51	46.27	6.31	--
0.2% EMS	50.76 ± 0.71	-0.73	7.12	6.57	85.25	16.02	1% = 3.124
0.3% EMS	58.61 ± 1.60	+7.12	13.88	13.00	87.71	32.15	5% = 1.884
0.02% SA	56.74 ± 0.99	+5.25	8.76	8.12	85.94	19.88	1% = 0.915
0.03% SA	57.81 ± 0.84	+6.32	7.31	6.83	87.30	16.86	5% = 0.551

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

recorded high values of genetic advance and it varied from 16.02% to 32.15% whereas, in control population it was only 6.31%.

(4) Primary Branches / plant

The data presented in tables 37 and 38 reveals the effect of various treatments on primary branches/plant. Even though, a positive shift in mean values was observed in the treated population, only the EMS treatments in the variety Ujwala succeeded to induce significant increase in this character. An increase of mean values was recorded, at 0.1% and 0.2% EMS treatments in the variety Ujwala.

However, the values of phenotypic and genotypic coefficient of variation increased in all the treatments. In the variety Ujwala, the phenotypic coefficient of variation varied from 16.13% to 18.64%. In Co -1, it varied from 13.54% to 17.85%. The genotypic coefficient of variation varied from 12.05% to 15.21% in the variety Ujwala and 9.91% to 15.03% in Co -1.

Heritability values also increased after mutagenic treatments in both the varieties. In Ujwala, it varied from 51.07% to 71.20% with the highest value at 0.1% EMS treatment. In Co -1, it varied from 53.47% to 92.89% with highest value at 0.2% EMS treatment.

Genetic advance increased in the treated population over the control. It varies from 23.76% to 33.88% in treatments whereas, in control population it was 7.39% in Ujwala. In the variety Co -1, it varied from 19.15% to 38.24% with a control value of 7.27%.

Table 37. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for primary branches/plant in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	4.93 ± 0.12	_	11.67	5.71	23.98	7.39	
0.1% EMS	5.50 ± 0.20	+0.57	18.03	15.21	71.20	25.19	1% = 0.662
0.2% EMS	5.33 ± 0.20	+0.40	18.64	13.33	51.20	33.88	5% = 0.399
0.01% SA	5.15 ± 0.20	+0.22	18.44	13.18	51.07	24.87	1% = 1.213
0.02% SA	4.93 ± 0.16	0.00	16.13	12.05	55.81	23.76	5% = 0.731

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 38. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for primary branches/plant in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	2.35 ± 0.04	--	9.12	5.00	30.20	7.27	--
0.2% EMS	2.49 ± 0.08	+0.14	15.56	15.03	92.89	38.24	1% = 0.276
0.3% EMS	2.44 ± 0.07	+0.09	13.54	9.91	53.47	19.15	5% = 0.167
0.02% SA	2.58 ± 0.09	+0.23	16.13	14.36	79.26	33.74	1% = 0.376
0.03% SA	2.46 ± 0.09	+0.11	17.85	14.69	67.71	31.91	5% = 0.227

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

(5) Secondary branches/plant

The data recorded on secondary branches/plant is presented in tables 39 and 40. It is clearly evident from the data that highly significant increase in mean values of the number of secondary branches/ plant was occurred in all the concentrations of the mutagens. The variety Co - 1 responded very significantly to this trait than the other variety Ujwala. It is also very clear that the EMS treatment was more effective in inducing such a desirable change, when compared to SA.

Low value of phenotypic and genotypic coefficient of variation was observed at 0.1% EMS treatment in the variety Ujwala, but in all other treatments, the phenotypic and genotypic coefficient of variation increased over control. In the variety Ujwala, the highest phenotypic and genotypic coefficient of variation *i.e.* 25.02% and 23.39% was recorded at 0.02% SA treatment. In the variety Co - 1, both the values increased in all the selected treatments and phenotypic coefficient of variation ranged from 13.80% to 19.49%, with the highest value at 0.3% EMS treatment. The genotypic coefficient of variation varied from 11.98% to 17.51%, with the highest value at 0.3% EMS treatment.

Heritability also increased in the treated population in both the varieties. In the variety Ujwala, it varied from 16.33% to 87.42% and in Co -1, it varied from 68.49% to 84.46%.

Table 39. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for secondary branches/plant in M_3 generation.

Var. Ujwala

Treatment	Mean ± S.E	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	11.12 ± 0.24	_	10.77	3.56	10.94	3.12	
0.1% EMS	14.58 ± 0.26	+3.43	8.42	3.40	16.33	3.63	1% =1.272
0.2% EMS	13.42 ± 0.55	+2.30	20.91	15.53	55.14	28.41	5% = 0.767
0.01% SA	13.55 ± 0.71	+2.43	22.27	18.67	70.24	41.30	1% = 3.336
0.02% SA	13.19 ± 0.66	+2.07	25.02	23.39	87.42	57.74	5% = 2.012

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 40. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for secondary branches/plant in M_3 generation.

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	15.07 ± 0.25	--	8.12	4.58	31.80	6.81	--
0.2% EMS	21.22 ± 0.61	+6.15	14.73	12.19	68.49	26.63	1% = 0.733
0.3% EMS	22.43 ± 0.86	+7.36	19.49	17.51	80.69	41.53	5% = 0.442
0.02% SA	19.35 ± 0.53	+4.28	13.80	11.98	75.36	27.46	1% = 1.848
0.03% SA	18.45 ± 0.64	+3.38	17.77	16.33	84.46	39.63	5% = 1.114

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Genetic advance values were increased in treatments than the controls. The highest value of genetic advance, i.e. 57.74% was observed at 0.02% SA treatment in the variety Ujwala. Where as, in the variety Co - 1, it was 41.53% at 0.3% EMS treatment.

(6) Number of Fruits/ Plants

The most important yield contributing trait, the number of fruits/plant showed highly significant increase after mutagenesis and selection (Table 41 and 42). The mean values of treated population shifted towards the positive direction and it was very pronounced in EMS treatments.

The phenotypic coefficient of variation varied from 8.02% to 15.12% in the variety Ujwala while, in Co -1, it varied from 10.26% to 24.73%. The genotypic coefficient of variation also showed an increase in the treated population, with a range of 6.73% to 13.94% in the variety Ujwala and 6.99% to 23.35% in Co -1. In the variety Ujwala, the response of both the mutagens to this trait was somewhat similar but in Co -1, EMS showed high value of both phenotypic and genotypic coefficient of variation.

Heritability also increased in the treated population and it varied from 64.13% to 86.31% in the variety Ujwala, in Co -1, it was 46.49% to 89.16%.

The genetic advance values were increased very much in treated population than the control. In Ujwala, it varied from 14.91% to 34.19%, with highest values at higher concentration of the mutagens. The genetic

Table 41. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for number of fruits/plant in M_3 generation.

Var. Ujwala

Treatment	Mean ± S.E	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	67.90 ± 0.79	--	5.57	2.89	26.89	3.95	--
0.1% EMS	96.14 ± 1.51	+28.24	8.02	6.73	70.44	14.91	1% = 4.882
0.2% EMS	84.58 ± 2.50	+16.68	15.12	13.88	84.42	33.67	5% = 2.944
0.01% SA	81.88 ± 1.64	+13.98	10.24	8.20	64.13	17.33	1% = 6.803
0.02% SA	78.30 ± 2.33	+10.40	15.01	13.94	86.31	34.19	5% = 4.102

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 42. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for number of fruits/plant in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	32.91 ± 0.38	--	5.86	2.60	19.69	3.05	--
0.2% EMS	41.99 ± 1.86	+9.08	22.74	19.63	74.52	44.74	1% = 3.664
0.3% EMS	42.72 ± 2.07	+9.81	24.73	23.35	89.16	58.20	5% = 2.209
0.02% SA	36.78 ± 0.80	+3.87	11.06	8.10	53.65	15.66	1% = 2.773
0.03% SA	34.77 ± 0.75	+1.86	10.26	6.99	46.49	12.59	5% = 1.672

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

advance varied from 12.59% to 58.20%, in the variety Co -1 and the EMS treatments showed very high values than SA treatments.

(7) Fruit weight

The data recorded on fruit weight is presented in tables 43 and 44. In the variety Ujwala, the mean values shifted to the positive direction after the mutagenic treatments. The increase in fruit weight was very significant in all the treatments and highest mean value (50.91) was observed at 0.02% SA treatment. In the variety Co -1, the mean shifted to both positive and negative directions. Significant positive shift in mean values was estimated at 0.02% and 0.03% SA treatments whereas, the EMS treatments failed to induce significant variation in fruit weight.

In the variety Ujwala, the phenotypic and genotypic coefficient of variation increased in the treated population, except at 0.2% EMS treatment. The highest values were observed at 0.1% EMS treatment. The highest values of phenotypic and genotypic coefficient of variation were recorded at 0.2 % EMS in the variety Co -1 *i.e.* 13.45% and 12.81%. The range of phenotypic variation was 7.55% to 13.45% with a control value of 3.68% and genotypic coefficient of variation, 5.70% to 12.81%, with a control value of 1.99%.

In the variety Ujwala, a decrease in heritability values was recorded with EMS treatments whereas, the SA treated population exhibited high values of heritability over control *i.e.* 89.30% and 63.17% at 0.01% and

Table 43. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for weight of twenty fruits (gms) in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	41.67 ± 0.63	--	7.73	5.26	46.33	9.45	--
0.1% EMS	47.30 ± 0.52	+5.63	31.41	12.88	16.81	14.91	1% = 4.930
0.2% EMS	46.96 ± 0.80	+5.29	7.59	2.55	11.24	2.25	5% = 2.973
0.01% SA	45.64 ± 0.83	+3.97	9.15	6.26	89.30	21.57	1% = 2.203
0.02% SA	50.91 ± 1.19	+9.24	11.97	9.51	63.17	19.96	5% = 1.329

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 44. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for weight of twenty fruits (gms) in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	85.81 ± 0.67	_	3.68	1.99	29.17	2.83	
0.2% EMS	84.29 ± 2.21	-1.52	13.45	12.81	90.73	32.21	1% = 7.818
0.3% EMS	87.67 ± 1.91	+1.86	11.13	8.91	64.11	18.84	5% = 4.714
0.02% SA	90.60 ± 1.34	+4.79	7.55	5.70	56.88	11.34	1% = 5.292
0.03% SA	91.84 ± 1.53	+6.03	8.49	7.04	68.64	15.39	5% = 3.191

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

0.02% SA treatments respectively. Heritability values considerably increased after the mutagenic treatments in the variety Co -1. It varied from 56.88% to 90.73%, with 29.17% in the control population.

Genetic advance increased after mutagenesis, except in the 0.2% EMS treatment in the variety Ujwala. The highest values of genetic advance were recorded in SA treatments in the variety Ujwala. Whereas, in the variety Co -1, EMS treatments caused high values of genetic advance. It varied from 11.34% to 32.21%, with 2.83% in the control population.

(8) Fruit length

The data summarized in tables 45 and 46 shows that the mean values of the treated population shifted towards the negative direction in the variety Ujwala however, the deviation was not significant, except at 0.02% SA treatment. In the variety Co -1, except at 0.03% SA treatment, the mean values shifted towards the positive direction. Significant increase in mean fruit length was noticed only at 0.2% EMS, *i.e.* 9.08 cm while, in control it was 8.92 cm.

A glance at the data shows that the phenotypic and genotypic coefficient of variation slightly increased with SA treatments in the variety Ujwala. However, in the variety Co -1, the coefficient of both phenotypic and genotypic variation increased with all the treatments. The maximum value of phenotypic and genotypic coefficient of variation was recorded at 0.3% EMS treatment *i.e.* 11.80% and 11.00% respectively.

Table 45. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit length (cm) in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	5.69 ± 0.08	--	6.83	4.44	42.31	7.62	--
0.1% EMS	5.57 ± 0.06	-0.12	5.13	4.00	60.88	3.14	1% = 0.160
0.2% EMS	5.67 ± 0.03	-0.02	2.42	1.70	49.10	8.24	5% = 0.096
0.01% SA	5.58 ± 0.08	-0.11	7.07	6.38	81.31	15.18	1% = 0.219
0.02% SA	5.22 ± 0.08	-0.47	7.34	5.60	58.15	11.28	5% = 0.133

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 46. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit length (cm) in M_3 generation.

Var. Co - 1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	8.92 ± 0.05	_	2.93	0.40	1.86	0.14	
0.2% EMS	9.08 ± 0.14	+0.16	7.74	7.06	83.15	16.99	1% = 0.328
0.3% EMS	8.93 ± 0.21	+0.01	11.80	11.00	86.96	27.08	5% = 0.066
0.02% SA	9.17 ± 0.12	+0.25	6.69	4.93	54.11	9.57	1% = 0.482
0.03% SA	8.77 ± 0.12	-0.15	6.96	6.38	84.16	15.46	5% = 0.290

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

The heritability values increased in all the mutagen doses in both the varieties. Compared to control values, the variety Co -1 showed high heritability values than the other variety Ujwala. In the variety Ujwala, the control value was 42.31% and in treatments it varied from 49.10% to 81.31%. In the variety Co -1, in treated population it varied from 54.11 to 86.96% while, in control it was 1.86%.

The values of genetic advance also increased in all the mutagen doses of both the varieties, except at 0.1% EMS treatment in the variety Ujwala. The maximum value, 15.18% was recorded at 0.01% SA treatment in the variety Ujwala whereas, in Co -1, it was 27.08% at 0.3% EMS treatment.

(9) Fruit width

The data recorded on fruit width is presented in tables 47 and 48. Though, the mean values shifted towards the positive direction in all the mutagenic treatments, significant variation in this trait was found only in the 0.03% SA treatment in the variety Co -1.

The phenotypic coefficient of variation increased at 0.2% EMS and 0.01% SA treatments in the variety Ujwala whereas, in Co -1 all the mutagen doses, except 0.2% EMS, showed high values. The values of genotypic coefficient of variation were higher than the control, except at 0.02% SA, in the variety Ujwala. In Co -1, a low value of genotypic coefficient of variation

Table 47. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit width (cm) in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	0.93 ± 0.008	_	4.01	2.63	42.95	4.56	
0.1% EMS	0.96 ± 0.008	+0.03	3.97	3.50	77.77	8.16	1% = 0.032
0.2% EMS	0.94 ± 0.009	+0.01	5.05	3.72	54.43	7.25	5% = 0.019
0.01% SA	0.96 ± 0.011	+0.03	6.28	3.47	30.56	5.06	1% = 0.088
0.02% SA	0.94 ± 0.007	+0.01	3.69	1.93	27.5	2.68	5% = 0.053

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 48. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit width (cm) in M_3 generation.

Var. Co-1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	1.14 ± 0.01	_	5.94	3.00	25.43	3.99	
0.2% EMS	1.17 ± 0.01	+0.03	5.70	1.34	5.48	0.83	1% = 0.692
0.3% EMS	1.19 ± 0.02	+0.05	7.69	3.54	21.12	4.29	5% = 0.417
0.02% SA	1.21 ± 0.02	+0.07	7.62	5.01	43.30	8.71	1% = 0.094
0.03% SA	1.24 ± 0.02	+0.10	7.83	5.67	52.53	10.85	5% = 0.057

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

was observed at 0.2% EMS treatment whereas, all other mutagen doses showed higher values than the control.

The values of heritability increased in EMS treatments *i.e.* 77.77% and 54.43% at 0.1% and 0.2% EMS respectively in the variety Ujwala. Whereas, in Co -1, the SA treatments showed high values of heritability *i.e.*, 43.30% and 52.53% at 0.02% and 0.03% SA treatments respectively.

Genetic advance also increased in treated population except at 0.02% SA treatment in the variety Ujwala and 0.2% EMS treatment in the variety Co -1. The maximum value of genetic advance was recorded in the variety Ujwala at 0.1% EMS *i.e.*, 8.16%. In the variety Co -1 it was 10.85% at 0.03% SA treatment. In control, it was 4.56% and 3.99% in the variety Ujwala and Co -1 respectively.

(10) Fruit yield / plant

The data (Tables 49 and 50) reveal that the treated population in M_3 generation was superior to the control with regards to the fruit yield/plant. The mean values in the treated population shifted significantly towards the positive direction in both the varieties. The population treated with different doses of EMS exhibited the maximum variation in mean values in both varieties.

The phenotypic and genotypic coefficient of variation also increased after the mutagen treatments. The highest value of phenotypic (21.02%) and genotypic (18.15%) coefficient of variation in the variety Ujwala was

Table 49. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit yield/plant (gms) in M_3 generation.

Var. Ujwala

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	161.30 ± 2.82	--	8.83	3.82	18.71	4.36	--
0.1% EMS	227.53 ± 4.79	+66.23	10.72	8.10	57.21	16.18	1% = 27.434
0.2% EMS	197.95 ± 5.61	+36.65	13.72	11.00	64.31	23.29	5% = 16.542
0.01% SA	186.31 ± 3.99	+25.01	10.75	6.57	37.31	10.59	1% = 17.365
0.02% SA	199.75 ± 8.23	+38.45	21.02	18.15	74.55	41.37	5% = 10.470

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

Table 50. Estimates of mean value ($\overline{X}$), shift in mean, coefficient of variation, heritability (h^2) and genetic advance (Gs) for fruit yield/plant (gms) in M_3 generation.

Var. Co -1

Treatment	Mean ± S. E.	Shift in $\overline{X}$	CVp (%)	CVg (%)	h^2 (%)	Gs (%)	L. S. D.
Control	138.62 ± 1.61	--	5.58	3.81	46.74	6.88	--
0.2% EMS	173.94 ± 9.25	+35.32	27.15	22.33	67.68	48.50	1% = 25.805
0.3% EMS	180.04 ± 9.30	+41.42	26.20	23.88	83.08	57.46	5% = 15.559
0.02% SA	159.99 ± 4.16	+21.37	13.13	10.86	68.45	23.72	1% = 23.776
0.03% SA	158.69 ± 6.32	+20.07	19.67	10.32	27.49	14.28	5% = 14.336

CVp = Coefficient of phenotypic variation.
CVg = Coefficient of genotypic variation.

recorded at 0.02% SA treatment. In the variety Co - 1, EMS treatment was found to be more effective in inducing both phenotypic and genotypic variation than SA. The phenotypic coefficient of variation was highest at 0.2% EMS treatment *i.e.* 27.15% in the variety Co - 1 while, the maximum genotypic coefficient of variation was observed at 0.3% treatment *i.e.* 23.88%. The phenotypic and genotypic coefficients of variation in control population were 5.58% and 3.81% respectively.

Heritability values also increased after mutagen treatments in both the varieties. It varied from 37.31% to 74.55% in the variety Ujwala, with 18.71% in control population. In Co -1, it varied from 27.49% to 83.08% in treatments and 46.74% in control. The maximum heritability values were recorded at 0.02% SA treatment, *i.e.* 74.55% and at 0.3% EMS, *i.e.* 83.08% in Ujwala and Co-1 respectively.

Similarly, the values of genetic advance increased over control in all the treatment doses. The highest value in the variety Ujwala, *i.e.* 41.37% and in Co -1, *i.e.* 57.46% was recorded at 0.02% SA and 0.3% EMS treatments respectively.

CHAPTER - 5

DISCUSSION

Among the modern methods of plant breeding the experimental induction of mutations occupies a more and more significant and established place. Mutations enhance genetic variability and broaden genotypic adaptability and increase genotype - environment interaction. Thus they enlarge the arena and scope of effective selection. Mutations produce variability that is either new or that was lost during the evolutionary course. Since genetic variability is created in an already improved and well-adapted genetic background, induced mutation has proffered excellent breeding material that has led to 'gene' and 'green' revolution (Kaul, 1989). When there is complete exploitation of genetic variation by means of selection of better genotypes from the mixed growing population, which resulted total consumption of genetic variability. At this juncture induction of mutation is of great value. Induced mutagenesis has become an important tool for the induction of genetic variability at a broad scale for the improvement of the crop plants.

The sensitivity of two chilli cultivars *viz.*, Ujwala and Co - 1, to various concentrations of two potent chemical mutagens *i.e.* ethyl methanesulphonate and sodium azide was assessed by studying the biological damage/changes. Various mutagenic treatments used in the present study brought about reduction in seed germination, seedling height and pollen fertility (Tables 1, 2, 3 and 5). Such reductions were found to be

dose dependent. Similar results have also been reported in various other crops (Jana, 1964; Nilan *et al.*, 1968; Khan, 1979; Khan and Siddiqui, 1987).

In the present study, seed germination is delayed after mutagenic treatments in both the varieties. Both cytological and physiological disturbances induced by the mutagens might have led to the arrest of mitotic proliferation of cells in the embryo, which in turn is liable to cause delay in germination. Delayed germination after seed treatment with different mutagens has been established by various workers (Siddiq and Swaminathan, 1968; Kleinhofs *et al.,* 1974; Khan *et al.*, 2000).

Chaudhary (1978) recorded a germination delay, when the seeds of wheat were treated with physical and chemical mutagens. Favret (1963) reported that delayed seed germination caused by various mutagens might be due to the depression in the rate of mitotic proliferations. The denatured DNA caused by mutagenic treatment, after sometime may be repaired resulting in the activation of biological processes involved in germination and thus delayed germination is observed (Hutterman *et al.*, 1978). In EMS treated seeds, the delayed germination might be due to the disturbances in the initial metabolic process and / or chromosomal aberrations whereas, in SA treatments, in addition to initial metabolic disturbances, gene mutations might have played a major role. Sander *et al.* (1978) observed that sodium azide induces no chromosome aberrations in barley after seed treatment.

A linear reduction in germination, with the increasing concentration of both the mutagens was observed but the extent of decrease in germination differed in the different mutagenic treatments (Table 1). Seed germination was found to be more adversely affected in different concentrations of SA than EMS. Varietal difference with regard to germination was also recorded after the treatment with the both mutagens. The variety Co-1 was found to be more sensitive to both of the mutagens than the other variety Ujwala (Table 1). The dependence of seed germination on dose was also reported in rice (Siddiq and Swaminathan, 1968), in mung bean (Subramanian, 1980; Khan, 1990), in safflower (Ramachandran and Goud, 1983) and in barley (Nilan *et al.,* 1973; Kleinhofs *et al.,* 1974).

Reduction of seed germination in SA treatments may be mainly due to the disturbances in the physiological activity such as respiratory process in the cell. Physiological effects of SA may be due to decline in assimilation mechanism, inhibition of catalase, peroxidase and cytochrome oxidase (Kleinhofs *et al.*, 1978). However, in EMS treatments both chromosomal and physiological imbalances might have been responsible for the reduction in germination. The decrease in the germination is generally attributed to inhibitory effects of mutagen on physiological and biological processes necessary for seed germination, which include altered enzyme activity (Kurobane *et al.*, 1979) and hormonal imbalance (Chrispeels and Varner, 1967). Varietal differences in seed germination observed in the present investigation, may be related to metabolic activity and most probably the

capability of some genotypes to reach the mutagen sensitivity stage than the other. Varietal differences were also reported earlier with respect to mutagen sensitivity in wheat (Goud, 1967 b), *Sorghum* (Ramulu, 1970), pea (Levy and Ashri, 1973) and castor (Athma and Reddy, 1985).

The mean height of seedlings decreased with the increasing concentrations of the mutagens used (Tables 2 and 3). Such reduction in seedling height was also observed by Konzak *et al.* (1961) in *Hordeum*, Iqbal (1969) in *Capsicum*, Rajput (1970) in *Triticum*, Fowler and Stefansson (1972) in *Brassica*, Singh *et al.* (1978) in *Pennisetum* and Khan (1990) in *Vigna*. The extent of decrease in seedling height was not the same in the two varieties of *Capsicum annum* studied. The variety Co - 1 was more resistant to both the mutagens (Tables 2 and 3). This kind of differential response has also been reported by Bhatia (1960), Nirula (1961), Singh *et al.* (1978) and Cheralu and Reddy (1985).

There are various opinions regarding the reduced seedling growth after mutagen treatments. Reduction of seedling growth was attributed to inhibition or change in enzyme activity (Blinks, 1952), impaired mitosis in the meristematic zone of the growing seedlings (Cherry and Hageman, 1961) and gross injury caused at cellular level, either due to gene controlled biochemical process or acute chromosomal aberrations or both (Narayana and Konzak, 1969). Sparrow *et al.* (1961) are of the opinion that the chief causes of reduced seedling growth are extra chromosomal damages or inhibition of cell division. Goud and Nayar (1968) demonstrated that seedling

growth depression basically might be due to inhibition of auxin synthesis. Cherry *et al.* (1962) suggested that the changes occurring in the specific activity of several enzymes by the treatments of different mutagens have a considerable adverse effect on growth rate. The chromosomes carry the genes for various types of metabolic activities at the initial stage of the growth of the plant. Any type of disturbances, which is either chromosomal or physiological, bring about various metabolic disorders that will cause several morphological and growth abnormalities in plant or plant organs.

In the present study, various kinds of cotyledonary abnormalities were observed in the treated population in the M_1 generation (Table 4). Such abnormalities were more pronounced after EMS treatments in the variety Co -1. Occurrence of seedlings with abnormal cotyledons are confirming the results obtained in various crops by several workers such as in rice (Yamagata, 1966; Bose and Chaudhary, 1968), *Sorghum* (Ramulu, 1970), lentil (Sharma and Kant, 1975) and mung bean (Chaturvedi and Singh, 1981).

Inhibition of ATP production, reduction in DNA synthesis and mitotic index due to chemical mutagens, as reported by Kleinhofs *et al.* (1978), may also be partly responsible for seedling injury. The formation of an extra cotyledonary leaf indicates the formation and involvement of additional leaf primordia of the embryonal tissue. Splitting of cotyledonary leaves may be due to acute cytochemical disturbances leading to the death of leaf primordia or of the embryonal cells responsible for leaf development. The

chlorophyll deficient spots on the cotyledonary leaves can be attributed to the failure of chlorophyll development in cells due to acute metabolic disturbances. The observation of cotyledonary leaf spots in EMS treated seedlings in the present study (Table 4) is in agreement with the findings of Lee and Halloran (1982). They also interpreted that the spotting is likely to be the result of plastid mutation than chromosome breakage or dominant or double recessive mutation.

The reduction in pollen fertility was dose dependent for the two mutagens in both the varieties used in the present study. Such reduction was more pronounced in the variety Ujwala than the variety Co -1 in both the mutagens (Table 5). The dependence of fertility on dose was also reported by Yamaguchi (1963), Sharma (1970), Goud *et al.* (1970) and Khan (1990). The pollen sterility induced by EMS appears to be either due to gene mutations, invisible deficiencies or both (Sato and Gaul, 1967). Chromosomal aberrations following EMS treatment have also been reported by Natarajan and Upadhya (1964), Grover and Virk (1986) and Kumar *et al.* (2003). However, most of the heritable pollen sterility after azide treatment must be attributed to gene changes since chromosome breaks are negligible following azide treatment (Kleinhofs *et al.*, 1978). According to Katayamma (1963), the pollen and seed fertility correspond approximately to the frequency of translocations showing adjacent segregation. The two varieties showed a differential response to both the mutagens. Similar influence of genic status on pollen sterility was also reported by Asha and Nayar (1986)

in *Capsicum*. The differential response of two varieties with regards to seedling injury and pollen sterility in the present study is in agreement with the observation of Singh *et al.* (1978) in *Pennisetum*. The genetic architecture of the organism is a potent factor in determining its response to mutagens (Chandola, 1968; Goud *et al.*, 1970).

The various types of meiotic chromosomal abnormalities observed in the PMC's of both the varieties of *C. annum* include, multivalents, stickiness, clumping, bridges, laggards, micro nuclei, non – synchronous separation, tripolar orientation and pentads. Such abnormalities were observed only in EMS treatments and exhibited a dose dependent increase in both the varieties (Table 6, 7 and Plate 3, 4). The dose dependent increase in meiotic abnormalities was earlier recorded in cereals (Swaminathan *et al.*, 1962; Reddy *et al.*, 1991) in mung bean (Ignacimuthu and Babu, 1989a) and in chilli (Sharma and Anis, 1994).

The mutagen induced structural alterations leads to various gene rearrangements and formation of multivalants. Reduction in chromosome pairing has been attributed to mutations in the genes governing homologous chromosome pairing and / or chromosomal structural changes (Acharia and Sinha, 1975; Reddy *et al.*, 1991). The univalent and multivalent formation was possibly the outcome of non or irregular pairing of chromosomes due to translocations (Katiyar, 1978).

Besides multivalents, chromosomal abnormalities such as laggards, bridges, micronuclei and non – synchronous separation were also observed.

The presence of bridges and laggards suggest that fragmentation and rejoining of broken ends of the chromosomes have occurred due to the acute effect of the mutagens. The formation of bridges can be due to the failure of chiasmata in a bivalent to terminalize and the chromosomes get stretched between the poles (Saylor and Smith, 1966).

The stickiness of chromosomes was also observed in both the varieties of *C. annum* in the present study. Similar results have also been reported by Abbasi and Anis (2002) in *Trigonella.* Stickiness could be due to depolymerization of nucleic acids caused by mutagenic treatment or due to partial dissociation of the nucleoproteins and alterations in their pattern of organization (Kumar *et al.*, 2003). Bhattacharya (1974) attributed the formation of laggards to chromosome spindle inhibition.

The non – synchronous movement of chromosomes was attributed to severe disturbance in spindle mechanism (Minija *et al.*, 1999). The chromatin bridges were known to occur when individual was either heterozygous for paracentric inversions or when chromosome breakage took place during the course of meiosis. The presence of laggards and fragments in high proportions has been attributed to the involvement of translocations in the formation of multivalents (Singh *et al.*, 1999). The occurrence of various types of meiotic aberrations in EMS treatments indicates the ability of this mutagen to induce structural changes in chromosomes in addition to gene mutations. The lack of chromosomal aberrations in SA treatments in the present study is in agreement with the findings of Sander *et al.*, (1978).

They reported that azide induced no chromosome aberrations in embryonic shoots of barley seeds, microspores of barley and human leucocytes. Sodium azide appears to be a point – mutation mutagen.

The occurrence of chlorophyll deficient seedlings in M_2 generation is very common in mutation studies. Though, the chlorophyll mutations are largely of theoretical interest in crop plants, the presence of such mutations will be useful for evaluating the genetic effects of mutagenic treatments. In the present study the chlorophyll mutants such as, albina, xantha, viridis, striata and maculata were recorded in the mutagen treated population (Table 8 and Plate 5). The frequency of such mutations was more in higher concentrations of mutagens. The EMS treatment was more effective in inducing high frequency of chlorophyll mutations with the increase in mutagen dose was reported by Gaul (1964) in barley, Nerker (1970) in *Lathyrus sativus*, Ignacimuthu and Babu (1988) and Khan and Siddiqui (1993) in mung bean. Occurrence of wider spectrum and high frequency of chlorophyll mutations at higher mutagenic level is perhaps due to the fact that both chromosome damage and somatic selection do not reach an optimum level (Campbell, 1966). Higher frequency of chlorophyll mutants in EMS could be explained on the basis of preferential action of EMS on chlorophyll development genes located near centromere (Swaminathan *et al.*, 1962) and / or preferential effect of EMS on Guanine in the G: C rich chloroplast genome (Lawley and Brooks, 1961). The spectrum of chlorophyll mutations was wider in the variety Co -1 than Ujwala. It indicates the

genotypic effect of induction of chlorophyll mutation. Goud *et al.* (1970) and Gupta and Yashvir (1975) reported that the genetic architecture of the organism is a potent factor in determining its response to mutagens. The low frequency and spectrum of chlorophyll mutations in SA treatments may be due to the lack of chromosomal aberrations or inefficiency of SA to act on specific sites of genes, which control chlorophyll development.

Various kinds of phenotypically visible, easily perceivable genetic alterations were observed after mutagen treatments. These morphological mutants were isolated on the basis of altered form, phenotype or morphological architecture. The frequency and spectrum of morphological mutants differed in different mutagenic treatments and also between both the varieties (Table 9 and Fig. 33, 34, 38, 40 - 48). The frequency and spectrum of such mutants were highest in the treated population of variety Co -1. It indicates a close relation between the chlorophyll mutations and morphological mutations in M_2 generation. Thakur and Sethi (1995) observed that the frequency of morphological mutants were 2-3 times more in chemical mutagenic treatments than physical mutagenic treatments in barley. Rajam and Subhash (1995) isolated different kinds of morphological mutants in M_2 generation of *Capsicum annum* and found that mutations induced by various treatments are of significance, since variants affecting almost every feature of the plant were observed in the treated population. Though, some of the mutants were associated with undesirable traits such

as reduction in yield, it could be overcome by trans-specific crosses with other cultivars or other mutants.

In the present study, the variety Co - 1 produced more frequency of morphological mutants than the variety Ujwala (Table 9). The variation of mutants produced by the two varieties indicates the role of genetic make up of plants in determining the mutational events. The difference in mutational events in two varieties could be due to the differential sensitivity of the mutant alleles responsible for various morphological characters.

Various workers isolated different types of morphological mutants of chilli. These include multilocular ovary (Rajam and Subbash, 1983) clustered bud mutant (Subhash *et al.*, 1981) erect fruit mutant (Rajam and Subbash, 1984), glossy-spinach, dwarf yellow and dwarf chlorotic mutant (Alcantara *et al.*, 1996). Raghuvanshi and Singh (1982) isolated five viable morphological mutants in chemical mutagen treated M_2 generation of *Capsicum annum*. Many such morphological mutants have been extensively studied in different crop plants such as *Phaseolus vulgaris* (Marghittu, 1972), *Pisum sativum* (Blixt, 1972) and *Cajanus cajan* (Chary and Bhalla, 1988). According to Gottschalk (1987), an agronomically useful trait is part of a pleiotropic pattern, so it cannot be used for breeding purposes in those cases where a negative trait belongs to the same pattern. However, the morphological mutants with negative selection value in the present study can be modified through cross breeding or selection by eliminating some undesirable traits.

The quantitative trait variations are due to cryptic gene mutations that influence form and / or function of organisms but such alterations can be initially detected and subsequently fixed through biometrical procedures. As majority of such mutations are in polygenically controlled traits, they are of greatest value to breeders since most of the economically useful traits are polygenically controlled. Frequency of useful induced mutants is higher via micro mutations than mega mutations (Kaul, 1989).

The assessment of the nature and extent of induced variations in yield contributing characters is very useful for the improvement of crops through selection process. In the present study, data on ten important quantitative characters were recorded and statistically analyzed. The study of shift in the mean value of a quantitative trait is important for ascertaining whether mutation can be restored for the improvement of the character under reference.

The data on ten quantitative characters were recorded and statistically analyzed to assess the extent of induced variability in M_2 generation (Tables 11 to 30) and M_3 generation (Table 31 to 50) of the two varieties of *Capsicum annum*. They include days to flowering, days to maturity, plant height, primary branches/ plant, secondary branches / plant, number of fruits / plant, fruit weight, fruit length, fruit width and total plant yield. Although, the mean shifted on either side of the control mean, the mean values of most of the yield contributing characters such as secondary branches / plant (Tables 19, 20, 39, 40), number of fruits / plant (Tables 21,

22, 41, 42) and yield / plant (Tables 29, 30, 49, 50) shifted towards the positive direction in the treated population. The increase in yield / plant is most probably related to the increase in the production of secondary branches. Patil *et al.* (1997) found a significant shift of mean values in a positive direction for secondary branches, number of fruits, length of fruits and fruit yield / plant after chemical mutagenesis in *Capsicum annum*, indicating tremendous scope for improving these characters. Mutations for polygenically controlled traits are obviously of the greatest importance in improving the desirable character. Brock (1965) postulated that in their direction, induced polygenic variation follows a path, which is opposite to that of the previous selection history. Gaul and Aastveit (1966), on the other hand, suggested that mutations are indeed random but that the mean shift in a direction is often associated with reduced vitality.

The increase in mean values of the important yield contributing characters may be due to the selection of normal looking plants, which might have resulted in high yielding mutants (Figs. 32, 49). . Scossiroli *et al.* (1966) also found an increase in the mean values of quantitative characters in irradiated populations of *Triticum* in M_2 and M_3 generations. They explained this change as a consequence of elimination of 'bad' genes. The induced variation in *Capsicum* after mutagenesis was earlier reported by Siddiqui and Azad (1998), Rajam and Subhash (1984), Saccardo and Vitale (1983), Ramalingam (1977a) and Bansal (1973).

The earliness in flowering in some of the treatments (Tables 11,12, 31, 32) can be attributed to the action of mutagens on genes, which regulate the metabolic pathway of floral hormone production. A decrease in the mean height of the plants is observed in the treated populations of the variety Ujwala (Table 15, 35). Whereas, the increase in mean height of the plants observed mainly in the treated population of the variety Co – 1 (Table 16, 36) is probably related to the increase in growth hormone synthesis. Earlier studies on mutation breeding in crop plants such as soybean (Papa *et al.*, 1961), wheat (Swaminathan, 1963) and barley (Gaul, 1967) brought out a reduction in mean values for plant yield when compared to the control. However, Tickoo and Jain (1979), Chaturvedi and Singh (1980) and Khan *et al.* (1999) reported a positive shift in mean in various crop plants. Similarly, Mehta *et al.* (1999) found a shift of mean in the positive direction in fruit yield and some other characters in *Capsicum annum.*

Brock (1965 and 1970) proposed a general hypothesis for the behavior of induced mutations in quantitatively inherited traits, according to which random mutations are expected to increase the variance and shift the mean away from the direction of previous selection history. The response of unselected character depends not only on its previous selection background but also on its genetic association with selected characters. Therefore, as per the hypothesis the mutagenic treatments are most likely to shift the mean in a negative direction. The shift in mean values in the positive direction for some characters indicates that the highest genetic potential for

these characters is to improve after the mutagenesis. The random shift in mean values also substantiates the fact of random nature of mutation in polygenically controlled traits.

Ramulu (1974) exercised selections for normal looking plants in M_2 generation of *Sorghum* and found an increase in mean values of the treated population in M_3 and M_4 generations in comparison with the control mean. The superior performance of most of the M_3 plants in the present study (Tables 31 – 50) may be due to the elimination of undesirable genes as a result of selection in M_2 generation (Table 11 – 30). Borojevic (1965), opinioned that an increase in M_3 means, is possibly in consequence of the purposeful elimination of all mutants, which produce abnormal spike morphology and fertility prior to M_3 generation.

Most of the genetic variability induced through mutagenesis, especially for quantitative characters are heritable and performance of selected genotypes are promising. The assessment of the variability in a mutated population is very much essential for better screening of desirable and economically beneficial mutant genes from the variable population. The quantitative characters studied in the present investigation showed a wide range of phenotypic variation. The magnitude of the phenotypic variation, however, does not reveal the relative amounts of heritable and non-heritable components of the variation. The estimates of genotypic coefficient are essential (Falconer, 1960; Kaul and Garg, 1979) since they indicate the degree of stability to the environmental fluctuations and the potential

transmissibility of a character from parent to offspring and from generation to generation. The estimation of genetic parameters such as phenotypic and genotypic coefficient of variation is one of the most acceptable methods to assess the magnitude of variability induced by the chemical mutagens in mutation experiments. These two genetic parameters showed an increase trend in almost all the mutagen treatments (Tables 11 – 30 and 31 – 50). The magnitude of such increase was prominent with regards to the most significant yield contributing traits like secondary branches/plant (Tables 19, 20, 39, 40) and number of fruits/plant (Tables 21, 22, 41, 42). It indicates the scope of further improvement of the varieties used in the present study through chemical mutagenesis. The intensity of variation in the genotypes was not same in the same treatment, such difference of variation was probably due to different genes or set of genes governing such characters. Such varietal variation caused by chemical mutagens in *Capsicum* was earlier reported by Solomatin (1976). The role of induced mutation to create useful genetic variations in quantitatively inherited traits had been well documented by Johnson *et al.* (1955), Tanisaka and Yamagata (1985) and Bhamburker and Bhalla (1983). The increase in genotypic variability and other genetic parameters were not dose related in both the varieties. Such response can be attributed to the random nature of mutation targeted at different gene loci. Lack of a consistent dose response relationship may be due to an additional uncontrolled environmental variation (Conger *et al.*, 1966). The pattern of induced variability may vary from character to character, primarily dependent upon the nature of inheritance and number of

genes involved (Khan, 1983). The low genotypic variance in some of the treatments was because of the recessive nature of the induced mutations and if wild type genes determine a parental character, the induced variation will be largely in the reverse direction (Tickoo and Chandra, 1999).

Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone. However, it is not necessary that a character showing high heritability will also exhibit high genetic advance (Johnson *et al.* 1955). The heritability is the heritable portion of phenotypic variance. It is a good index of the transmission of characters from parents to their offspring (Falconer, 1960). High heritability values were observed, especially in yield contributing traits in almost all the mutagenic treatments in both the varieties (Tables 19, 20, 21, 22, 29, 30, 39, 40, 41, 42, 49, 50). The increase in heritability values in the treated population is not related to the dose of mutagen, which may be due to polydirectional random nature of mutation. Moreover, the values of heritability depends on the magnitude of all the components of variance, a change in any one of these may affect it. The decrease in the heritability in some of the treatments (Table 50) can be attributed to the uncontrolled environmental influence or the changes in genotypic variance was not at par with the total phenotypic variance. The higher heritability values for yield and yield components were earlier reported by Rajput (1974), Khan (1979) and Khan and Siddiqui (1995). The higher estimated heritability for various

desirable characters in M_2 and also in M_3 generation indicates the further scope of selection for the genetic improvement of the two varieties of *C.annum* namely, Ujwala and Co – 1.

Genetic advance is the improvement in the mean genotypic values of selected plants over the parental population. It is the measure of genetic gain under selection. Johnson *et al.* (1955) emphasized that heritability estimates along with estimates of genetic advance are usually more helpful than heritability alone in predicting the resultant effects of selection. The estimated value of genetic advance in percentage of mean did not exhibit a definite trend with the increase in dose of mutagen. It varied in two varieties and also in different treatments (Tables 11 to 50). In general, the characters like plant height (Tables 15, 16, 35, 36), primary branches/plant (Tables 17, 18, 37, 38), secondary branches/plant (Tables 19, 20, 39, 40), number of fruits/plant (Tables 21, 22, 41, 42) fruit weight (Tables 23, 24, 43, 44) and fruit yield (Tables 29, 30, 49, 50) showed comparatively higher values of genetic advance than the other characters under consideration. In general, EMS treatment gave the maximum values of genetic advance however, few exceptions can also be found. The high heritability values along with high genetic advance are more responsive to the effective selection and improvement. The high values of heritability and genetic advance suggested that mutations have mostly occurred at the loci having additive effects (Lawrence, 1975). The high values of genetic advance are indicative of additive gene action and low values are indicative of non-additive gene

action. Genetic advance is indicative of the genetic progress for a particular trait under suitable selection procedure (Kaul, 1980).

The active principle for pungency in chilli is mainly capsaicin. The average capsaicin in Indian chilli varies about 0.2 to 0.3 percent. There are totally seven closely related alkyl anillyl amides, which are responsible for pungency in chilli (Hosmani, 1993). Tezpur variety (*Capsicum fruitescens* var. Nagahari) of chilli contains maximum capsaicin, which seems to be the hottest chilli known so far (Mathur *et al.*, 2000). The site of capsaicinoid synthesis is the fruit placenta (Iwai *et al.*, 1977).

In the present study, out of the five mutants analyzed for capsaicin content (Table 10), Tall with short fruit mutant contain comparatively high percentage of capsaicin (0.3%). Small sized fruits contain high concentration of capsaicin while long fruits contain low concentration of capsaicin. Fruits with thin pericarp contain higher capsaicin content as compared with varieties having thick pericarp (Hosmani, 1993). In the present investigation, the long thick fruit mutant contains comparatively low percentage of capsaicin (0.05%). The high capsaicin content of tall with short fruit mutant is most probably related to fruit size and nature of pericarp. The capsaicin content is also dependent upon placental content of chili fruits, more the placental tissue higher the capsaicin content (Ramanujan and Thirumalachar, 1966). Raghuvanshi and Kumar (1991) selected four chilli mutants with high capsaicin content after physical and chemical mutagenesis. The change in capsaicin content in chilli is also affected by

change in climate (Tiwari *et al.*, 2005). The degree of pungency varies, probably due to the presence of genes modifying the factor of pungency. Inheritance of capsaicin content in placental tissue is observed to be controlled by single dominant gene (Sharma and Saini, 1980). Whereas, Ramanujam and Thirumalachar (1967) were of the opinion that the inheritance of capsaicin was of polygenic nature.

In the light of above discussion, it can be concluded that various biological damages induced by the chemical mutagens *viz.* ethyl mathanesulphonate and sodium azide depend on the dose, type of mutagen and genotype. The various mutagenic treatments used in the present study were found to be very effective in inducing genetic variation, which create new gene combinations in a number of desirable traits. This may be probable reason for the selection of one potential mutant *viz.* talll with short fruit mutant in the M_3 generation having higher content of capsaicin (table 10). Therefore, the induction of mutation through chemical mutagens could be employed as the most effective tool to create quantitative changes in the genetic architecture of *Capsicum annum*.

CHAPTER - 6

SUMMARY

Capsicum, which belongs to the family Solanaceae, has a unique place in human diet as a condiment and as a vegetable. Considering the economic importance of this crop both in domestic and international market, an attempt was made to induce genetic variability by using two potent chemical mutagens *viz.*, ethyl methanesulphonate (EMS) and sodium azide (SA) in two varieties namely, Ujwala and Co – 1. The main objectives of this investigation are (1) to study the biological damage like inhibition in seed germination, seedling injury, cotyledonary abnormalities and pollen sterility in M_1 generation, (2) to analyze the frequency and spectrum of morphological and chlorophyll mutants in M_2 generation, (3) to select mutants having desirable characters in M_2 generation, (4) to isolate mutants in M_3 generation having superior characters and to estimate capsaicin content of some selected mutants and (5) to calculate mean, standard deviation, phenotypic and genotypic coefficient of variability, heritability and genetic advance of both control and treated populations in M_2 and M_3 generations in order to assess the extent of genetic variability induced by the mutagens.

A dose dependent increase in biological damage in M_1 generation as the decrease in seed germination, seedling height and pollen fertility was observed in both varieties after treatment with varying doses of EMS and SA, under study. Various types of meiotic chromosomal aberrations like multivalents, stickiness, clumping, bridges, laggards, tripolar orientation,

micronuclei, pentads and non synchronous separation of chromosomes were observed in PMC's of M_1 plants treated with different doses of EMS. The frequency of aberrations were also dose dependent and increased with the increase in mutagen doses. The variety Ujwala was more sensitive to such aberrations than the other variety Co – 1. The SA treatments could not induce any meiotic chromosomal aberrations in PMC's.

Various types of cotyledonary abnormalities such as seedlings with splitted cotyledonary leaves, three cotyledonary leaves, and cotyledonary leaves with white spots and cotyledonary leaves with yellow streaks were also recorded in M_1 generation.

These abnormalities were recorded in EMS treated populations of both the varieties and SA treated population of variety Co – 1. The frequency of such cotyledonary abnormalities was not dependent on the dose of mutagen.

A wide spectrum of chlorophyll mutants was obtained in M_2 generation. They include Albina, Xantha, Viridis, Striata and Maculata. All the chlorophyll deficient mutants were lethal except viridis, which showed stunted growth and produced few fruits. The variety Co – 1 has shown the greater frequency of chlorophyll mutation as compared to the variety Ujwala. The frequency of chlorophyll mutations was found to be higher at higher concentrations of both the mutagens. It was also found that EMS is very effective in inducing chlorophyll mutations than SA.

Twelve different types of morphological mutants were isolated from the M_2 and M_3 populations based on their easily detectable morphological alterations. These mutants were recovered from the mutagen treated population of both the varieties and their frequency of occurrence was not related to the dose. However, the frequencies of such mutants were comparatively high in the variety Co – 1. Though most of them are of negative selection value, a few of them can be utilized as a gene resource for further breeding programme by eliminating undesirable traits associated with them.

The capsaicin content of some selected mutants were estimated by HPLC method. The tall with short fruit mutant contain maximum capsaicin and pungency. There was no much increase in capsaicin content in other mutants. The long thick fruit mutant and viable chlorophyll deficient mutant contain very low percentage of capsaicin.

The assessment of the nature and extent of induced variations in yield contributing characters is very useful for the improvement of crops through selection process. In the present study, data on ten important quantitative characters in M_2 and M_3 generations were recorded and statistically analyzed. They include days to flowering, days to maturity, plant height, primary branches / plant, secondary branches / plant, number of fruits / plant, fruit weight, fruit length, fruit width and total plant yield. Although, the mean shifted on either side of the control mean, the mean values of most of the yield contributing characters such as secondary branches / plant,

number of fruits / plant and yield / plant shifted towards the positive direction in the treated population.

The quantitative characters studied in the present investigation showed a wide range of phenotypic and genotypic variation. These two genetic parameters showed an increasing trend in almost all the mutagen treatments. The magnitude of such increase was prominent with regards to the most significant yield contributing traits like secondary branches/plant and number of fruit/plant. The increase in genotypic variability and other genetic parameters were not dose related in both the varieties.

Heritability and genetic advance are important selection parameters. High heritability values were observed, especially in yield contributing traits in almost all the mutagenic treatments in both the varieties. The increase in heritability values in the treated population is not related to the increase in dose of mutagen.

Genetic advance is the improvement in the mean genotypic values of selected plants over the parental population. The estimated value of genetic advance in percentage of mean did not exhibit a definite trend with the increase in dose of mutagen. It varied in two varieties and also in different treatments. The characters like plant height, primary branches/plant, secondary branches/plant, fruits / plant and fruit yield showed comparatively higher values of genetic advance than the other characters under consideration. In general, EMS treatment gave the maximum values of genetic advance however, few exceptions can also be found.

The various mutagenic treatments used in the present study were found to be very effective in inducing genetic variation, which create new gene rearrangements in a number of desirable traits. The higher capsaicin content observed in tall with short fruit mutant in the M_3 generation substantiates this statement. The induced genetic variation was not related to the increase in dose of the mutagen whereas, the lethality and genotoxicity exhibited a linear increase with the dose. Therefore, the induction of mutation through chemical mutagens could be employed to create quantitative changes in the genetic architecture of the varieties of *Capsicum annum*.

CHAPTER - 7

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