IMPROVEMENT OF CHICKPEA (*CICER ARIETINUM* L.) THROUGH MUTATION BREEDING

Ph. D. THESIS

BY

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SEPTEMBER, 1997

DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA
DHAKA - 1000

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A THESIS
SUBMITTED TO THE UNIVERSITY OF DHAKA
IN FULFILMENT OF THE REQUIREMENTS OF
THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
PLANT BREEDING

SEPTEMBER, 1997

DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA
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DEDICATED TO THE MEMORY OF MY PARENTS

Acknowledgements

I wish to acknowledge my gratefulness to Professor M. Akhtaruzzaman, Department of Botany, University of Dhaka, Dr. M. A. Q. Shaikh, Director General, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh and Professor Dr. R. H. Sarker, Department of Botany, University of Dhaka for their constant help, guidance and encouragement during this research work.

I wish to thank the Chairman, Department of Botany, University of Dhaka and the authority of the University for their kind approval of my enrolment as a Ph. D. student. I would also like to thank Dr. M. Imdadul Hoque, Associate Professor and Dr. Sk. Shamimul Alam, Assistant Professor, Department of Botany, University of Dhaka for their helpful suggestions and inspirations.

I also express my sincere gratitude to Dr. M. Idris Ali, Director Research, BINA, Mymensingh for providing facilities to carry out the work at the Institute. I also express my sincere gratitude to Mr. Md. Ataur Rahman, Director (Admn.), Dr. M. A. Hamid, Chief Scientific Officer and Head, Plant Breeding Division, BINA, Mymensingh.

I am specially indebted to my colleagues, Dr. M. Ali Azam, Dr. M. L. Das, M. A. Majid, Principal Scientific Officer, Dr. M. A. Samad, Senior Scientific Officer, and Z. U. Ahmed Chief Scientific Officer, BINA who have assisted me cheerfully by reading and offering valuable comments on the entire manuscript.

The understanding and infinite amount of patience of my wife, Zakia Mursheda also made a significant contribution to this effort. I am also grateful to her.

My sincere thanks are due to many individuals on the staff of Plant Breeding Division of BINA, who have been most helpful during the entire period of work.

September, 1997

K. M. Shamsuzzaman

Certificate

This is to certify that the research work embodying the results reported in this thesis has been carried out under our guidance in the experimental fields of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, BINA sub-station, Ishurdi and BINA sub station, Magura.

It is further certified that the research work presented here is original and suitable for submission for the award of Ph. D. degree.

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Chapter I
INTRODUCTION

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important pulse crops in Indo-Pak-Bangladesh sub-continent being used as *dal* by majority of the people. Among all the continents, Asia contributes about 89% to the world production of chickpea (Jodha and Subba Rao, 1986). It has been well recognized as a valuable source of dietary protein for many peoples in the world. It is the third-most important pulse crops in Bangladesh (BBS,1996) and also the third-most important grain legumes in the world (van Rheenen, 1991). In many countries like, Algeria, Burma, Bangladesh, Iran, India, Mexico, Morocco, Pakistan, Spain, Syria, Tanzania, Tunisia and Turky, chickpea is an important source of protein in human diets, animal feeds and plays a significant role in the farming systems in these countries (Nour, 1986). It occupies a unique position in the world agriculture by virtue of its higher seed yield , higher seed protein content, capacity of fixing nitrogen from atmosphere and its adaptability to a wide range of environments. Chickpea has become absolutely necessary since it (a) provides the much needed protein to the vast majority of the people of the developing countries like Bangladesh, (b) supplements nutritious fodder to the mainly straw-based animals, (c) is an intregal part of the highly intensive cropping system, (d) is suitable for marginal farmers due to its low input requirements and (e) can fix nitrogen, thereby reducing the cost of production and improving the already depleted soil environment. In spite of its importance, the national productivity and per capita availability is very low, 7 g/day in Bangladesh (Khan *et al.*, 1981) compared to 49 g/day in India (Johl, 1984).

Increased demand for rice and wheat as a consequence of rapid population growth has been reducing the total acreage, production and even the per hectare productivity of grain legumes during the last decades (Shaikh *et al.*,1978; Shaikh,1988). Due to long crop duration, excessive vegetative growth and susceptibility to diseases and pests of chickpea are the main causes against its adaptation as a popular crop (Shamsuzzaman *et al.*, 1994). This is why, like other

pulses, chickpea production has been declining in Bangladesh (BBS, 1996) because of the problems similar to other pulse crops.

The rice-based diet of the people of Bangladesh contains minimal amounts of proteins and more so lysine and threonine (Oram *et al.*, 1987). Chickpea seeds contain considerable amount of proteins and vitamins (Singh, 1986), (Appendix-1). Its protein content is 3 to 4 times higher than the cereals. Chickpea proteins are nutritionally valuable for lysine and threonine (Williams and Singh, 1986). Therefore, the declining trend of its production is alarming because any further reduction in the lysine-rich legumes in the diet of the rural people may lead to serious amino acid imbalance (Oram *et al.*, 1987).

Generally lower yield of pulses than cereals (Jain, 1975) led to the assumption that the former may have a lower genetic potential for yield (Boulter, 1973; Swaminathan, 1973). But Jain (1975) reported that grain legumes have higher genetic potential for yield than the cereals. Therefore, unless its competitiveness in price and yield is substantially improved, it is likely to remain poor crop for poor people in poor environments (Saxena and Singh, 1986).

Yet chickpea had a long history of cultivation because of its economic importance. Among the pulses chickpea had been found in archeological collections. Even *ca.* 5450 BC it was cultivated at Hecilar near Burdur in Turky (Helbaek, 1970). This crop was mentioned in some ancient manuscripts. In Mesopotamia as long as 3000 BC chickpea was cultivated and it had local name *halluru* (van der Maesen, 1986). Thus it becomes that chickpea has been cultivated for at least 7,000 years (van der Maesen, 1972).

Man consciously or unconsciously, selected improved types of this plant as they have done with other crops. However, no recorded history of selection is available to indicate when, where or how the breeding efforts began. It is known that early in the

20th century, a planned breeding programme was initiated, perhaps at the Imperial (now Indian) Agricultural Research Institute, Pusa, India, around 1905 (Singh,1986).

In Bangladesh till now very little attention has been given to improve its yield potential. That is why it is facing high competition with the easily available HYVs of cereals, particularly *boro* rice and wheat. There is a tremendous diversion of land from winter pulses to cereals. The low yield potential of the existing varieties is one of the important constraints for poor production of chickpea. Further, it has been traditionally grown under marginal conditions of moistures stress, low fertility and poor management. Therefore, in this country it has been selected mainly for adaptation to low levels of management rather than for yield potential with better management.

Jain (1975) assumed that natural selection played a major role over human selection in determining their morphological, physiological and agronomic characters in pulses like chickpea. He pointed out that in view of the past history of selection, pulses have lost useful alleles as far as yield is concerned. On the other hand, Ho (1974) suggested that still enough genetic potential is present in most of the pulses. This may have been the case with chickpea. Therefore, the lost variability of this crop should be regenerated through extensive hybridization among genetic stocks collected from different parts of the world and also through induced mutations breeding techniques. In this connection it may be noted that through the later technique, 1790 varieties of 158 plant species were released in 52 countries in the world (Maluzynski *et al.*,1995).

Some previous workers tried to induce mutants of chickpea. For example, Ekbote (1942) reported tinny leaved, simple leaved, flower colour and seed coat colour mutations in chickpea. Balasubramanian (1951) reported 13 mutants which arose spontaneously in it. Chaudhuri *et al.* (1958) observed bronze foliage colour, chlorophyll and carotene mutant in this crop. In chickpea, Athwal (1963) and Enken (1963) used X-rays and gamma-rays respectively, to create variability. Among the chemical mutagens, sodium azide has been recently used (Nilan *et al.*, 1973 ; 1976;

Kleinhofs *et al.*, 1974; 1978; Shaikh *et al.*, 1983 and Shamsuzzaman *et al.*, 1994). Mutation breeding techniques were used in developing improved chickpea varieties (Shaikh *et al.*, 1982; Haq, 1983). Many induced mutant varieties of this crop have been released in the last few years through mutation breeding techniques (Anonymous,1991).

Hybridization is the most common tool for plant improvement. But it is difficult in chickpea, due to small flower size. Hence the approach of mutation breeding is more advisable in this crop. Induced mutagenesis may be of special use in adding qualitatively inherited characters (Swaminathan, 1968) as well as in broadening the genetic variability of quantitatively inherited characters (Gregory, 1955; Scossiroli, 1968; Krull and Frey, 1961; Brock, 1967; Gaul, 1961; Oka *et al.*, 1958; Mandal, 1974; Sarma, 1975; Sharma, 1977; Gupta *et al.*, 1996; Srivastava and Singh, 1996).

Thus it appears that planned mutagenesis can create more variability to cause a desirable shift in the mean values of the characters under consideration. Therefore, present investigation on chickpea has been undertaken to induce genes mutations, using physical and chemical mutagens, particularly early maturing, large seed size and higher seed yield.

The design of this work has the following aspects:

(i) To study the effect of mutagens in M_1 generation,

(ii) To analysis of M_2 population and selection of mutants with desirable characters,

(iii) To study the inheritance patterns of selected mutants in the M_3 generation and selection of true-breeding mutants, and

(iv) To evaluate the agronomic performances of the selected mutants in the preliminary observation trials in M_4 generation.

Chapter II
REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The literatures used in the present study is reviewed under the following heads:-

A. Concept of mutations

B. Methods of using mutations in plant breeding

C. Mutagenic agents used

D. Dose rate and effect of mutagens

E. Mutagenic effectiveness and efficiency

F. Application and scope of induced mutations

G. Macromutations

H. Micromutations

I. Heritability and genetic advance

J. Selection techniques of quantitative traits

K. Improvement in the existing cultivars through induced mutation

L. Selection of mutants in different generations

A. Concept of mutations

In 1901, the term mutation was first coined by Vries. Mutations are the ultimate source of variability in organisms. Mutations are of two types i.e., spontaneous and induced mutations. Later on, large number of physical and chemical mutagens have been found and their potency in lower and higher organisms have been described. Several mutagens proved to be effective for increasing genetic variability as a potent supplementary tool to develope new genotypes with desirable characters in plants. Direct use of mutations is present-day approach to plant breeding, particularly when it is desired to improve one or two easily identifiable characters in an well-adapted variety. Induced mutation has been intensively utilized by Muller (1927); Asseyeva (1927); Stadler (1928); Goodspeed (1929); Ekbote (1942); Gregory (1955); Gaul

(1961); Brock (1967); Mandal (1974); Shaikh *et al.* (1982); Hassan and Khan (1991) and many others in higher and lower organisms.

B. Methods of using mutations in plant breeding

Generally three methods of mutations are being used in the breeding procedure for improving lower and higher plants (Sigurbjornsson, 1977). These are (i) use of point mutations, (ii) use of chromosome mutations and (iii) use of mutagenic agents for special breeding problems.

C. Mutagenic agents used

Physical and chemical mutagens are being widely used to improve crop plants:

(i) Physical mutagens

Besides ultra-violet light, there are several types of ionizing radiations, namely X-rays and gamma-rays, alpha and beta particles, protons and neutrons. Each of these has in common the property of being able to discrete releases of energy called ionization or ion pairs as they pass through matter (Briggs and Constantin, 1977). The mechanism of interaction of ionizing radiation with the biological material has been known since the discovery of X-rays. Ionizing radiations lead to inhibition of mitosis, induce chromosomal aberrations. Point mutations effect like oxidation of double bonds, enzymatic inhibition and molecular polymerization. Because of the short wave length, gamma-ray possesses very high penetration power.

(a) Chemical effects of ionizing radiation

In the ionizing process positive radical ions and free electrons are produced. In biological systems the electron is trapped in the polar radical ion, which is both unstable and reactive, to react with other molecules or pass through interval rearrangement. In water solution the free electrons can polarize a number of water molecules and is called

a hydrated electron. If oxygen is present, hydrated electron can react to form H_2O_2 radicals. All the radical species and H_2O_2 can react with solute molecules and result in chemical changes similar to those taking place after a direct ionization of a molecule (Ahnstrom, 1968a; Ahnstrom, 1968b).

(b) Reproductive and interphase death

Lethal action of ionizing radiations on cell usually measured as the loss of mitotic activity. Indirect evidence that this is the result of chromosomal aberrations has been obtained in several systems. A direct proof was given by Grote and Revel (1972) who has seen under the microscope followed the fate of irradiated single *hamster* cell. Interphase death is accompanied by a leakage of various components out into the medium; for example, one can detect the release of histrones and nuclear enzymes from the nucleus of irradiated cells.

(c) Damage and repair of DNA

Different types of chemical changes are induced by ionizing radiations and this has made it difficult to sort out which are the biologically most important changes. Irradiation of DNA either in solution in solid state, produces a number of chemical changes, the same ones as can observed after irradiation of the sample nucleotides. Release of phosphate and bases can be observed. The bases are attached by deamination, by breaking up of the cycle structure and formation peroxides. In deoxiribose the oxidation of the alcohol groups can take place, breakage of carbon bonds is also common (Ahnstrom, 1968; Ahnstrom, 1968). The direct effect of ionizing radiations on the DNA molecule includes the rupturing of hydrogen bonds linking the base pairs, induction of breaks in one or both the DNA chains and cross linking within as well as between the two DNA strands and protein which damage the genetic apparatus viz., chromosomes, ribosomes, mRNA and some enzymes. For long time it has been clear that the primary damage induced by ionizing radiation is modified in

enzymatic repair processes and only a small fraction of the damage remains to realized in a biological chain (Lea, 1955).

The indirect effects of radiations results from the fact that the living tissues contain about 75% water generating free radicals, which are considered to be a major cause for mutation induction.

(ii) Chemical mutagens

During the Second World War, discovery of chemical mutagen was another milestone in the history of induced mutations. Though there were few earlier reports but Auerbach (1943), Oehlkers (1943) and Rapoport (1946) at first demonstrated the mutagenic effect of some chemicals. The number of chemical mutagens is much greater than physical mutagens and is continuously increasing. However, for practical purposes of mutation induction in cultivated plants, so far only a few have proved really useful. Ethyl methanesulphonate (EMS), diethyl sulphonate (dES), ethyleneimine (EI), ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH) and methyl nitroso urea (MNH) and azides are also common and effective mutagens (Heslot, 1959; 1962; 1977). Azide is an effective mutagen under certain treatment conditions. It is possible to obtain high mutation frequencies, most of the apparently gene mutations with negligible frequency of chromosome aberrations (Kleinhofs *et al.*, 1974). Although a potent mutagen, it is relatively safe, non-persistant (Smith, 1966) and inexpensive. Azide in acid solution was found to be very effective in inducing chlorophyll- deficient as well as morphological mutations it seems ineffective (Kleinhofs *et al.*, 1974). Presoaking in O_2 bubbled water also enhanced mutagenicitiy. The exact mode of azide as a mutagen is not well understood yet (Nilan, *et al.*, 1976; Heslot, 1977). On the other hand, the mutagenic action of sodium azide appears to be unique among the mutagens at present known (Kleinhofs *et al.*, 1978). At low P^H

uncharged hydrogen azide (HN_3) molecule forms, which can penetrate cell membrane with greater extent than the N^-_3 ion, thus producing more mutations.

Azide induced few, chromosome aberration in barley (Choudhary and Kaul, 1976; Nilan *et al.*,1973; Sideris *et al.*,1973) and in soybean (Vig, 1973). Sodium azide induced more micromutations in mungbean at low P^H (Mahapatra, 1983). It also induced flower colour mutations in chickpea (Shaikh *et al.*, 1983).

D. Dose rate and effect

The dose rate of radiation and chemical mutagens have significant effect in both qualitative and quantitative characters. The dose of a chemical mutagen treatment is made up of concentration, duration of treatment and temperature during treatment. Dose effect of physical and chemical mutagen on germination, growth, sterility and survival has been demonstrated by Rajput and Siddiqui (1982) in soybean, Nalampang and Jan-on (1982) in blackgram, Balaravi (1982) in pigeonpea, Sharma and Sharma (1983) in lentil, Patil and Bohra (1961) in peanuts, Santos (1965) in *Phaseolus aureus* and Shaikh *et al.*(1983) in pulses. Germination decline was reported after treatment with X-ray in pea (Blixt and Mossberg ,1967) NEU in oat (Shevtsov, 1969), gamma rays in pigeon pea (Balaravi, 1982), NMU and gamma-rays in lentil (Sharma, 1977) and sodium azide in mungbean (Mahapatra, 1983). Blixt (1964) observed positive correlation between seedling injury and survival in pea following treatment with different mutagens.

E. Mutagenic effectiveness and efficiency

Effectiveness relates to the rate of mutation as related to dose while efficiency is refereed to the mutation rate in relation to damage. Extensive studies on mutagenic effectiveness and efficiency of various mutagen have been carried out by Konzak *et al.* (1965). Sharma and Sharma (1979) reported that NMU was almost three times more

effective than gamma rays in lentil. Its efficiency was also 1.5-2.0 times higher than that of gamma rays when chlorophyll and morphological mutations or both together were considered. Prasad (1972) compared the mutagenic effectiveness and efficiency of gamma rays, EMS, NMU and NMG in *Triticum durum* and concluded that NMU was the most reasonably effective mutagen. Nerkar (1977) observed a different situation for both the parameters in *Lathyrus sativus*. Effectiveness was found to be the highest with NMU and the order NMU > EMS > gamma-ray. But in respect of efficiency, gamma -rays was superior than all other mutagens and the order was gamma-ray > EMS > NMU. Rathnaswamy and Kamalanathan(1978), in contrast with the views of some earlier works, observed that for both the parameters, gamma rays were better than EMS. According to Mahapatra (1983) sodium azide (low P^H) was more effective and efficient than gamma rays, EMS and NMU.

F. Application and scope of induced mutations in plant breeding

In plant breeding there are two possibility for using induced mutations. These are (a) the propagation of a desired mutant without further genetic manipulation and (b) the use of such mutant in a cross breeding programme. Gustafsson (1947) indicated that most induced mutations are of negative selection value. However, several artificial mutants have proved to be superior to the parent.

(i) Mutations are particularly of aids to the domestication of natural species. Many genes are disadvantageous in agricultural situations. Where they occur as dominant alleles in the wild plant their activity is easily destroyed by mutation to recessive alleles or suppressed by modification of the other genes (Brock, 1977).

(ii) In adding simply inherited characters to a well-adapted genotype (Pal and Swaminathan,1960; Varughese and Swaminathan, 1968).

(iii) In diploidization of artificial polyploid by promoting preferential pairing arising from differential affinity (Stebbins, 1960).

(iv) In breaking up close undesirable linkages or gene blocks (Sears, 1956).

(v) In changing the pleotropic gene effect, thereby modifying the association of undesirable correlated characters (Falconer, 1981).

(vi) In adding entirely new, unknown hereditary variability when natural resources are exhausted (Swaminathan, 1968).

(vii) In enhancing the genetic variance for quantitative characters, thereby creating scope for selection advance (Gregory, 1955; Scossiroli, 1968; Krull and Frey, 1961; Gaul, 1961; 1965 and Rawlings *et al.*, 1958). Gaul (1961; 1965) classified mutations into macro-mutations and micro-mutations. Swaminathan (1963; 1965) broadly grouped induced mutations into following four types according to the degree of changes in phenotype and method of detection.

(a) Macro-mutations: They involve change in a whole constellation of characters and have a visible phenotypic expression. These can be recognized at single plant level.

(b) Mutations affecting single character: The change brought about an effects an easily detectable single trait, thus rectifying defects in a variety without disturbing the otherwise desirable genetic architecture of the plant.

(c) Systematic mutations: Macro-mutations which either simulate an already existing taxon or necessitate the creation of a new taxonomic unit.

(d) Micro-mutations: These involve changes in quantitative characters and are utmost important to plant breeders. Such mutations affecting one or several genes can be detected only through the use of suitable biometrical procedure.

G. Macro-mutations

The easily identifiable mutations are often called "macro-mutations" (Swaminathan, 1965). It occurs when major genes of a character are mutated. Many attempts have been made from time to time to induced and select beneficial major gene mutations. Some typical macro-mutants of self-pollinating crops are erectiodes, dwarfs and semi dwarfs, speltoids, early, barbed, smooth awned, disease resistant types and

other drastic changes. Delauny (1934) and Sapehin (1936) reported short straw and physiological mutations in wheat and also showed that mutation of major gene have direct application in crop improvement. Early ripening, early flowering and high yielding mutant in chickpea by Ivannikov and Moraru (1968). Dwarf, compact upright and gigas mutant were selected in chickpea and carried further for the development of pure lines (Kharkwal, 1981). High yield-high protein chickpea mutant variety Hyprosola was released by Shaikh *et al.*(1982). Shamsuzzaman and Shaikh (1991) obtained early maturing and high yielding mutant of chickpea.

(i) Chlorophyll mutations

Chlorophyll mutants or colour mutants serve as a phenotypic test in judging mutagenic efficiency. It has importance in horticultural crops and ornamental plants. Tallenaar (1938) was first to isolate a light green tobacco mutant after X-ray treatment. Very few chlorophyll mutants were released for commercial cultivation. Frequency study of chlorophyll mutation has been carried out by many workers in chickpea (Lysikov *et al.*, 1967; Bhapkar and Patil, 1962). Athwal (1963) obtained 55% chlorophyll mutants, most of them survived a few days; other developed chlorophyll as they green , while some appearing normal at first and than lost chlorophyll in chickpea through X-ray irradiation. Athwal *et al.*(1970) reported that several albino and sterile mutants were found in the M_2 generation from gamma ray irradiated seeds of chickpea.

(ii) Viable mutations

The polyploids give rise to a much higher frequency of viable mutations than their diploid analogues (Mackey, 1959; Swaminathan, 1963). In autotetraploids the frequency of viable mutations increases in M_3 and later generations.

H. Micro-mutations

Mutation occuring in a character which is controled by polygenes i.e., where affect of minor genes is of very little contribution towards the pleiotropic expression is known as micro-mutation. Micro-mutants can be detected through the analysis of variance in the M_2 and subsequent generations with the help of biometrical methods. Morgan (1912) stressed that mutation with minor effects occur in *Drosophila.* Johannsen (1913) first described micromutations in bean plants. However, the applied value of micromutation in plant breeding was shown by Knapp (1950) in barley. Many authors reported that induced mutations could shift the mean value of the quantitative characters towards negative direction. It was observed that the reduction in mean yield and increase in genetic variance depend on the dose applied in barley (Gaul, 1965). Goud (1967) reported the effect of physical and chemical mutagens on tiller number and yield in six varieties of wheat. Bhatti *et al.* (1970) showed that gamma-ray irradiated populations increased some quantitative characters, pods/plant, seeds/plant and seed yield/plant which could be exploited in the crop improvement. Mandal (1974) reported that reduction in per cent of germination and plant survival and increase in pollen sterility and different types of plant mutations were observed. It had been also reported that combined treatment was more effective than chemical mutagen alone. Dry seeds of chickpea cultivar C727 were irradiated with 20 kR gamma rays, very early flowering mutant was identified by Haq *et al.* (1989). The trait was stable in M_3- M_6. Shaikh *et al.* (1982) developed a high yielding and high protein mutant variety of chickpea. Oram *et al.* (1987) reported isozyme similarity and genetic differences in morphology between a mutant variety, Hyprosola and its parental cultivar, Faridpur-1.

Dhopte *et al.* (1974) used gamma rays in gram and reported stomatal frequency per square changes, stomatal index, length of epidermal layers, thickness, leaflet area, respiration, chlorophyll content and extra colour and water content. It also changed in

seed protein content, 100-seed weight and seed yield. Five selected lines of chickpea were exposed to different doses of gamma rays and a series of treatment with N-nitroso-N-methyl Urethane and N-methyl-N-nitroso quinidine. Desirable mutants like erect, multiseed, increased podding and improved grain quality were scored and reported by Kalia *et al.* (1981). Rao (1988) noted various effects in the M_1 and M_2 an early flowering and high yield mutant in chickpea through different doses of gamma rays. Hassan and Khan (1991) obtained semi erect growth habit, two weeks early maturing, moderately resistant to *ascochyta* blight and produced higher seed yield through 10 kR gamma-ray radiation of chickpea variety, 6153.

I. Heritability and genetic advance

The heritability potentiality of an individual to inherit a particular trait in the offspring. It may be measured in two ways. In broad sense, the fraction of the total phenotypic variance that remains after exclusion of the variance due to environmental effects. In narrow sense, the ratio of the additive genetic variance to the total phenotypic variance. So, the heritability can be defined as ability of a variety to inherit to the progenies by ignoring the environmental effects (Allard, 1960). Heritability and genetic advance are very important parameters from breeding point of view. They can give an idea of expected effectiveness of selection in the material to be carried out. Yield is highly dependent on the other characters. All these characters are influenced more or less of the surrounding environment. Therefore, the observed variability are partitioned into heritable and non-heritable components by mean of estimation of genetic parameters (Kandasamy *et al.,* 1989). Patil and Phandis (1977) reported in chickpea that eight quantitative characters showed high heritability (broad sense) and high expected genetic advance for pods per plant, pod weight per plant and 100-seed weight. Adhikari and Pandey (1982) reported in 36 genetically diverse lines of chickpea that yield and 14 yield related characters were showed high heritability and genetic

advance. Johnson *et al.* (1955) suggested that estimates of heritability along with genetic advance are normally more useful than heritability alone for predicting the progress of selecting the best individuals.

J. Selection practices for improvement in quantitative parameters

In general, creation of variability in a population and its selection are the prime objectives of any plant breeding work. Particularly in mutation breeding work, selection practices showed be done with an appropriate material (Robbelen, 1981).

(i) Selection of parents

During the selection of parent variety in which mutations will be induced following points should be considered (a) best adapted variety in a location, (b) a recently released variety, (c) advanced promising lines going to be released and (d) introduced variety having restriction for release by specific limitations e.g. susceptibility to a specific disease, pest, lodging etc. (Sigurbjornsson and Micke, 1974).

(ii) Selection of mutagens, doses and replications

Physical and chemical mutagen are generally used to create variability in a particular crop variety. Concentrations or doses should be selected about 20% higher and lower of the estimated dose range (Konzak and Mikaelson, 1977). At least two replications are suggested to minimize the error.

(iii) Selection methods for mutants

Method for mutant selection are generally suggested three types i.e., (a) Visual method: Visual selections are the most effective and efficient methods for identifying mutant phenotypes, (b) Mechanical or physical method: This method can be used in mutation breeding for selecting seed size, shape, weight etc., (c) Other selection

method: Chemical and biochemical, physiological and other screening methods are needed for selecting different kinds of mutants.

K. Improvement in the existing cultivars through induced mutation

(i) Yield

Yield is a quantitative characters and highly dependent on others and is also influenced by environmental factors. It shows continuous variation. In spite, positive yield mutants are very rare. But a large number of high yielding mutant varieties has been developed and released for commercial cultivation in many countries (Sigurbjornsson and Micke, 1974; Maluszynski *et al.*, 1995)

(ii) Plant maturity

Mutants with early flowering or ripening time have been reported in numerous experiments (Rao, 1988) and number of early maturing mutant cultivars have been released (Sigurbjornsson and Micke, 1974; Shamsuzzaman and Shaikh, 1991). Early flowering or maturity mutations are less frequent than late ones (Gustafsson, 1947; Yagamata and Syakudo, 1960; Scarascia-Mugnozza *et al.*, 1961; Kawai, 1963a; 1963b). It has been reported that correction between total yield (grain and straw) and ripening time is positive but correlation between proportion of grain yield to total yield is negative in early ripening barley mutants (Scholz, 1957; Aastveit, 1965). In the evaluation of the practical value of early maturing mutations, yield per day during their growth period should be taken into account.

(iii) Plant type and growth habit

The most distinct change of growth habit observed in mutant is ageotropism or laziness. Ageotropic or lazy mutants have been found in lupins (Tedin and Hagberg,

1952), in peas (Blixt *et al,* 1958; Gelin, 1960; Blixt, 1970) and in chickpea (Kharkwal, 1981). Prostrate, spreading, sparse and rossete-like types, which are more or less similar to lazy type in appearance are induced in many plants. Erect and upright mutants have been also reported in numerous plants.

(iv) Disease and pest resistance

Disease resistance varieties are key factors in increasing yield and keeping crop production safe from epiphytotic attack. The development of resistance varieties is a means of controlling many diseases and, therefore, has become a common objectives in plant breeding programmes. The first report on the induction of mutations for disease resistance is reported in *Haisa* barley to three races of powdery mildew in Germany through X-ray treatment (Freisleben and Lein, 1942). Many disease resistant varieties/ lines of chickpea were developed through mutation breeding (Malik and Tofail, 1981; Haq *et al.,* 1988; Hassan and Khan, 1991). Information about mutation induction for resistance to insects and other plagues is very rare, in spite of the fact that the genetic variability in the host is important (Painter, 1951). Few workers reported in seasame resistant to galls fly (Jacob, 1955), cotton resistant to *Emposea devantans* (Swaminathan, 1965), barley. Resistant to root-knot nematode *Meloidogyne javanica* was obtained in chickpea through induced mutations (Bhatnagar *et al.,* 1988)

L. Selection of mutants in different generations

Donini and Mugnozza (1968) selected of mutants of *durum* wheat in M_2 and M_3 generations. According to their procedure, selection of mutants in M_3 generation was dictated by the observation that in same experiments, especially with chemical mutagens, the M_1 mutated sector size was too small to allow the segregation in M_2 of recessive mutants. It was ascertained that the number of mutations first isolated in M_3 equaled or sometimes exceed the number of mutations recorded in M_2 generation.

Palenzona (1965) reported that the selection in M_3 generation of wheat is more efficient than in M_2 generation. But Scossiroli *et al.* (1966) did not find any difference between M_2 and M_3 generations.

Rutzer *et al.* (1976) practiced plant wise harvesting in M_1 generation. In the following generation (M_2) selection was started for short-statured and early maturing rice

plants with high yield potentials. In M_3 generation few promising mutants were obtained which bred true like M_2 generation. Ultimately, a semi dwarf stable rice cultivar was developed with 15% higher yield than the control. In addition, the first mutant cultivar, Reimei was released in Japan and 21 additional cultivars were developed (Kawai and Amano, 1991). Robbelen (1981) also indicated that mutations in M_2 generation.

Kharkwal (1983) gave priority on the selection of micro-mutations in M_2 generation. He opined that the selected families should have higher co-efficient of variation and higher mean values as well. Using the mean and variance at interfamily and intrafamily levels most of the induced variability was rigorously rejected in M_2 itself. Only the families showing definite superiority over the control were retained for advancing generation. Sarker (1985) followed similar selection techniques for improvement of quantitative characters in lentil.

Kamala and Sasikala (1985) detected four gamma-ray induced high yielding mutants of sesame in M_2 generation. These mutants gave 3-30% more seed yield and 5-13% more oil content than the control. One mutant had whirled-capsule phyllotaxy and another had normal alternate capsule set up. The breeding behavior of these mutants was studied up to M_4 from single-plant progenies in each generation. High seed yields were recorded in all the generations. Reciprocal crosses involving the mutants and control did not differ much, showing lack of any maternal influence on seed yield. Similarly, Murty (1980) started selecting mutants in M_2-M_4 generations. All the selected mutants of M_2 and M_4 were grown in plant to progenies and true breeding

behavior of these mutants were evaluated. Ozbek *et al.* (1991) selected 4320 single plants of cotton in M_2 generation. They further screened these mutants and selected 160 mutants in M_3 generation and made a comparative study between the mutants and control in M_4 generation and finally evaluated 48 mutants as promising for further trial.

Pathirana (1990) used to practice collection of seeds from the first formed five capsules of each surviving M_1 plants and bulked the seeds treatment-wise. In M_2 generation plants were grown on the plant-progeny basis along with control. The surviving plants were harvested individually in the M_3 and M_4 generations, and on an M_2 plant progeny basis, were grown following randomized complete block design with replications. Pathirana (1992) also reported that seed colour of sesame was inherited by single gene and he obtained brown, cream and white coloured seeds by treating black coloured seeds of a local sesame variety with gamma-rays.

Rahman *et al.* (1992) reported that two yellow seeded mustard varieties were developed by using gamma rays treatment. Seeds from the first formed 10 pods of each M_1 plants at harvest were collected to grow M_2 population in the next year. They selected mutants with increased number of pods/plant, resistance of *Alternaria* disease and high seed yield in M_2. The selected plants were screened for seed-oil content, determined by NMR techniques in the laboratory and further selection was made by studying the breeding behavior of the plant population in M_3 generation. Three most promising mutants were tested in M_4 generation. Chauhan and Kumar (1987) obtained chocolate coloured seed mutant with improved seed yield and yield contributing characters from a yellow seeded mother variety of *Brassica campestris* at 50 kR dose in M_1 generation which bred true in the subsequent generations. Induced mutation could also change the flower colours (Shaikh *et al.*, 1983; Abo-Hegazi, 1991; Datta, 1991). Ivannikov and Moraru (1968) isolated morphological mutants in M_1 and M_2 generation after treatment with gamma rays and NMU (N-nitro-methyl urea).

Shaikh *et al.* (1982) started selection of mutants of chickpea in M_2 generation. Seed yields and protein contents of the true breeding, high yielding mutant types were monitored during the M_3, M_4 and M_5 generations. A chickpea mutant was identified with higher protein content, i.e., it had 4% more proteint and also with 19% higher seed yield than the mother variety, Faridpur -1. It produced 45% more protein per unit area than the mother variety. Haq and Shakoor (1980) selected mutants in M_2 generation of chickpea for screening against *Ascochyta* blight. Chickpea mutants were found in X_2 generation, especially after treatment with 3000r (Athwal, 1963).

M. Chickpea varieties released through mutations in Indo-Pak-Bangladesh

So, far it was known that a total of 7 mutant varieties of chickpea were released in Bangladesh , Pakistan and India through mutation breeding techniques (Table 2.1).

Table 2.1. Varieties of chickpea released through mutation breeding upto 1990 (Source: Anonymous, 1991)

Mutant Varieties	Country	Year	Mutagens	Main characters
Hyprosola	Bangladesh	1981	Gamma-rays	High-yield-high-protein
CM 72	Pakistan	1983	Gamma-rays	Blight resistance
Kiran	India	1984	Neutrons	Erectoid types
Pusa 408	India	1985	Gamma-rays	Higher yield
Pusa 413	India	1985	Gamma-rays	Higher yield
Pusa 417	India	1985	Gamma-rays	Higher yield
NIFA 88	Pakistan	1990	Gamma-rays	Blight resistance

Chapter III
MATERIALS AND METHODS

III. MATERIALS AND METHODS

The work presented in this dissertation are based on the study for improving chickpea genotypes through induced mutation breeding techniques. The investigations were carried out at the Plant Breeding Division of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during 1992-1996.

A. Materials

(i) Seed

The experimental materials were the seeds of two exotic strains G-319 and G-97 obtained from the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), India, and a mutant variety, Hyprosola developed at BINA, Mymensingh, Bangladesh. These seeds were purified by controlled inbreeding and single plant selection. The seeds had 98-100% germinability before treatment. Morphological characteristics of the strains and variety are described below.

Strain G-319 **(Plates 1a and 2a)**

Plant habit : Annual, semi errect, plant height 58-63 cm (at normal soil and climatic conditions), herbacious, stem hard and tap root with many lateral roots. The strain is not well adapted in Bangladesh climatic conditions.

Branch : Profusely branched.

Leaf : Light green in colour, allternete, leaflet deeply toothed, ovate-shaped with acute tip.

Flower : Papilionaceous and zygomorphic, infloresence two -flowered raceme developing in the axil of leaf.

Sepals : Calyx tube (5) gamocepalous, lanceolate, green .

Petals : Corolla 5, vaxilary in aestivation, colour white background with shades of purple, blue and pink.

Androecium : Stamens 10, diadelphous (9) + 1, anthers bilobed, orange coloured and basifixed.

Gynoecium : Stigma 1, ovary superior, sessile and oval with a terminal, slightly bent style.

Fruit : Pod shape roughly oval, large size, surface wheat-straw colour.

Seeds : Beak shaped, semi round and wrinkled with attractive seed colour and large seed size (100-seed weight= 26.0- 26.5 gm).

Maturity : It matures about 19-20 weeks after sowing at normal soil and climatic conditions.

Strain G-97 **(Plates 1b and 2b)**

Plant habit : Annual, errect, plant height 60-65 cm (at normal soil and climatic conditions), herbacious, stem hard and well developed tap root with many lateral roots.

Branch : Less branched.

Leaf : Deep green in colour, allternate, leaflet deeply toothed, ovate shaped with acute tip.

Flower : Papilionaceous and zygomorphic, infloresence two or three flowered raceme developing in axil of leaf.

Sepals : Calyx tube (5) is gamocepalous, lanceolate, green .

Petals : Corolla 5, vaxilary in aestivation, colour-white background with shades of blue and pink.

Androecium : Stamens 10, diadelphous (9) + 1, anthers bilobed, orange coloured and basifixed.

Gynoecium : Stigma 1, the ovary superior, sessile and oval with a terminal, slightly bent style.

Fruit : Pod shape roughly oval, medium size, surface smoot with wheat- straw colour.

Seeds : Beak shaped, semi round and wrinkled with attractive seed colour and medium seed size (100-seed weight= 17.0- 17.5g).

Maturity : It matures about 19-20 weeks after sowing at normal soil and climatic conditions.

Variety Hyprosola **(Plates 1c and 2c)**

Plant habit : Annual, bushy, (plant height 50-55 cm at normal soil and climatic conditions), herbacious, annual, stem hard and well developed tap root with lateral roots.

Branch : Profusely branched.

Leaf : Deep green in colour, allternete, leaflet deeply toothed, ovate-shape with acute tip.

Flower : Papilionaceous and zygomorphic, infloresence two-flowered raceme developing in the axil of leaf.

Sepals : Calyx tube (5) is gamocepalous, lanceolate, green .

Petals : Corolla 5, vaxilary in aestivation, colour-white back ground with shades of purple, blue and pink.

Androecium : Stamens 10, diadelphous (9) +1, anthers are bilobed, orange colour and basifixed.

Gynoecium : Stigma 1, the ovary superior, sessile and oval with a terminal, slight bent style.

Fruit : Pod shape roughly longer, very small size, surface of the friut is wheat-straw colour.

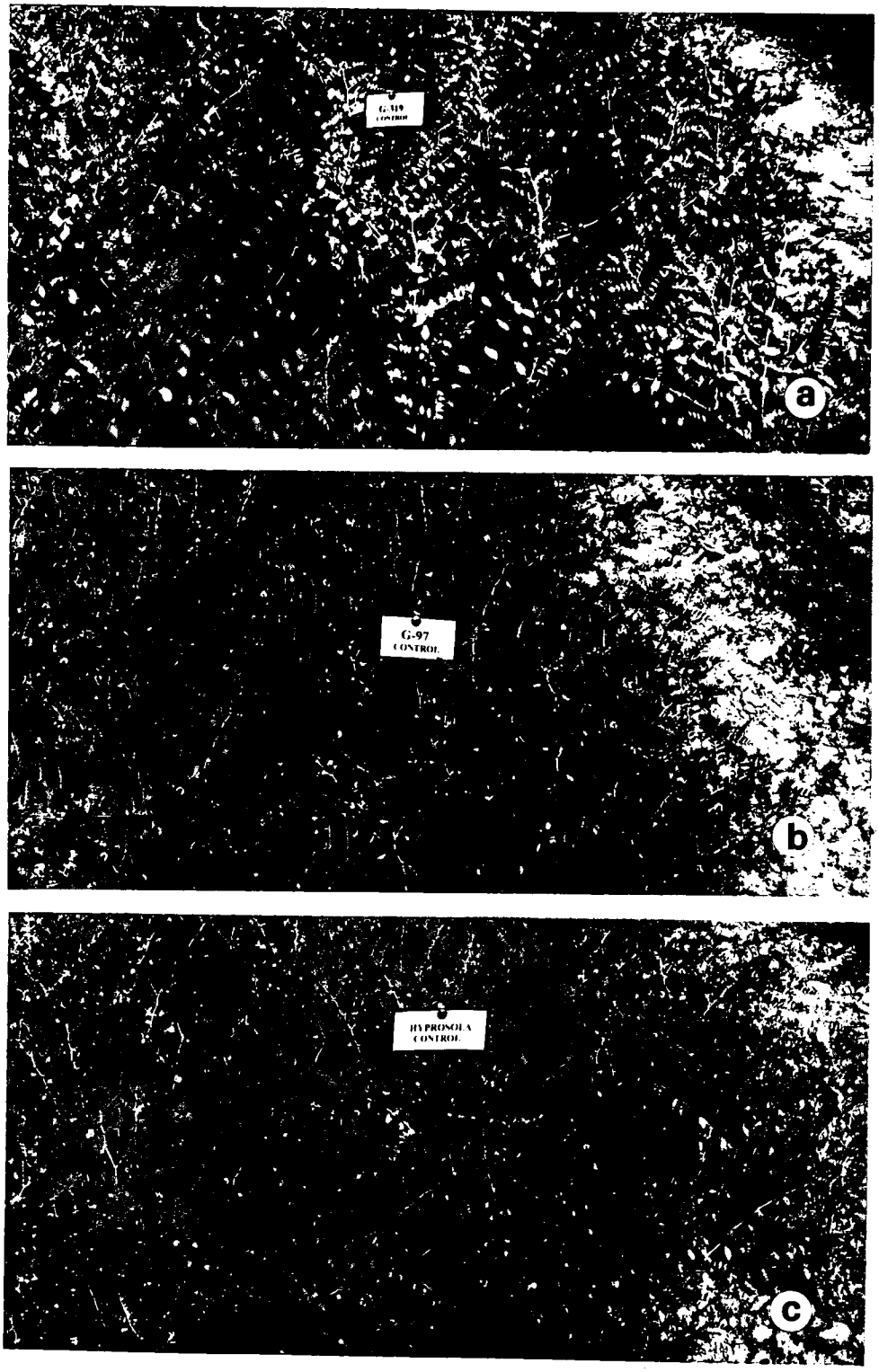


Plate 1. Field view of three chickpea genotypes: a (G-319), b (G-97) and c (Hyprosola)

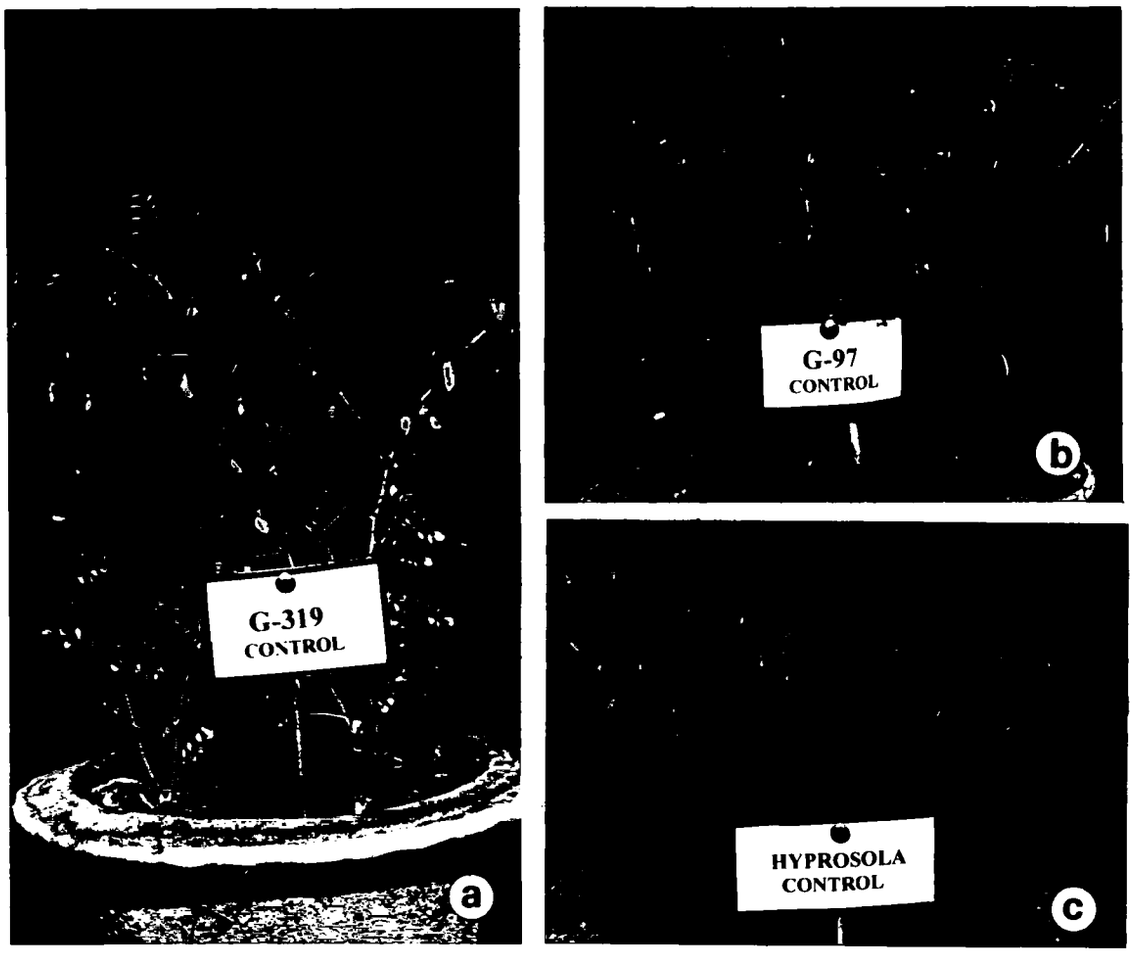


Plate 2. Chickpea plants grown in pots: a (G-319), b (G-97) and c(Hyprosola)

Seeds : Beak shape, semi round and wrinkled with dark seed colour and small seed size (100-seed weight= 7.0-7.5 g).

Maturity : It matures about 18-19 weeks after sowing at normal soil and climatic conditions.

(ii) Mutagens

Two mutagenic agents in different doses/concentrations were used in this investigation. These were a physical mutagen, gamma rays from ^{60}Co source and a chemical mutagen, sodium azide (NaN_3). Sources and mode of action of the mutagens are given in Table 3.1.

Table 3.1 . Sources and characteristics of the mutagens

Mutagen	Source	Description	Functional group	Mode of action
Gamma rays	IFRB, Savar	Short wave length	----	Ionization
Sodium azide	BINA, Mymensingh	Monofuctional NaN_3	Azide	Base modification

B. Methods

Dry, clean and homogeneous air-dried seeds with about 12% moisture content were used. The details of the treatments with mutagens are summarized in Table 3.2.

(i) Treatment procedures

(a) Gamma rays (Physical mutagen)

For each treatment four hundred air dried seeds (about 12% moisture content) of G-319, G-97 and Hyprosola were irradiated with 100, 200, and 300 Gy ^{60}Co gamma rays from the Institute of Food and Radiation Biology, Savar, Dhaka in 1992.

(b) Sodium azide (Chemical mutagen)

For each treatment 400 seeds with about 12% moisture content were treated with 0.2, 0.3 and 0.4mM concentration of sodium azide for inducing mutations. Optimum doses of sodium azide for chickpea were worked out by Shaikh *et al.* (1983). The seeds were first soaked in distilled water for 6 hours. The soaked seeds were removed from the flasks, adsorbed water was removed by pressing under folds of blotting paper and were immediately transferred into the freshly prepared sodium azide solutions. The treatment continued for 240 minutes. The seeds were removed from the mutagen and washed thoroughly in running tap water for one hour with occasionally stirring. These were sown in the field soon after treatment.

Table 3.2. Different doses/concentrations of gamma rays and sodium azide used for three strains/variety in this ivestigation

Variety/ Strains	Mutagen	Doses Gy/Conc.	Duration	Treatment condition
G-319	Gamma rays	100Gy	-	Dry
		200Gy	-	Dry
		300Gy	-	Dry
	Sodium azide	0.2mM	240 minutes	Presoaked
		0.3mM	240 minutes	Presoaked
		0.4mM	240 minutes	Presoaked
G-97	Gamma rays	100Gy	-	Dry
		200Gy	-	Dry
		300Gy	-	Dry
	Sodium azide	0.2mM	240 minutes	Presoaked
		0.3mM	240 minutes	Presoaked
		0.4mM	240 minutes	Presoaked
Hyprosola	Gamma rays	100Gy	-	Dry
		200Gy	-	Dry
		300Gy	-	Dry
	Sodium azide	0.2mM	240 minutes	Presoaked
		0.3mM	240 minutes	Presoaked
		0.4mM	240 minutes	Presoaked

(ii) Growing of M_1 generation

The treated and untreated control seeds were sown in a well prepared field at the Bangladesh Institute of Nuclear Agriculture (BINA) farm, Mymensingh, Bangladesh. The experiment was conducted in a randomized complete block design with three replications during rabi season of 1992-93. Unit plot size was 5m x 0.6m. Distances between rows and plants within rows were 30 and 6-7 cm, respectively. Closer spacing was maintained for less branching to obtain minimum seeds for growing M_2 generation (Konzak and Mikaelson, 1977).

(iii) Observations recorded in M_1 generation

(a) Germination of seeds: The emergence of coleoptile was taken as an indication of germination of seeds. It was recorded daily and since the germination tends to be late in the treated materials, observations were continued up to 30 days after sowing. Finally, the germination percentage of seeds was calculated.

(b) Seedling height: Seedling height at 30 days was recorded from 10 randomly selected plants of each treatment.

(c) Plant survival: Survival percentage was calculated as the percentage of plants surviving at maturity based on the total number of seedlings' germinated in each treatment.

(d) Pollen grain fertility: Percentage of fertile pollen grains was counted under microscope. These were collected at 7 a.m. to 8 a.m. from each treatment. Ten observations were taken from each dose/concentration.

(e) Pod fertility: Ten pods were taken from randomly selected plants and fertile pods were counted. It was measured as the ratio of the pods having seeds to the total number of pods in a plant arranged over these 10 plants in each treatment.

(f) Collection of seeds from M_1 plants: The M_2 generation was raised as plant-progeny- rows. Early maturing seeds of individual M_1 plants were collected separately which constituted the M_2 family.

(iv) Growing of M_2 generation

Seeds of the individual M_1 plants were sown in the field of BINA sub-station, Ishurdi in separate M_2 plant-progeny- rows during rabi season of 1993-94. Varying number of M_2 families were grown in each treatment of each strain/ variety. The spacing between rows and plants within rows were 40 and 15 cm, respectively. One

light irrigation was given at the time of flowering. Normal cultural practices were followed as and when required.

(v) Observations recorded in M_2 generation

(a) Macro-mutations

Chlorophyll mutations: Both treated and control population were carefully observed for chlorophyll mutations for four weeks from the time of emergence of seeds. The identification and classification procedures proposed by Gustafsson (1940) were followed. The frequency of chlorophyll mutations in each treatment was calculated.

Screening of morphological mutations: In general, visual selection methods for identifying mutant phenotype are the most effective and can be very efficient. Through the entire growth period , all M_2 progenies were examined for visible morphological mutations. Viable mutations affecting different morphological characters such as plant height, leaf size, pod size, erect plant habit and seed coat colour were recorded. The frequency of morphological deviant types in each treatment was calculated.

Estimation of mutagenic effectiveness and efficiency: Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen, while mutagenic efficiency gives an idea of proportion of the mutations in relation to the total biological damage measured through lethality, sterility etc. Both of these parameters were determined as suggested by Konzak *et al.* (1965) as follows:

Mutagenic effectiveness = Mf/tc or Mf/kR

Mutagenic efficiency = Mf/L

Where, Mf = percentage of families segregating for total mutations (chlorophyll and viable)

t = duration of treatment with chemical mutagen

c = concentration of chemical mutagen in terms of percentage

kR = kilorads of physical mutagen

L = percentage of lethality in M_1

(b) Micro-mutations

Number of M_2 families were large at sowing time but the number of families harvested was less in each treatment. The families had no uniform plant population pressure and those with non-uniform population were discarded. Only families having uniform plant population were included in the study. Five plants from each M_2 family were randomly selected to collect data on seven quantitative characters viz., days to first flowering, days to maturity, plant height, number of pods/plant, number seeds/plant, 100-seed weight and seed yield/plant. The above mentioned characters are briefly described below:

Days to flowering : This is the total number of days taken by a plant from sowing date to onset of flowering .

Days to maturity : This is the total days taken by a plant from its sowing date to when 95% pods are in yellowish or straw colour.

Plant height (cm) : This is the length of a plant from soil surface to the tip of the plant.

Number of pods/plant : Total number of effective pods produced by a plant which was counted at maturity .

Number of seeds/plant : Total number of seeds produced by a plant.

Hundred seed weight (g) : The total seed weight of each single plant was divided by total seed number/plant, and then multiplied by 100.

Seed yield/plant (g) : The total weight of sun dried seeds harvested from a plant.

(vi) Growing of M_3 generation

Only the selected mutants in M_2 generation were advanced to raise M_3 generation along with their corresponding parents. The experiment was conducted at BINA sub-station, Ishurdi during 1994-95. In this generation selected plants were grown plant-progeny-rows to select true-breeding desirable mutants for further study. Distances between rows and plants were 40 and 15 cm, respectively. Recomended cultural practices were followed to ensure normal growth and developement of plants.

(vii) Observations recorded in M_3 generation

Selection of mutants in this generation were restricted to micro-mutations only. In the M_2 generation, both interfamily and single plant selections were made by comparing the mean performance of a mutant with the mean of the control. In case of days to flowering, days to maturity and plant height with lower means than control were prefered during selection. On the other hand, number of pods/plant, number of seeds/plant, 100-seed weight and seed yield/plant were compared during selection with the higher means of the control. Salient features of macro-mutations were large pod size compared with small pod size, tall with dwarf and erect plant with bushy type.

Micro-mutants were selected on the basis of better performance of the normal looking plants. M_3 plants were compared with those of M_2 mutants/ variants. Pattern of transmission of character from M_2 to M_3 was also established.

Ten plants were taken randomly from each family for recording data on plant height, pods/plant, seeds/plant, 100-seed weight and seed yield/plant. Days to flowering and days to maturity were recorded from a plot of each treatment.

The mean values of each recording data were analysed statistically for estimation of coefficient of variation (CV%), genotypic and phenotypic coefficient of variation (GCV & PCV) , heritability (H%) and genetic advance (GA%). The mutants, which bred true and showed superiority over the control in respect of 100-seed weight,

seed yield /plant and some agronomic characters, were selected for raising in M_4 generation. Mutants with poor performance were discarded drastically in M_3 generation. The population strength of each treatment in M_2 and M_3 generations are shown in Table 3.3.

(viii) Growing of M_4 generation

The M_4 generation was raised from the selected M_3 families along with their corresponding parents during 1995-96. Eighteen M_3 families were advanced to M_4 generation except those showing poor performance in respect of yield and other agronomic characters. Pedigree of the 18 selected mutants are shown below:
CPM9, CPM18, CPM21, CPM26, CPM34, CPM35, CPM41, CPM57, CPM58, CPM63, CPM73, CPM80, CPM96, CPM97, CPM111, CPM112, CPM116 and CPM123.

The rejected families, however, were also grown to observe their performance but not included in this study. Two experiments were conducted with the promising mutants at two locations, i.e., at BINA sub-stations, Ishurdi and Magura. The experiments were laid out in a randomized complete block design with three replications. Unit plot size was 5m x 2m= $10m^2$. Distances between rows and plants within rows were 40 and 15 cm, respectively. Fertilizers were applied @ 20 N, 60 P_2O_5 and 30 K_2O kg/ha. Intercultural management were practised to ensure normal growth and development of plants.

Table 3.3. Population strength in M_2 and M_3 generations

Strains/ Doses/ Conc.	No. of seeds treated	M_2 population		M_3 population	
		No. of families	No. of plants	No. of families	No. of plants
G-319					
Control	400	10	300	10	600
100 Gy	400	28	840	10	600
200 Gy	400	30	900	13	780
300 Gy	400	31	930	14	840
0.2mM	400	26	780	04	240
0.3mM	400	25	750	05	300
0.4mM	400	24	720	06	360
G-97					
Control	400	10	300	10	600
100 Gy	400	25	750	06	360
200 Gy	400	25	750	15	900
300 Gy	400	25	750	13	780
0.2mM	400	26	780	03	180
0.3mM	400	27	810	06	360
0.4mM	400	26	780	06	360
Hyprosola					
Control	400	10	300	10	600
100 Gy	400	25	750	04	240
200 Gy	400	25	750	04	240
300 Gy	400	25	750	07	420
0.2mM	400	25	750	02	120
0.3mM	400	25	750	03	180
0.4mM	400	25	750	02	120
Total	8,400	498	14,940	153	9,180

(ix) Observations recorded in M_4 generation

Agronomic data were collected from 10 randomly selected plants of each plot. Plot seed yield was converted to kg/ha. Breeding behaviour of the mutants were carefully studied. The mutants showed early maturity with larger seed size and higher yield compared to the better parent were selected, while the remaining mutants were discarded again. So, the number of selected mutant was again reduced drastically in M_4

generation. Mean values, obtained for each of the characters were computed for statistical analysis.

C. Statistical analysis of micro-mutation

Data on different quantitative characters were collected from M_2, M_3 and M_4 generations. Those parameters were statistically analysed using the procedures of the MSTATC PC packages (Fried, 1992).

(i) Aanalysis of variance

Creation of variability is the prime objective of breeders. So, the breeders are interested to estimate the component of variance. It can be obtained through Analysis of Variance (ANOVA). Variation observed in this study can be partioned into two, one is the variation within mutants and another within mutant families. The analysis of variance was done following pattern as presented in Table 3.4.

Table 3.4. Pattern of analysis of variance

Source of variation	Degrees of freedom	Sum of square	Mean sum of square	F value
Replication	r-1			
Family	f-1		M1	
Error	(r-1) (f-1)		M2	
Total				

(ii) Analysis of induced variability in M_2 generation

Range and mean were calculated on family and population basis in each treatment and control. The parameters of variability such as variance and coefficient of variation were calculated on family and population basis using the above packages and

the standard statistical procedures (Snedecor and Cochran, 1967). The induced variability was analysed by estimating the following components of mean and variance:

(a) Family mean : The mean of a character based on the data collected from five plants in a family.

(b) Population mean: It was the average mean value over different families in a particular treatment.

(c) Interfamily variance: It was the variance for a character between different families within a treatment.

(d) Intrafamily variance: This was the variance of a character among the randomly selected plants within a family.

(e) Population variance: This was interplant variance of a character which was averaged over all the families in a treatment.

(f) Intrafamily coefficient of variability: The intrafamily coefficient of variability of a particular character was estimated as the following method:-

$$\textbf{Intrafamily CV\%} = \frac{\text{Intrafamily variance}}{\text{Population mean}} \times 100$$

(g) Population coefficient of variability : It was calculated using the population variance and population mean for a character of a particular treatment.

$$\textbf{Population CV \%} = \frac{\text{Population variance}}{\text{Population mean}} \times 100$$

(iii) Estimation of component of variation and genetic parameters

According to Burton (1952), the phenotypic variance (Vp) and genotypic variance (Vg) are used to compute the components of variation and genetic parameters which are given below :

(a) Component of variation :

$$\text{Phenotypic coefficients of variation (PCV)} = \frac{\sqrt{Vp}}{\bar{X}} \times 100$$

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{Vg}}{\bar{X}} \times 100$$

Where, $\bar{X}$ is the mean of a character.

(b) Genetic parameters:

Heritability : Heritability (h^2) in broad sense was estimated as the ratio obtained by dividing the genotypic variance to the phenotypic variance which is given below : -

$$\text{Heritability } (h^2) = \frac{Vg}{Vp} \times 100$$

Genetic advance : The expected gain in selection (GA) was calculated as percentage of mean which is given below :

$$GA\ \% = \frac{K\ \sqrt{Vp} \times h^2}{\bar{X}} X\ 100$$

Where, K =2.06 (selection intensity at 5% level), $\bar{X}$ = Mean of a character

(iv) Analysis of induced variability in M_3 generation

The analysis of induced variability was done by estimating the components of mean and variance as in M_2 generation.

(v) Performance of mutants in M_4 generation

The formula used calculating the Critical Difference (CD) for compare the mean performance of the selected mutants and control is given below :

$$C.D. = S.E. \times t$$

Where, S.E. = Standard Error of the difference of the mutant means to be compard and is equal to :

$$S.E. = (2.MSe/r)^{1/2}$$

Where, MSe = Error MSS

r = No. of replications

t = Tabulated value at 5% or 1% level of significance for DF of the EMS.

If the mean difference between any two mutants is greater than the calculated value of C.D. then the difference is taken to be significant.

(vi) Selection of promising families

The CV value for each of the seven characters of each M_2 family was compared with the corresponding highest value observed in the control families. An M_2 family having CV value higher than the highest value observed in the control families was considered as the family carrying induced mutation. In M_3 generation, as the variance was expected to come down, the comparison of mean values was considered to be most important to see the effectivess of selection made in M_2 generation. Thus, the mean for each of the M_3 families was compared with the highest family mean observed in the

control. An M_3 family having mean value higher than the highest value observed in the control families was considered as promising M_3 family to carry forward to M_4 generation for confirmation of true breeding behaviour of the selected mutants. All other mutants of no economic value were discarded drastically.

Chapter IV
EXPERIMENTAL RESULTS

IV. RESULTS

The chickpea strains G-319, G-97 and Hyprosola were treated with different doses of gamma-ray and with various concentrations of sodium azide. The treated materials had been studied in M_1, M_2, M_3 and M_4 generations. Results obtained in this study of yield and yield contributing characters are described and presented generation-wise.

A. Observation in M_1 generation

The germination of seeds (%), seedling height (cm), plant survival at 30 days after sowing and at maturity as well as pollen grain fertility (%), were studied to observed the effect of the mutagenic treatment for the plants of M_1 generation.

(i) Germination of seeds

Both gamma rays and sodium azide had significant effect on germination of chickpea seeds (Table 4.1). Seed germination was found to decrease with increase of doses of gamma rays and concentrations of sodium azide. Seed germination percentages ranged from 66.9 to 92.8 in different doses/ concentrations of the mutagens in the three chickpea strains used in the present investigation (Fig.1a). It was also observed that the different doses of gamma rays caused higher deleterious effect on germination of seeds than the concentrations of sodium azide. For example at higher doses, the embryos showing signs of germination failed to produce shoots. The strain G-319 was found to be more sensitive to both the mutagens than the other two strains, G-97 and Hyprosola when compared on the basis of their seed germination.

(ii) Seedling height

The average height of 30 days old seedling was found to decrease significantly with the increase of doses/concentrations of gamma rays and sodium azide in all the genotypes (Table 4.1). The mean seedling heights following gamma-ray treatments ranged from 12.6 to 23.4 cm compared to that of the control seedlings of G-319, G-97 and Hyprosola recorded as 24.1, 23.6 and 22.5 cm, respectively. It was observed that the 300 Gy treatment caused maximum reduction in seedling height in all the genotypes. The highest reduction in seedling height (56.6%) was observed in Hyprosola at 300 Gy of gamma rays compared to its control (Fig.1b). On the other hand, in case of sodium azide treated materials of 0.4 mM concentration showed maximum reduction of seedling height (60.8%) in Hyprosola.

(iii) Plant survival

It was also found that the percentages of plant survival, both at 30 days old seedling and at maturity decreased significantly with the increasing doses/concentrations of the mutagens in all the genotypes (Table 4.1). The lowest survival percentages of seedling at 30 days old and at maturity were observed as 66.6% and 64.4%, respectively in G-97 at 300 Gy of gamma rays (Figs.2a and 2b).

(iv) Fertility of pollen grains

The fertility of pollen grain studies revealed that all the mutagenic treatments caused considerable decreasing effect on this character (Table 4.1). Fertility of pollen grains ranged from 93.4 to 99.7% (Fig.3). The higher dose of gamma-ray (300 Gy) caused more deleterious effect on the fertility of pollen grains.

Effect of seed germination, seedling height at 30 days old seedling, plant survivals at maturity and pollen fertility (%) in M_1 generation are presented in Appendix 2.

Table 4.1. Effect of gamma-ray and sodium azide on germination of seeds, seedling height, plant survivals and pollen grain fertility in M_1 generation of three varieties of chickpea

Strain/ Doses/ Conc.	Germination (%)	Seedling height at 30 days (cm)	Plant survival at 30 days (%)	Plant survival at maturity (%)	Pollen grain fertility (%)
G-319					
Control	96.0	24.1	84.6	80.0	97.0
100 Gy	84.6	23.4	76.0	71.6	94.3
200 Gy	75.0	20.1	70.6	65.0	94.0
300 Gy	64.3	16.5	60.3	57.0	90.6
0.2 mM	85.3	20.7	74.6	71.0	96.3
0.3 mM	79.0	16.1	69.0	65.6	94.6
0.4 mM	72.0	14.8	61.3	58.6	92.0
G-97					
Control	95.6	23.6	90.0	85.3	98.0
100 Gy	87.3	22.1	81.0	76.0	94.6
200 Gy	75.6	20.7	73.0	70.0	93.0
300 Gy	69.3	16.3	60.0	55.0	91.6
0.2 mM	86.6	19.7	80.3	74.6	97.3
0.3 mM	81.3	17.4	70.0	63.0	94.0
0.4 mM	75.6	14.9	64.0	60.0	92.6
Hyprosola					
Control	98.3	22.5	90.0	83.0	97.3
100 Gy	88.6	20.1	83.0	75.0	96.3
200 Gy	79.3	18.6	70.6	66.3	94.0
300 Gy	70.6	12.6	65.3	60.0	94.0
0.2 mM	91.3	19.2	80.0	72.0	97.0
0.3 mM	85.3	16.3	71.0	67.0	96.3
0.4 mM	81.0	13.7	65.0	61.0	93.6
LSD at 5%	3.07	1.72	2.34	2.64	2.71

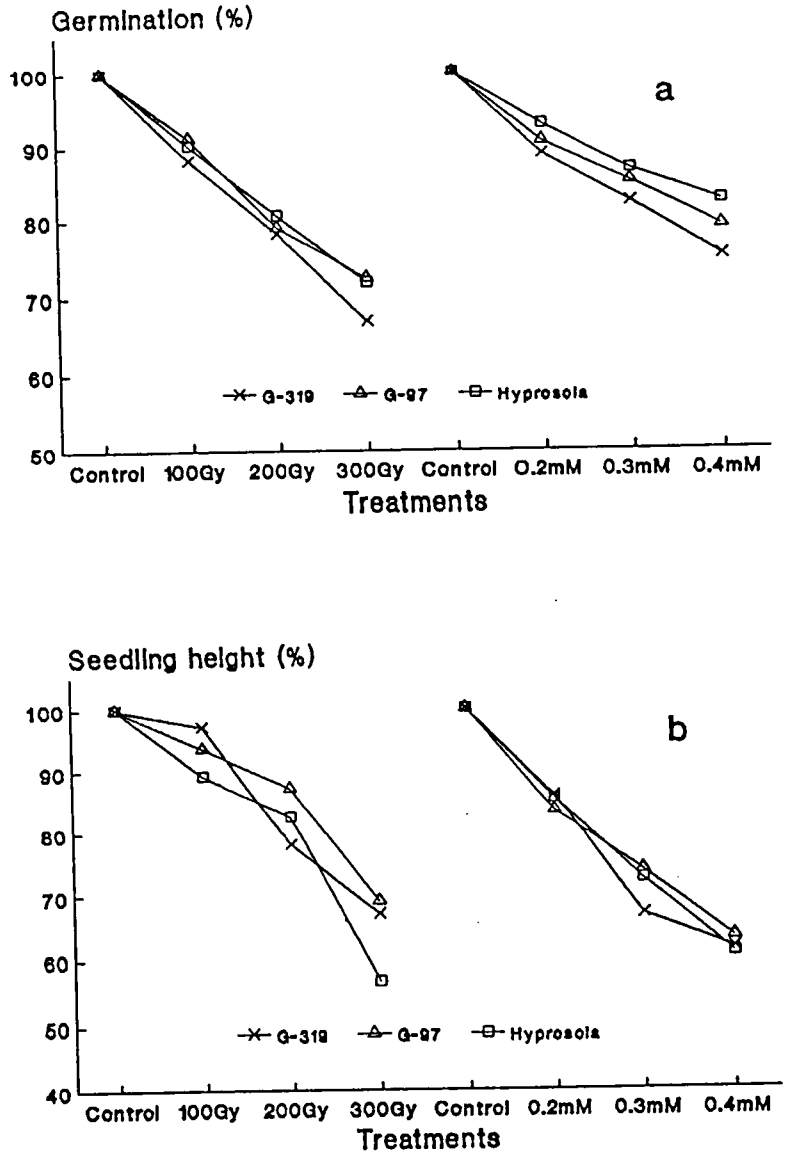


Fig. 1. Effect of mutagens on germination (a) and seedling height (b)

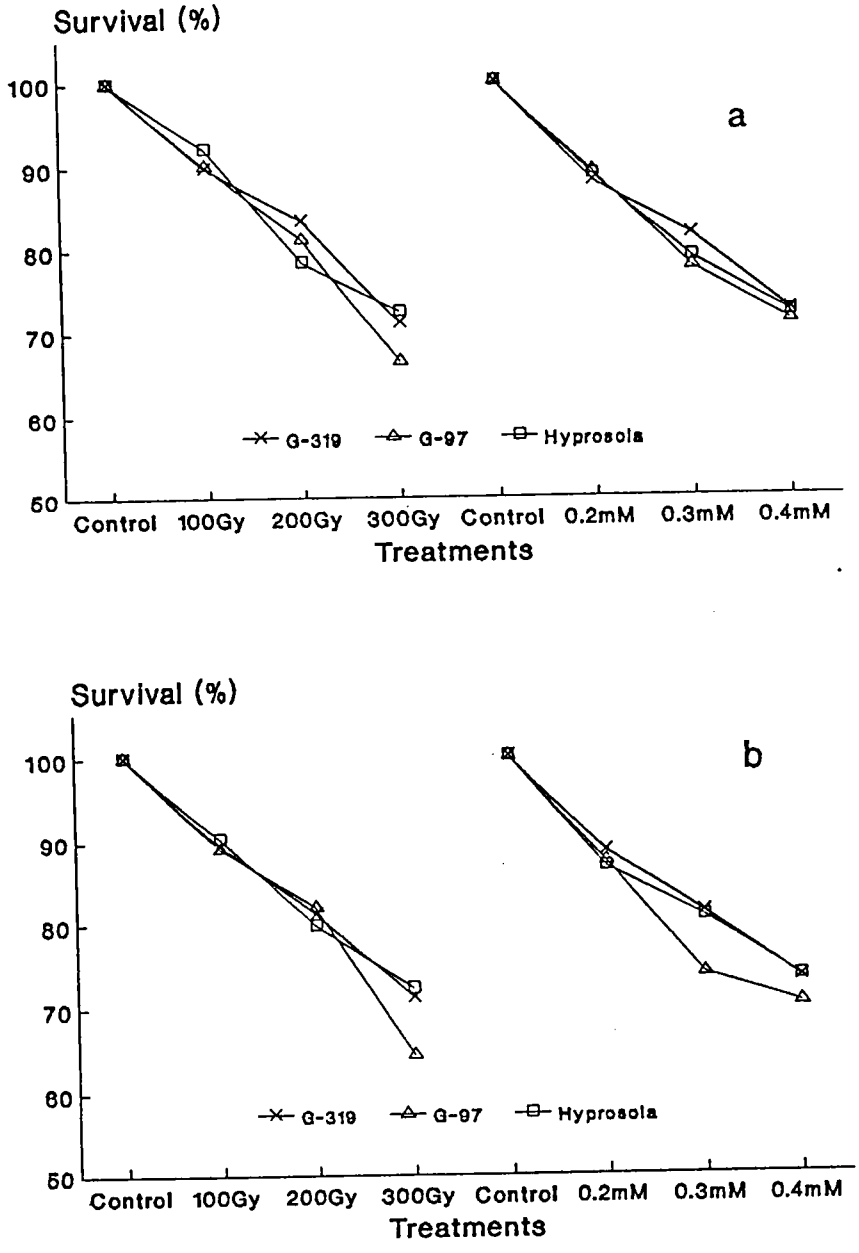


Fig. 2. Effect of mutagens on plant survival at 30 days (a) and at maturity (b) of three chickpea genotypes

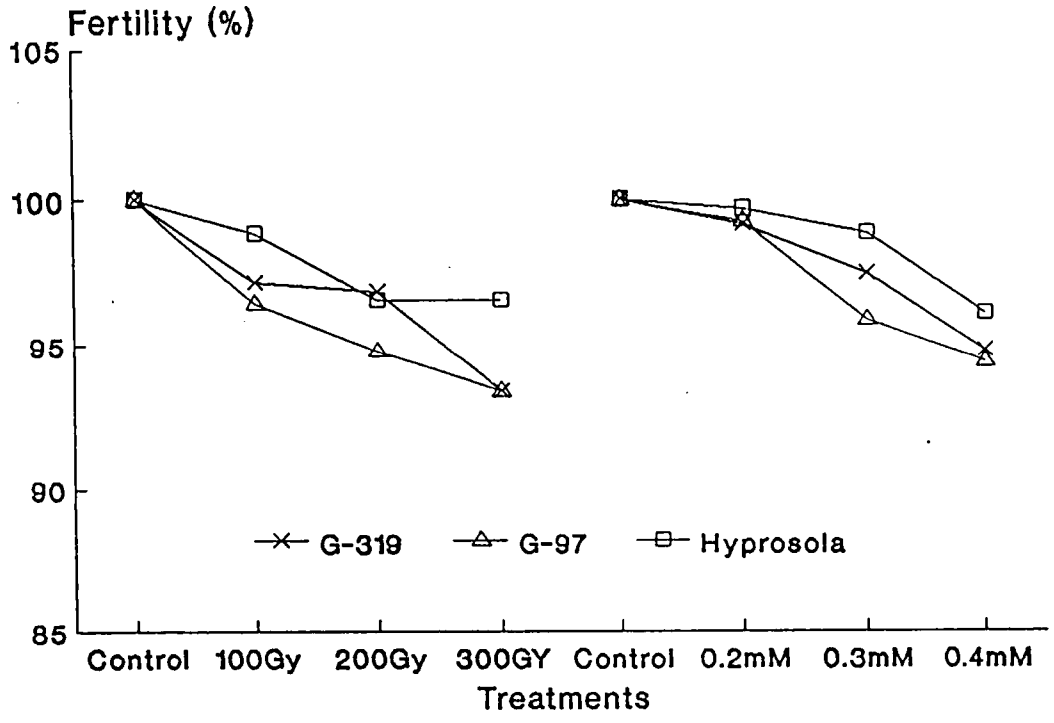


Fig. 3. Effect of mutagens on pollen fertility

B. Observation in M_2 generation

(i) Macro-mutation

The M_2 families were screened for phenotypically detectable mutation at different stages of growth which are described below.

(a) Chlorophyll mutations

The mutated M_2 families which segregated for chlorophyll mutations, were classified into Albina, Chlorina, Viridis and Xantha according to Gustafsson (1940).

Albina: The seedlings emerged completely white and died within 10-12 days of germination. This was a complete lethal mutation.

Xantha: Seedlings were straw-yellow, pale yellow, or with orange tinge. They started dying within a few days after emergence. Some of them survived upto 30 days. A few spots of greenish tissues were present in some seedlings which lived longer, but ultimately they also died. All such mutants were lethal.

Chlorina : Seedlings were pale-yellow or yellowish green, lethal or semi-lethal, most of them died after 3-4 weeks. Some plants even reached flowering stage but most withered with small flowers.

Viridis : The mutants were mostly semi-lethal with light green colour. They showed reduced vigour but normal fertility. The plants remained light green colour upto maturity.

The frequencies of chlorophyll mutations in different treatments (mutated M_2 families and plants) have been summarized in Table 4.2. Out of 24,900 M_2 plants only 77 chlorophyll mutants of different types were observed. The highest per cent of chlorophyll mutations were observed in the strain G-319. In this variety, 16.12% families and 1.09% plant mutated following 300 Gy gamma rays treatment. On the other hand, there was no chlorophyll mutation in control families and 0.2 mM treatments of sodium azide in all the three strains of chickpea. Similarly, treatment with 0.4 mM concentration of NaN_3 in the strain G-319 and 0.3 mM of sodium azide in the variety Hyprosola failed to produce any chlorophyll deficient plants. Chlorophyll

Table 4.2. Frequencies of chlorophyll mutants in the M_2 generation of three chickpea varieties following treatments with gamma-ray and NaN_3

Strains/ Doses/ Cone.	M_2 families			M_2 plants		
	Total	Mutated	Mutated %	Total	Mutated	Mutated %
G-319						
Control	10	0	0.00	500	0	0.00
100 Gy	28	2	7.14	1400	7	0.50
200 Gy	30	3	10.00	1500	8	0.53
300 Gy	31	5	16.12	1550	17	1.09
0.2 mM	26	0	0.00	1300	0	0.0
0.3 mM	25	1	4.00	1250	3	0.24
0.4 mM	24	0	0.00	1200	0	0.00
G-97						
Control	10	0	0.00	500	0	0.00
100 Gy	25	1	4.00	1250	3	0.24
200 Gy	25	2	8.00	1250	8	0.64
300 Gy	25	4	16.00	1250	13	1.04
0.2 mM	26	0	0.00	1300	0	0.00
0.3 mM	27	1	3.74	1350	3	0.22
0.4 mM	26	1	3.84	1300	3	0.23
Hyprosola						
Control	10	0	0.00	500	0	0.00
100 Gy	25	0	0.00	1250	0	0.00
200 Gy	25	1	4.00	1250	3	0.24
300 Gy	25	3	12.00	1250	5	0.40
0.2 mM	25	0	0.00	1250	0	0.00
0.3 mM	25	0	0.00	1250	0	0.00
0.4 mM	25	2	8.00	1250	4	0.32
Total	498	26	(5.22)	24900	77	(0.30)

Figures in parentheses indicate percentages of mutated family

mutations gradually increased with the increase of the doses of gamma rays and sodium azide concentrations. In general, chlorophyll mutation frequency of different strains was in the order of G-319> G-97> Hyprosola.

A large spectrum of chlorophyll mutations were observed in M_2 generation. The relative frequencies of different types of chlorophyll mutations observed in different mutagenized populations are shown in Table 4.3. Four types of chlorophyll mutations were found following treatment with both the mutagens, i.e., albina, chlorina, xantha and viridis. The gamma-ray treated population produced a higher chlorophyll mutations (83.1%) than the sodium azide treated population (16.8%). Chlorina (45.4%) was shown to be more frequent than Xantha (28.5%), Viridis (14.2%) and Albina (11.6%) in the M_2 population. No Viridis type of mutant was found in the variety Hyprosola. The highest number of chlorophyll mutants were found in the strain G-319 (41.5%) followed by the strain G-97 (31.1%) and Hyprosola (10.3%) in gamma rays treated population. Most of the chlorophyll mutants died within 3-4 weeks after germination. Few of them were recorded for chlorophyll deficiency but they died at maturity without producing any pods.

(b) Morphological mutations

The mutations showing morphological changes in plant type and leaf structure were recorded. The frequencies of morphological mutations observed in M_2 generation are presented in Table 4.4. Out of a total of 498 M_2 families consisting of 24,900 plants, only 24 families and 82 individual plants were found to be morphological deviant types. The highest percentage of morphological deviant types were found in the strain G-97 treated with 300 Gy (16.0% families and 1.1% of plants mutated). On the other hand, no morphological mutant was observed in the control families as well as in the treatment of 0.2 mM of sodium azide in all the three strains. Beside these, 0.3 mM concentration of sodium azide in the strain G-97 and Hyprosola did not produce any morphological deviant plants. The frequency of morphological mutation, ranging from

Table 4.3. Spectrum of chlorophyll mutations in M_2 generation of three chick-pea varieties following gamma-ray and NaN_3 treatments

Treatments	Total mutations	Different types of chlorophyll mutations			
		Albina	Chlorina	Xantha	Viridis
Gamma rays					
G-319	32	3	13	10	6
	(41.5)	(3.8)	(16.8)	(12.9)	(7.7)
G-97	24	3	10	7	4
	(31.1)	(3.8)	(12.9)	(9.0)	(5.1)
Hyprosola	8	1	5	2	-
	(10.3)	(1.2)	(6.4)	(2.5)	(0.0)
Sub Total	64	7	28	19	10
	(83.1)	(9.0)	(36.3)	(24.6)	(12.9)
Sodium azide					
G-319	3	1	2	-	-
	(3.8)	(1.2)	(2.5)	(0.0)	(0.0)
G-97	6	-	3	2	1
	(7.7)	-	(3.8)	(2.5)	(1.2)
Hyprosola	4	1	2	1	-
	(5.1)	(1.2)	(2.5)	(1.2)	(0.0)
Sub Total	13	2	7	3	1
	(16.8)	(2.5)	(9.0)	(3.8)	(1.2)
Grand Total	77	9	35	22	11
		(11.6)	(45.4)	(28.5)	(14.2)

Figures in parentheses indicate percentages of mutated families

Table 4.4. Frequencies of morphological mutants of chickpea in M_2 generation following treatment with gamma-ray and sodium azide

Strains/ Doses/ Conc.	M_2 families			M_2 plants		
	Total	Mutated	% Mutated	Total	Mutated	% Mutated
G-319						
Control	10	0	0.0	500	0	0.0
100 Gy	28	1	3.5	1400	3	0.2
200 Gy	30	1	3.3	1500	4	0.2
300 Gy	31	3	9.6	1550	11	0.7
0.2 mM	26	0	0.0	1300	0	0.0
0.3 mM	25	1	4.0	1250	3	0.2
0.4 mM	24	1	4.1	1200	3	0.2
G-97						
Control	10	0	0.0	500	0	0.0
100 Gy	25	1	4.0	1250	2	0.1
200 Gy	25	2	8.0	1250	7	0.5
300 Gy	25	4	16.0	1250	14	1.1
0.2 mM	26	0	0.0	1300	0	0.0
0.3 mM	27	0	0.0	1300	0	0.0
0.4 mM	26	2	7.6	1300	7	0.5
Hyprosola						
Control	10	0	0.0	500	0	0.0
100 Gy	25	1	4.0	1250	3	0.2
200 Gy	25	3	12.0	1250	13	1.0
300 Gy	25	2	8.0	1250	7	0.5
0.2 mM	25	0	0.0	1250	0	0.0
0.3 mM	25	0	0.0	1250	0	0.0
0.4 mM	25	1	4.0	1250	3	0.2
Total	498	24	(4.8)	24,900	82	(0.3)

Figures in parentheses indicate percentage of mutated families

0.0 to 1.1% on plant basis was found to increase with the increase of doses except 300 Gy of Hyprosola.

The different types of morphological mutants, i.e., narrow leaf, broad leaf, dwarf plant, tall plant, bushy plant, large and small podded plants are shown in Table 4.5. The spectrum of viable mutations varied and depended on treatments. The highest percentage of morphological mutants observed were of narrow leaf types (20.7%) in the M_2 population. On the other hand, per cent of the large podded (8.5%) mutant was the lowest in the M_2 population. A mutation rate on plant basis for the mutagens showed that the gamma rays treatment produced higher morphological mutations than sodium azide treatment.

(c) Mutagenic effectiveness and efficiency

The effectiveness and efficiencies of the doses/concentrations of the mutagens varied largely is shown in Table 4.6. Among all the concentrations of sodium azide in the variety Hyprosola, 0.4 mM was the most effective concentration (115.3). On the other hand, 0.2 mM concentration of sodium azide of the three genotypes proved to be the lowest effective dose among the treatments. Thus the most effective concentration of sodium azide varied from strain to strain of chickpea.

The degree of efficiency of various doses/concentrations mutagens varied within a range from 0.00 to 1.36 (Table 4.6). A 300 Gy of gamma-ray in the strain G-97 (1.36) showed the highest efficiency among all the doses/concentrations. From this study it is indicated that the gamma rays are more efficient than sodium azide.

(ii) Micro-mutation

(a) Days to flowering

The population means, ranges of family means, coefficients of variation (CV%) and the number and frequency of mutated families following each treatment along with

Table 4.5. Spectrum of morphological mutations in M_2 generation of chickpea following treatment with gamma-ray and NaN_3

Treatments	Total mutations	Plant types			Leaf types		Pod types	
		Dwarf	Tall	Bushy	Narrow	Broad	Small	Large
Gamma rays								
G-319	18 (21.9)	1 (1.2)	2 (2.4)	4 (4.8)	4 (4.8)	3 (3.6)	2 (2.4)	2 (2.4)
G-97	23 (28.0)	2 (2.4)	1 (1.2)	5 (6.0)	4 (4.8)	7 ( )	3 (3.6)	1 (1.2)
Hyprosola	23 (28.0)	4 (4.8)	1 (1.2)	3 (3.6)	7 ( )	3 (3.6)	3 (3.6)	2 (2.4)
Sub Total	64 (78.0)	7 (8.5)	4 (4.8)	12 (14.6)	15 (18.2)	13 (15.8)	8 (9.7)	5 (6.0)
Sodium azide								
G-319	8 (9.7)	1 (1.2)	2 (2.4)	3 (3.5)	-	1 (1.2)	-	1 (1.2)
G-97	7 (8.5)	- -	3 (3.6)	- -	1 (1.2)	1 (1.2)	1 (1.2)	1 (1.2)
Hyprosola	3 (3.6)	1 (1.2)	- -	- -	1 (1.2)	- -	1 (1.2)	- -
Sub Total	18 (21.9)	2 (2.4)	5 (6.0)	3 (3.6)	2 (2.4)	2 (2.4)	2 (2.4)	2 (2.4)
Grand Total	82	9 (10.9)	9 (10.9)	15 (18.2)	17 (20.7)	15 (18.2)	10 (12.1)	7 (8.5)

Figures in parentheses indicate percentages of family mutated

Table 4.6. Effectiveness and efficiency of different doses/ concentrations of gamma-ray and sodium azide on the M_1 generation of chickpea

Strains/ Doses/ Conc.	Lethality at 30 days in M_1 (%)	Mutated family in M_1 (%)	Mutagenic Effectiveness	Mutagenic Efficiency
G-319				
Control	00.0	00.0	0.0	0.00
100 Gy	10.2	10.7	1.0	1.04
200 Gy	16.6	13.3	0.6	0.80
300 Gy	28.8	25.8	0.3	0.89
0.2 mM	11.9	0.0	0.0	0.00
0.3 mM	18.5	8.0	105.2	0.43
0.4 mM	27.6	4.1	40.0	0.15
G-97				
Control	0.0	0.0	0.0	0.00
100 Gy	9.9	8.0	0.80	0.80
200 Gy	18.9	16.0	0.80	0.84
300 Gy	23.4	32.0	1.06	1.36
0.2 mM	10.8	0.0	0.0	0.00
0.3 mM	22.3	3.7	52.6	0.16
0.4 mM	28.9	11.5	10.8	0.39
Hyprosola				
Control	0.0	0.0	0.0	0.00
100 Gy	7.8	4.0	0.4	0.51
200 Gy	21.6	16.0	0.8	0.74
300 Gy	27.5	20.0	0.6	0.72
0.2 mM	11.2	0.0	0.0	0.00
0.3 mM	21.2	0.0	0.0	0.00
0.4 mM	27.8	12.0	115.3	0.43

their controls are shown in Table 4.7. The population mean of flowering time in all the treated populations has been compared with their parents (Fig.4.). The range of family means was found to be wider in treatments of both gamma-ray and sodium azide over the control in each strain of chickpea. It is clear that the range of family means of flowering time increased in all the M_2 populations over the controls (48.4-78.6 days). A more definite evidence of induced variability is the coefficients of variability (CV%). It increased in all the treated populations over the controls. The population CV% ranged from 1.11 to 6.47. The ranges of family CV% were found from 0.00 to 11.24 in the M_2 generation. Percentage of mutation frequency was determined with its number of family studied in each treatment. The frequency of such mutated family was the highest in 300 Gy of gamma-ray in the strain G-97 (16.0%) and the lowest value was 3.57% in 100 Gy of the strain G-319..

Analysis of variances (mean square values), components of variation and genetic parameters for days to flowering are presented in Table 4.8. The inter-family variances for all the treatments were significant, i.e., the M_2 families of different treatments were found to differ significantly among themselves. Genotypic coefficients of variation were higher in the gamma-ray treated populations which ranged from 2.84 to 7.74 compared to 1.05 to 5.00 in the sodium azide treated population. The lowest and the highest genotypic coefficients of variation were found in the control G-97 (0.77) and in 300 Gy of gamma-ray treatment in the strain G-319 (7.74), respectively. Similarly the highest phenotypic coefficient of variation (8.04) was found in the 300 Gy of gamma-ray in the strain G-319 treated population while the lowest phenotypic coefficients of variation (1.63) was observed in the control Hyprosola. The phenotypic coefficients of variation ranged from 3.48 to 8.04 in treatment with different doses of gamma-ray and 1.84-7.76 in the treatments with sodium azide. The estimates of heritability and genetic advance (% of mean) were also higher in the treated population than the controls. The ranges of heritability were estimated from 26.95 to 92.80. The ranges of genetic advance were

Table 4.7. Means, coefficient of variations and mutation frequencies for days to flowering in M_2 generation of chickpea after mutagenic treatment

Strains/ Doses/ Conc.	Ranges of family means	Population CV%	Ranges of families CV%	Frequencies of mutated families No.	%
G-319					
Control	69.2-71.4	1.47	1.00-1.81	5.0	0.00
100 Gy	67.4-71.8	2.01	0.62-3.39	1.0	3.57
200 Gy	61.0-77.2	1.80	0.78-4.98	2.0	6.66
300 Gy	55.0-73.0	2.16	0.76-4.76	3.0	9.67
0.2 mM	60.4-71.2	2.14	0.69-3.94	1.0	3.84
0.3 mM	56.2-70.2	3.32	0.77-7.22	1.0	4.00
0.4 mM	53.6-74.2	5.95	0.65-11.24	2.0	8.33
G-97					
Control	73.8-75.2	1.11	0.73-1.21	0.0	0.00
100 Gy	61.0-75.6	3.20	1.23-4.73	1.0	4.00
200 Gy	62.6-76.0	2.28	1.59-3.49	2.0	8.00
300 Gy	70.8-78.0	3.12	1.57-5.88	4.0	16.00
0.2 mM	68.6-77.6	2.81	1.57-5.24	1.0	3.84
0.3 mM	62.6-75.2	3.24	1.50-5.50	1.0	3.70
0.4 mM	59.6-78.6	2.18	0.73-4.04	2.0	7.69
Hyprosola					
Control	59.8-61.2	1.34	0.74-1.63	0.0	0.00
100 Gy	51.4-62.6	2.32	0.88-4.76	1.0	4.00
200 Gy	52.4-64.8	3.61	1.41-6.45	2.0	8.00
300 Gy	48.4-61.2	2.59	0.66-4.53	2.0	8.00
0.2 mM	58.8-62.0	1.51	0.00-2.83	1.0	4.00
0.3 mM	50.4-61.2	6.47	0.73-4.10	1.0	4.00
0.4 mM	54.4-63.8	2.03	0.00-6.46	1.0	4.00

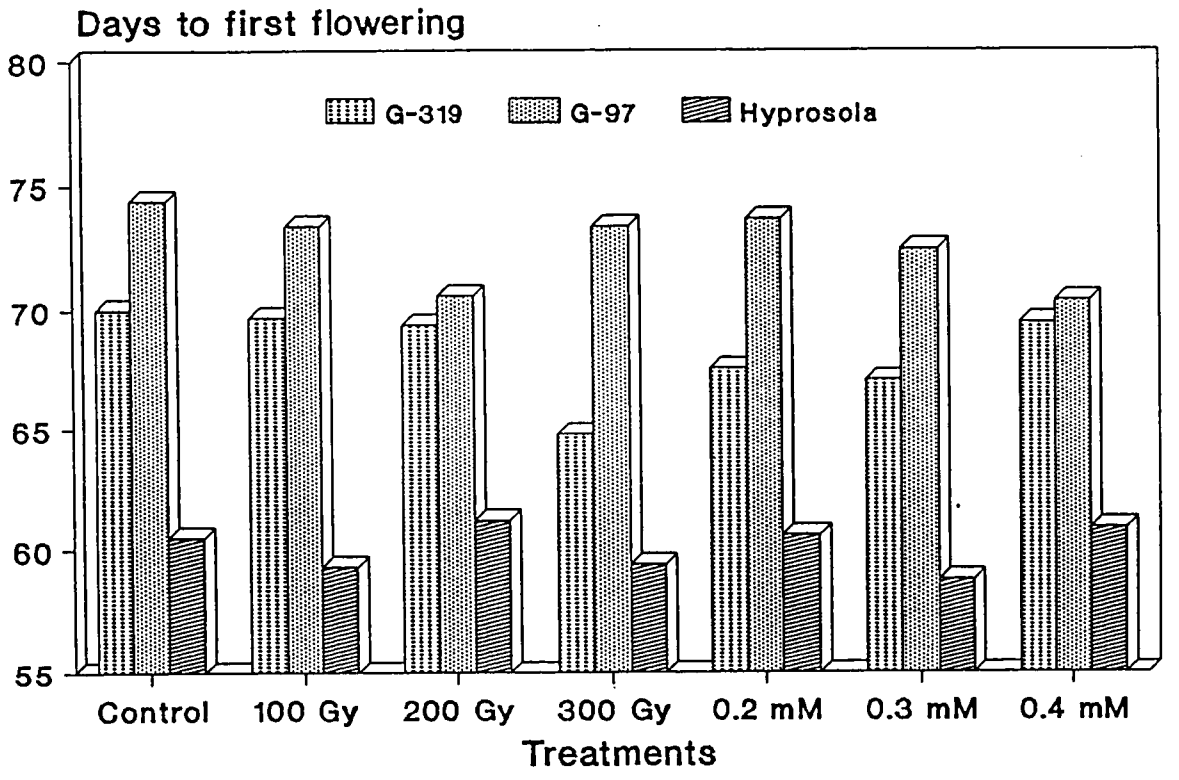


Fig. 4. Effect of different treatments of gamma rays and sodium azide on days to first flowering in M_2 generation.

Table 4.8. Analysis of variance, component of variation, heritability and genetic advance for days to flowering in M_2 generation of chickpea

Strains/ Doses/ Conc.	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	2.94	0.54	1.40	2.03	7.54	1.99
100 Gy	21.74**	1.79	2.84	3.48	66.66	4.78
200 Gy	76.15**	2.32	5.55	5.84	90.47	10.86
300 Gy	128.34**	7.84	7.74	8.04	92.80	15.37
0.2 mM	40.72**	1.08	4.10	4.62	78.61	9.15
0.3 mM	40.26**	11.15	3.95	5.06	62.07	6.37
0.4 mM	77.56**	8.68	5.00	7.76	41.43	6.63
G-97						
Control	3.00	1.54	0.77	2.58	8.33	0.45
100 Gy	74.06**	9.46	5.04	5.97	71.33	8.78
200 Gy	127.05**	2.42	7.06	7.42	90.51	13.84
300 Gy	59.15**	5.65	4.47	5.45	67.25	7.55
0.2 mM	17.01**	3.58	2.16	3.55	37.13	2.71
0.3 mM	52.34**	9.53	4.22	5.32	62.86	6.89
0.4 mM	53.38**	2.38	4.53	5.03	81.21	8.42
Hyprosola						
Control	2.26	0.16	0.93	1.63	7.65	1.10
100 Gy	20.81**	9.60	3.27	4.01	66.66	5.51
200 Gy	25.03**	23.06	3.27	4.87	45.06	4.52
300 Gy	66.05**	4.02	5.99	6.53	84.30	11.34
0.2 mM	2.89**	2.90	1.05	1.84	33.06	1.24
0.3 mM	41.26**	2.54	3.92	7.56	26.95	4.20
0.4 mM	12.89**	1.58	2.27	3.19	59.89	3.94

** , Significant at 1% level

found to be the highest in 300 Gy of gamma-ray in the strain G-319 (15.37) and the lowest in the control G-97 (0.45).

(b) Days to maturity

The overall population means, range of family means, population coefficients of variation, ranges of family CV% and frequencies of the mutated families are presented in Table 4.9. The population means for days to maturity in each doses/concentrations were compared with their respective parents (Fig.5). The range of family means was found to be wider in all the treatments of both mutagens over the control in each of the strains of chickpea. The ranges of family means for days to maturity increased in all the treatments over the control (118.8-140.4). Population CV% and ranges of family CV% were higher in the treated population than that of the control. The highest population CV% was found in 0.3 mM concentration of sodium azide in the strain G-97 (3.54). On the other hand, the lowest population CV% was observed in 0.4 mM concentration of sodium azide in the variety Hyprosola (0.75). The ranges of population CV% were higher in all the treatment (0.00-7.44). The frequencies of mutated families were also estimated treatment- wise. The highest mutation frequency (16.00) for days to maturity was observed in 300 Gy of gamma-ray in the strain G-97. On the other hand, the lowest mutation frequency (3.57) was found in 100 Gy of gamma-ray in the strain G-319.

Analysis of variance (mean square values), component of variation, estimates of heritability and genetic advance (% of mean) for the days to maturity are presented in Table 4.10. The inter-family variances were significantly different in each treatments. Genotypic and phenotypic coefficients of variation were comparatively higher in the treated population than the controls. The result revealed that the components of variation increased over the controls due to mutagenesis. The highest heritability value (97.31%) was found in 200 Gy of gamma-ray in the strain G-319. On the other hand,

Table 4.9. Means, coefficient of variations and mutation frequencies for days to maturity in M_2 generation of chickpea

Strains/ Doses/ Conc.	Ranges of family means	Population CV%	Ranges of family CV%	Frequencies of mutated families No.	%
G-319					
Control	133.4-135.0	0.82	0.41-1.21	0.0	0.00
100 Gy	127.8-139.4	1.10	0.40-2.02	1.0	3.57
200 Gy	129.0-139.6	1.14	0.33-3.96	3.0	10.00
300 Gy	125.6-139.4	1.10	0.40-1.82	3.0	9.67
0.2 mM	129.6-136.4	2.28	0.78-3.95	2.0	7.69
0.3 mM	125.2-137.4	1.52	0.77-7.22	1.0	4.00
0.4 mM	119.4-140.0	2.28	0.81-7.44	1.0	4.16
G-97					
Control	135.2-137.0	1.63	1.60-1.64	0.0	0.00
100 Gy	119.4-137.0	1.28	0.65-2.75	2.0	8.00
200 Gy	120.0-138.0	1.18	0.60-2.87	3.0	12.00
300 Gy	127.6-140.0	1.43	0.82-2.82	4.0	16.00
0.2 mM	132.6-140.4	1.66	0.42-2.99	2.0	7.69
0.3 mM	121.0-140.0	3.54	0.82-2.80	3.0	11.11
0.4 mM	126.4-140.4	1.43	0.32-2.63	2.0	7.69
Hyprosola					
Control	126.4-127.0	0.66	0.55-0.70	0.0	0.00
100 Gy	120.6-131.0	1.27	0.43-2.48	1.0	4.00
200 Gy	124.8-133.8	1.32	0.43-2.36	1.0	4.00
300 Gy	118.8-130.2	1.19	0.00-2.03	3.0	12.0
0.2 mM	125.0-130.8	0.98	0.35-2.16	1.0	4.00
0.3 mM	124.0-131.4	1.49	0.43-2.86	1.0	4.00
0.4 mM	124.8-129.4	0.75	0.00-1.06	1.0	4.00

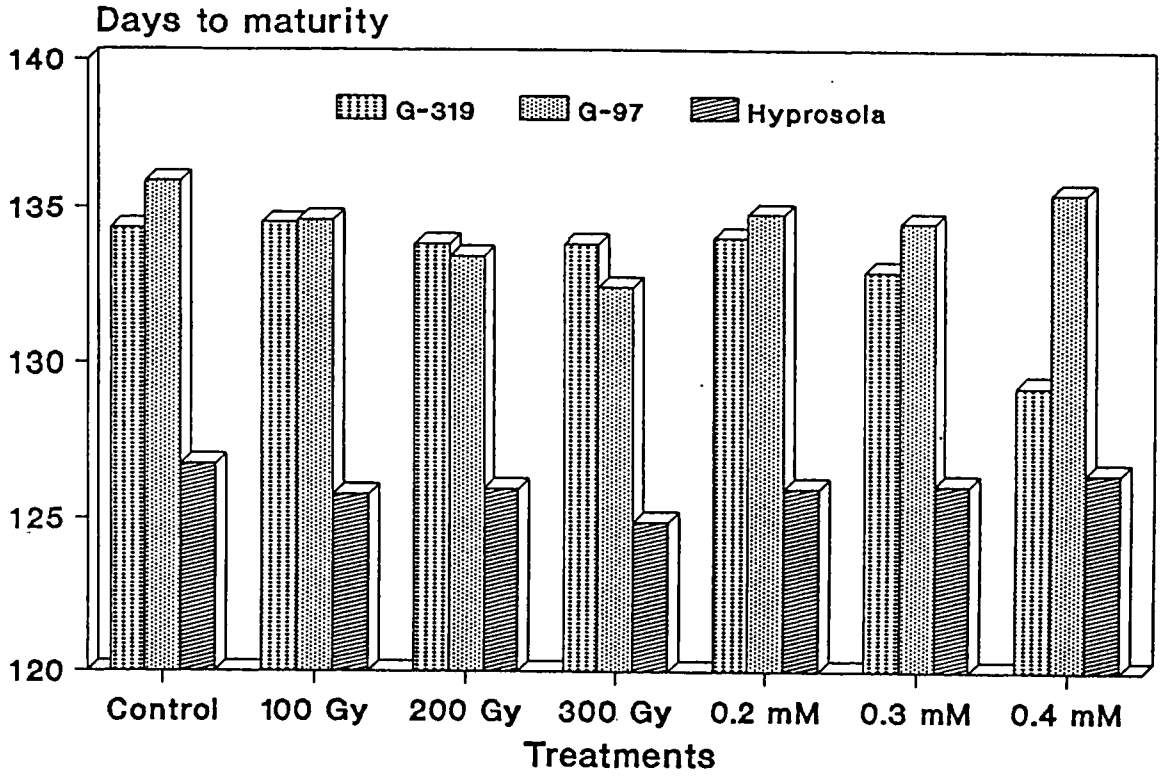


Fig. 5. Effect of different treatments of gamma rays and sodium azide on days to maturity in M_2 generation

Table 4.10. Analysis of variance, component of variation, heritability and genetic advance for days to maturity in M_2 generation of chickpea

Strains/ Doses/ Conc.	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	2.06	0.96	0.30	0.87	12.31	0.22
100 Gy	55.70**	1.43	2.43	2.66	83.01	4.56
200 Gy	75.45**	1.62	6.85	6.94	97.31	13.92
300 Gy	65.78**	5.04	2.66	2.88	85.42	5.07
0.2 mM	25.11**	7.56	1.30	2.63	24.72	1.33
0.3 mM	41.21**	16.13	2.08	2.57	65.29	3.46
0.4 mM	91.53**	32.23	3.14	3.88	65.67	2.25
G-97						
Control	2.34	5.34	0.52	1.71	9.11	0.32
100 Gy	95.12**	2.48	3.19	3.43	86.16	6.01
200 Gy	123.93**	4.14	3.66	3.85	90.67	7.19
300 Gy	26.58**	0.42	1.56	2.12	54.31	2.37
0.2 mM	14.18**	3.18	0.98	1.84	26.08	1.01
0.3 mM	147.77**	8.13	3.97	4.30	85.47	7.57
0.4 mM	24.47**	3.75	1.67	2.20	57.75	2.61
Hyprosola						
Control	0.40	0.30	0.04	0.31	7.75	0.24
100 Gy	17.42**	12.30	1.36	1.86	53.90	2.07
200 Gy	17.53**	6.36	1.36	1.89	51.48	2.01
300 Gy	58.60**	7.50	2.68	2.93	83.67	5.06
0.2 mM	6.75**	1.94	0.79	1.26	39.61	1.03
0.3 mM	11.58**	13.94	1.00	1.79	31.38	1.16
0.4 mM	10.24**	0.77	1.08	1.31	67.75	1.82

**, Significant at 1% level

the lowest heritability value (24.72%) was observed in 0.2 mM of sodium azide treatment in the same strain. The ranges of heritability percentages are much wider in the treated population. The highest genetic advance value (13.92%) was found in 200 Gy of gamma-ray in the strain G-319 and the lowest (1.01%) in 0.2 mM concentration of sodium azide in the strain G-97.

(c) Plant height

The population means, ranges of family means, population CV%, ranges of family CV% and number and percentages of semi-dwarf/dwarf plant height from mutated families in the M_2 generation are presented in Table 4.11. The population means of plant height in all the treatment were compared with their controls (Fig.6). Both the mutagens showed considerable effect on plant height. The ranges of family mean were found to be higher in the treated population than the control families. Reduction in plant height was observed with the increase in doses of gamma-rays and concentrations of sodium azide. In that case the plant height ranged from 40.8 to 70.6 cm. The range of population CV% was estimated to be wider in the mutagenized population and varied from 2.18 to 9.23% in the treated populations than that of the controls (2.17-2.42%). The families with the population mean at least 5 cm shorter than their respective parents were considered as dwarf mutants, having higher CV% than the control. These were estimated in the number and percentages of mutated families in different treatments of gamma-ray and sodium azide. The highest number of mutant families (16.00%) for plant height was observed in 300 Gy of gamma-ray in the variety Hyprosola.

Both intra-family and inter-family variances (mean square values), components of variation, heritability and genetic advance for plant height are presented in Table 4.12. The inter-family variances were significant in the treated population. Both the component of variations were considerably higher in the treated population. The

Table 4.11. Means, coefficient of variations and mutation frequencies for plant height in M_2 generation of chickpea

Strains/ Doses/ Conc.	Ranges of family means	Population CV%	Ranges of family CV%	Frequencies of mutated families No.	%
G-319					
Control	59.6-61.4	2.29	1.17-2.77	0.0	0.00
100 Gy	54.8-66.6	2.89	0.75-3.67	1.0	3.57
200 Gy	54.0-63.6	2.77	0.90-4.34	2.0	8.33
300 Gy	51.4-66.6	2.45	0.88-3.75	3.0	9.67
0.2 mM	51.6-64.2	4.68	0.74-5.49	1.0	3.84
0.3 mM	51.0-66.8	5.20	1.40-7.19	1.0	4.00
0.4 mM	51.6-60.4	3.76	1.19-9.60	2.0	8.33
G-97					
Control	60.4-63.0	2.42	1.85-2.59	0.0	0.00
100 Gy	54.4-63.6	2.65	1.79-4.48	1.0	4.00
200 Gy	51.6-67.8	3.57	1.35-8.74	2.0	8.00
300 Gy	57.0-70.6	3.23	1.34-5.55	2.0	8.00
0.2 mM	56.4-63.2	4.19	1.29-7.50	1.0	3.84
0.3 mM	57.0-64.4	3.54	1.77-6.04	3.0	11.11
0.4 mM	58.8-65.6	4.72	0.67-4.46	2.0	7.69
Hyprosola					
Control	50.4-51.0	2.17	1.08-3.04	0.0	0.00
100 Gy	45.4-54.6	3.03	1.08-7.04	1.0	4.00
200 Gy	44.8-55.2	9.23	0.81-4.66	2.0	8.00
300 Gy	40.8-59.6	4.06	0.00-7.17	4.0	16.00
0.2 mM	50.9-56.0	2.42	0.86-4.56	0.0	0.00
0.3 mM	48.4-54.6	4.21	1.00-5.58	1.0	4.00
0.4 mM	41.4-52.6	2.18	0.88-4.57	1.0	4.00

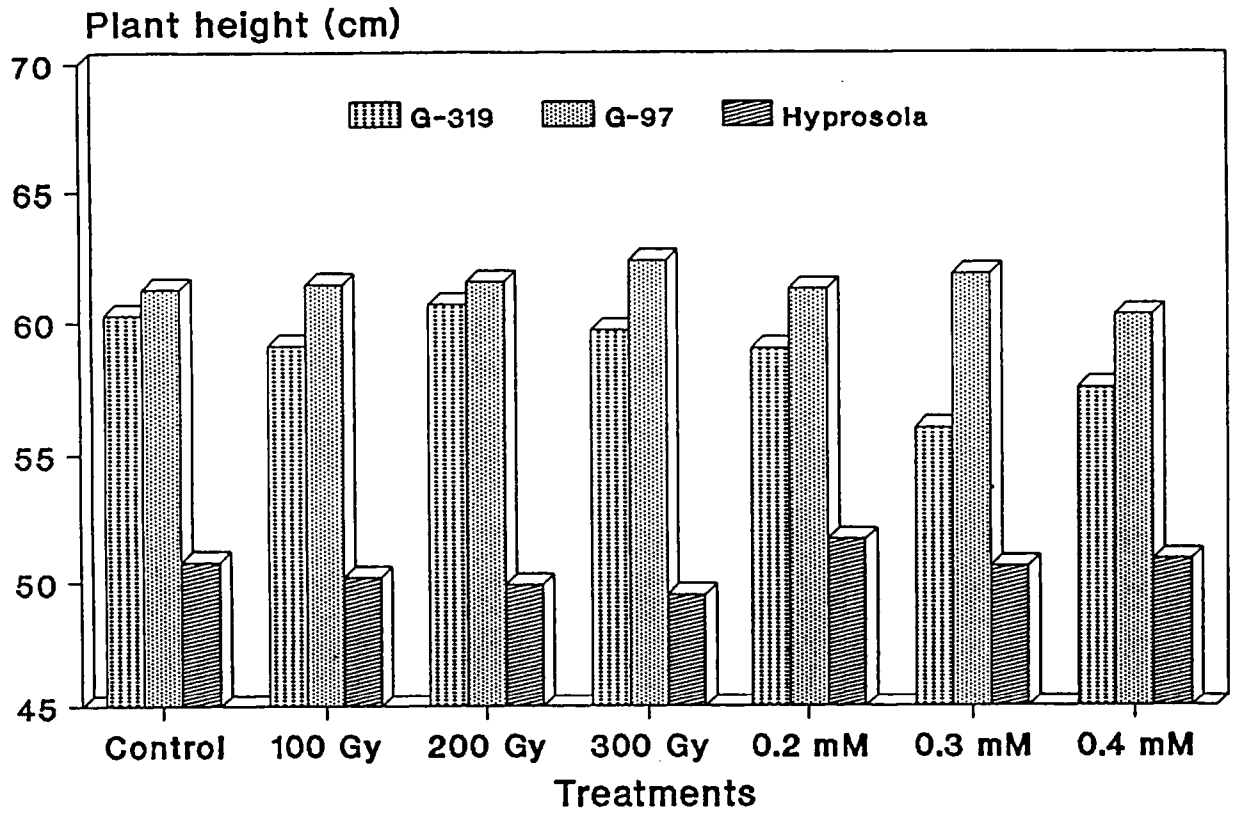


Fig. 6. Effect of different treatments of gamma rays and sodium azide on plant height in M_2 generation

Table 4.12. Analysis of variance, component of variation, heritability and genetic advance for plant height in M_2 generation of chickpea

Strains/ Doses/ Cone.	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	2.24	0.54	0.40	2.34	3.04	0.14
100 Gy	34.77**	1.20	4.37	4.76	84.27	8.27
200 Gy	22.67**	5.95	3.27	4.24	76.42	6.75
300 Gy	48.83**	4.36	5.10	5.66	81.34	9.44
0.2 mM	27.45**	3.02	3.76	4.76	61.77	6.05
0.3 mM	55.33**	10.65	5.45	7.53	52.40	8.13
0.4 mM	33.97**	34.63	4.19	5.64	55.19	6.42
G-97						
Control	2.06	2.21	1.23	2.72	7.50	1.15
100 Gy	38.82**	3.47	4.37	5.11	73.10	7.69
200 Gy	78.10**	4.83	6.21	7.16	75.09	11.08
300 Gy	85.61**	6.49	6.47	7.23	80.03	11.91
0.2 mM	11.82**	2.18	1.55	4.47	12.00	2.10
0.3 mM	18.41**	9.69	2.66	4.43	36.07	3.29
0.4 mM	19.21**	7.50	1.62	2.10	30.42	2.29
Hyprosola						
Control	2.14	0.34	1.21	2.48	5.75	1.21
100 Gy	12.82**	3.95	2.88	4.17	47.61	4.09
200 Gy	37.66**	22.49	3.63	10.07	23.02	2.70
300 Gy	56.88**	24.59	6.56	7.72	72.32	11.49
0.2 mM	6.31**	2.83	1.88	3.06	37.84	2.38
0.3 mM	10.14**	5.31	2.07	4.47	19.57	1.89
0.4 mM	22.49**	0.39	4.04	4.59	77.69	7.34

**, Significant at 1% level

genotypic coefficients of variation (GCV) ranged from 1.55 to 6.56 in the treated populations which was much higher than that of the controls (0.40-1.23). The highest GCV (6.56) was exhibited in 300 Gy gamma-ray in the variety Hyprosola. On the other hand, the highest phenotypic coefficients of variation (10.07) was found in 200 Gy of the same variety, Hyprosola. Heritability in the mutagenized population was recorded from 12.00 to 80.03%. All the populations showed higher heritability except 0.2 mM treatment in the strain G-97 than the controls. The genetic advance in different treatments ranged from 1.89 to 11.91 while it was found to be from 0.14 to1.21 in the controls.

(d) Number of pods/plant

It is evident from Table 4.13 that although the increase of doses/concentrations of both mutagens have resulted in a decreasing effect on the population in the number of pods/plant (Fig. 7), but there are some exceptions. The 0.3 mM concentration of NaN_3 in the strain G-97 and the same concentration in Hyprosola showed increasing trend of the population mean for number of pods/plant. The ranges of population mean was substantially greater than the controls. The ranges of family means in the treated populations were observed from 40.6 to 145.8. The highest CV% (5.97) was recorded in 300 Gy in the strain G-319 while the lowest value (1.37) was obtained in 200 Gy gamma-ray in the variety Hyprosola. The population CV% increased in the mutagenized population than in the controls. Regarding the mutation frequency, the highest frequency of mutagenized families was 12.90% in 300 Gy of gamma-ray in the strain G-319. On the other hand, lowest percentage was found to be 3.70% in 0.3 mM concentration of sodium azide in the strain G-97.

Significant inter-family differences for number of pods/plant in the treated progenies are shown in Table 4.14. Higher genotypic and phenotypic coefficients of variation were observed in the treated populations than the controls. These values

Table 4.13. Means, coefficient of variations and mutation frequencies for pods/plant in M_2 generation of chickpea

Strains/ Doses/ Cone.	Ranges of family means	Population CV%	Ranges of families CV%	Frequencies of mutated families No.	%
G-319					
Control	50.4-53.0	1.55	0.82-3.52	0.0	0.00
100 Gy	40.6-72.6	5.19	0.73-8.66	2.0	7.14
200 Gy	49.2-60.0	4.09	1.06-4.48	2.0	6.66
300 Gy	42.4-58.2	5.97	0.89-3.37	4.0	12.90
0.2 mM	42.8-60.2	2.95	1.10-9.62	1.0	3.84
0.3 mM	41.4-50.2	5.89	1.44-8.64	1.0	4.00
0.4 mM	47.0-54.8	3.69	1.64-9.38	2.0	8.33
G-97					
Control	72.0-75.4	1.69	1.21-2.44	0.0	0.00
100 Gy	66.2-95.6	2.61	0.57-5.76	1.0	4.00
200 Gy	69.6-82.0	2.68	1.59-5.38	3.0	12.00
300 Gy	66.4-98.0	2.55	1.08-4.37	3.0	12.00
0.2 mM	65.6-75.0	3.12	1.59-5.85	1.0	3.84
0.3 mM	70.4-87.0	2.45	1.31-3.79	1.0	3.70
0.4 mM	67.4-97.2	2.04	0.60-4.48	2.0	7.69
Hyprosola					
Control	99.6-104.2	1.68	0.54-2.15	0.0	0.00
100 Gy	96.6-112.0	1.51	0.42-3.75	1.0	4.00
200 Gy	99.6-112.0	1.37	0.54-2.93	3.0	12.00
300 Gy	79.4-117.6	2.02	0.80-3.38	2.0	8.00
0.2 mM	99.8--110.0	2.09	0.51-4.31	1.0	4.00
0.3 mM	100.0-134.4	4.12	0.40-4.76	1.0	4.00
0.4 mM	101.0-145.8	2.46	0.43-6.93	2.0	8.00

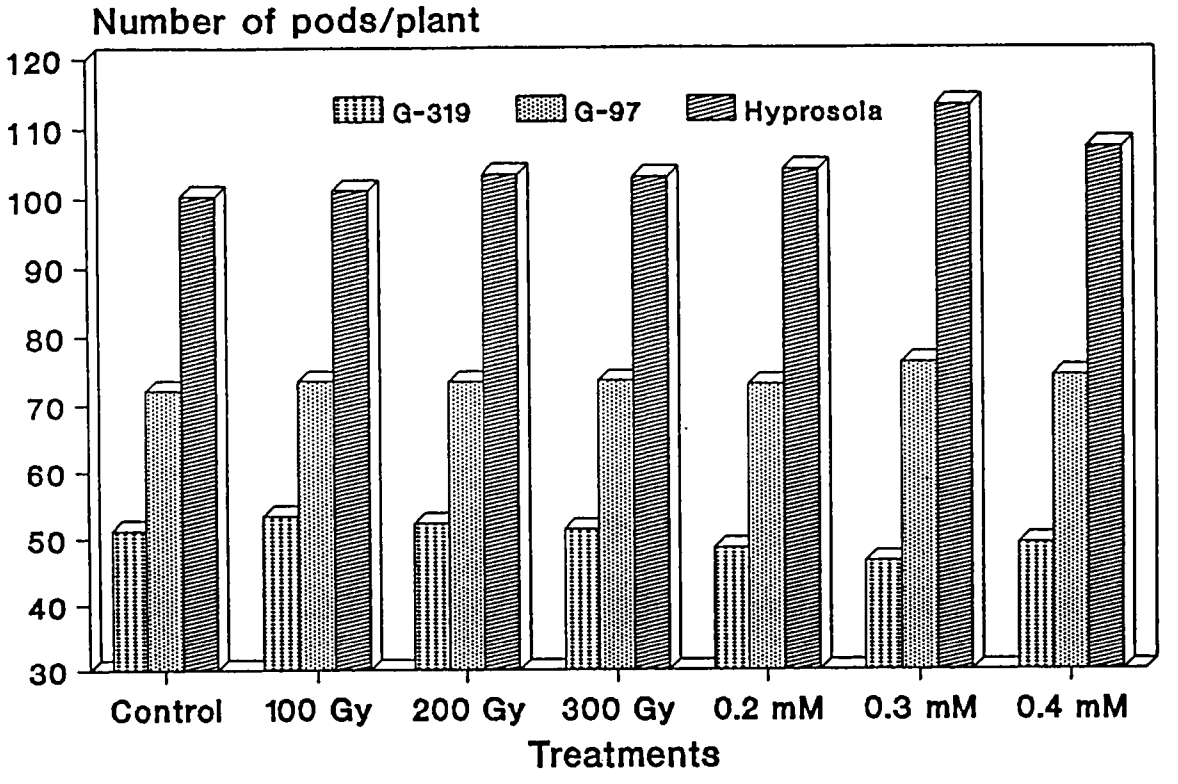


Fig. 7. Effect of different treatments of gamma rays and sodium azide on number of pods/plant in M_2 generation

Table 4.14. Analysis of variance, component of variation, heritability and genetic advance for number of pods/plant in M_2 generation of chickpea

Strains/ Doses/ Conc.	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	2.86	0.86	1.98	2.52	21.90	3.94
100 Gy	261.23**	8.73	13.35	14.32	85.96	25.37
200 Gy	65.35**	5.97	6.67	7.68	72.77	11.73
300 Gy	68.27**	0.71	6.67	8.95	55.60	10.25
0.2 mM	45.68**	5.00	5.79	7.36	61.91	9.39
0.3 mM	89.54**	10.29	8.67	10.48	68.46	5.18
0.4 mM	24.53**	16.13	4.17	5.56	56.15	6.24
G-97						
Control	2.04	1.14	1.32	2.14	27.92	1.68
100 Gy	306.77**	1.92	10.57	10.88	94.38	21.16
200 Gy	140.49**	1.72	15.12	15.30	97.79	30.83
300 Gy	324.55**	3.35	10.88	11.73	94.82	21.82
0.2 mM	21.37**	13.55	2.44	3.97	38.00	3.10
0.3 mM	159.14**	3.92	7.31	7.71	89.89	14.27
0.4 mM	285.10**	2.83	10.10	10.31	96.08	20.39
Hyprosola						
Control	1.86	1.36	0.77	1.84	17.44	0.66
100 Gy	53.49**	23.78	3.15	3.50	81.30	5.86
200 Gy	49.49**	16.14	2.97	3.27	82.45	5.59
300 Gy	259.88**	0.82	6.93	7.22	92.15	13.70
0.2 mM	29.66**	4.17	2.11	2.99	51.18	3.15
0.3 mM	508.51**	5754.21	8.43	8.78	92.77	16.73
0.4 mM	402.91**	19.22	8.28	8.60	91.90	5.16

**, Significant at 1% level

increased with the increase in doses/concentrations of mutagens. The highest GCV (15.12) was found in 200 Gy of gamma-ray in the strain G-97, while the lowest value (2.11) in 0.2 mM of sodium azide in the variety Hyprosola. The heritability estimates were higher in the mutagenized populations. It ranged from 51.18 to 97.79% as against from 17.44 to 27.92% in controls. The genetic advance in the treated population was much higher than the controls. The highest genetic advance (30.38) was found in 200 Gy of gamma-ray in the strain G-97.

(e) Number of seeds/plant

The ranges of family means, population CV%, ranges of population CV% and frequencies of the mutated families of this character are presented in Table 4.15. Population means increased in the treated populations than the controls (Fig. 8). The result showed that the range of family means in the treated population was observed to be wider (41.6-263.4) than the control families (60.8-195.2). Variation among the population means were observed. Greater range of CV% was found in the treated population than the controls. The highest CV% (9.23%) was obtained in 100 Gy of gamma-ray in the variety Hyprosola while the lowest (1.86) was found in 100 Gy of gamma-ray in the strain G-97. The highest percentage of mutated families (9.67%) was observed in 300 Gy of gamma-ray in the strain G-319. On the other hand, 100 Gy of gamma-ray in the strain G-319 showed the lowest value (3.57%).

Mean square values of the analysis of variance, component of variation and genetic parameters for number of seeds/plant are summarized in Table 4.16. The inter-family mean squares were significant in the mutagenized population at 1% level. The population under different treatments showed higher genotypic and phenotypic coefficients of variation over the controls. The highest genotypic coefficients of variation (14.59) was shown in 0.4 mM sodium azide in the strain G-97 while the lowest (1.55) was found in 0.2 mM treatment of sodium azide in the variety

Table 4.15. Means, coefficient of variations and mutation frequencies for number of seeds/plant in M_2 generation of chickpea

Strains/ Doses/ Conc.	Ranges of family means	Population CV%	Ranges of families CV%	Frequencies of mutated families No.	%
G-319					
Control	60.8-62.8	1.17	0.57-1.65	0.0	0.00
100 Gy	58.0-84.0	6.18	0.47-7.14	1.0	3.57
200 Gy	56.8-77.0	7.5	0.26-9.46	2.0	6.15
300 Gy	49.6-74.8	6.04	0.36-8.38	3.0	9.67
0.2 mM	49.2-71.4	7.39	1.10-11.59	1.0	3.84
0.3 mM	41.6-69.0	4.54	0.05-6.97	1.0	4.00
0.4 mM	55.2-60.6	3.71	0.52-8.66	1.0	4.16
G-97					
Control	104.0-113.0	2.63	1.02-2.72	0.0	0.00
100 Gy	102.8-110.8	1.86	0.45-4.02	1.0	4.00
200 Gy	98.4-152.0	6.49	0.46-8.02	2.0	8.00
300 Gy	73.6-146.6	7.78	0.46-9.56	2.0	8.00
0.2 mM	94.2-134.4	2.41	0.73-6.10	1.0	3.84
0.3 mM	90.4-119.0	4.78	0.73-8.76	1.0	3.76
0.4 mM	99.8-144.8	7.14	0.32-9.57	1.0	3.84
Hyprosola					
Control	179.9-195.2	1.78	0.49-7.58	0.0	0.00
100 Gy	160.0-195.4	9.23	3.40-9.21	1.0	4.00
200 Gy	187.4-208.6	5.10	1.00-7.85	2.0	8.00
300 Gy	185.0-245.4	7.78	2.31-9.12	1.0	4.00
0.2 mM	143.8-196.6	6.68	1.80-8.23	1.0	4.00
0.3 mM	182.8-263.4	5.07	1.41-7.94	1.0	4.00
0.4 mM	187.8-207.0	6.34	1.32-8.69	1.0	4.00

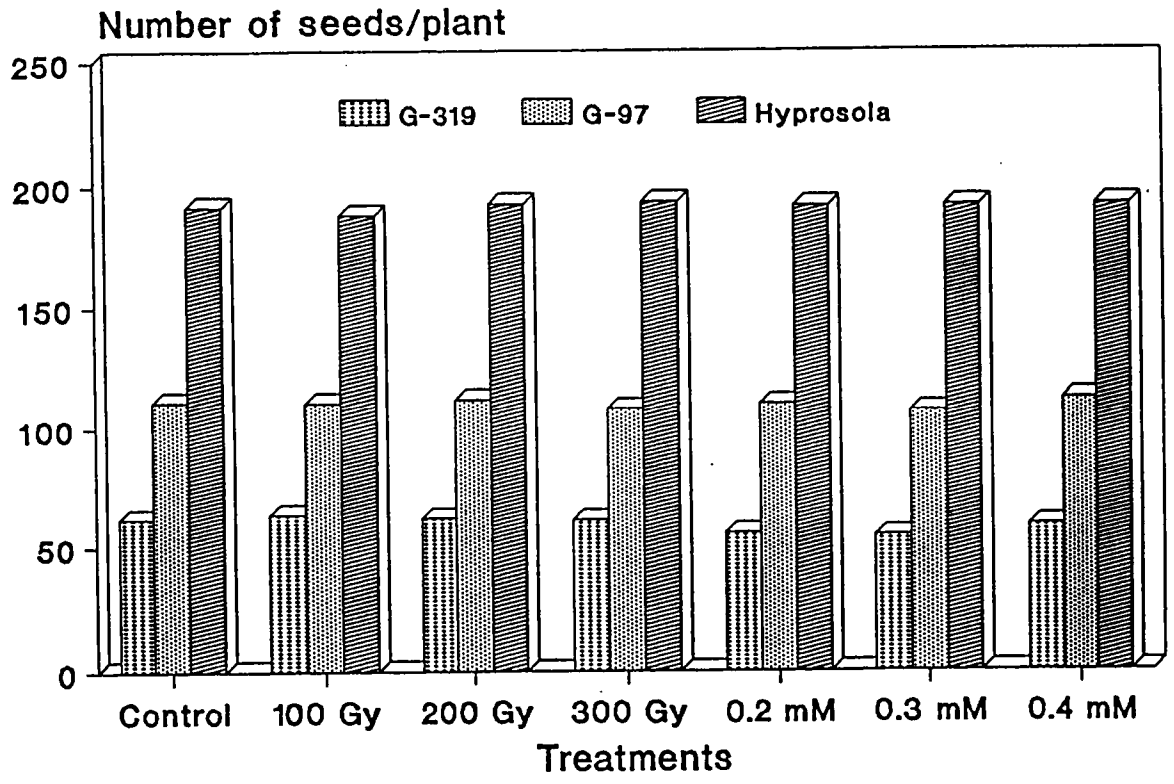


Fig. 8. Effect of different treatments of gamma rays and sodium azide on number of seeds/plant in M_2 generation

Table 4.16. Analysis of variance, component of variation, heritability and genetic advance for number of seeds/plant in M_2 generation of chickpea

Strains/ Doses/ Conc.	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	2.80	3.10	1.34	1.75	55.08	1.99
100 Gy	274.20**	3.08	11.58	11.78	96.57	23.45
200 Gy	100.88**	4.35	7.09	7.80	82.69	13.29
300 Gy	133.62**	1.84	8.37	8.62	94.28	16.75
0.2 mM	121.51**	4.48	8.22	11.04	55.32	12.59
0.3 mM	198.41**	12.96	11.39	12.26	86.29	21.80
0.4 mM	16.41**	20.43	2.58	4.52	32.62	3.03
G-97						
Control	2.46	5.96	1.53	3.15	30.09	1.95
100 Gy	898.60**	1.90	12.08	12.23	97.66	24.60
200 Gy	622.45**	0.35	9.94	10.06	97.77	22.26
300 Gy	795.79**	4.42	11.64	11.77	97.70	23.69
0.2 mM	548.49**	27.75	9.45	9.76	93.89	18.87
0.3 mM	124.06**	10.78	4.48	5.27	72.20	7.85
0.4 mM	340.22**	7.09	14.59	14.63	99.39	29.96
Hyprosola						
Control	2.36	3.46	1.38	1.45	68.07	1.94
100 Gy	272.08**	16.94	3.91	4.10	90.99	7.69
200 Gy	109.77**	8.45	2.37	2.62	82.35	4.46
300 Gy	569.10**	16.42	9.14	9.17	99.27	18.76
0.2 mM	54.95**	3.54	1.55	2.28	26.21	2.17
0.3 mM	623.49**	41.24	11.90	12.08	47.07	24.31
0.4 mM	102.92**	8.82	2.27	2.64	74.04	4.04

**, significant at 1% level

Hyprosola among the treated population. Similar results were found in phenotypic coefficients of variation. Heritability of the treated population was much higher than that of the control families. The highest heritability (99.39%) was recorded in 0.4 mM concentration of NaN_3 in the strain G-97. On the other hand, the lowest heritability values (26.21%) was found in 0.2 mM concentration of sodium azide in Hyprosola. Similar results were found in case of genetic advance being ranged from 2.17 to 29.96% in the treated population while 1.94 to 1.99 in the controls.

(f) 100-seed weight

The ranges of family means, coefficients of variation and the number and percentages of mutated families for each treatment along with their controls are shown in Table 4.17. The population means of 100-seed weight in all the treated populations are compared with their controls in Fig.9. The population means of 100-seed weight decreased with the increase of doses in the strain G-319. But the other two strains, G-97 and Hyprosola population means were not much influenced by the treatments.

The ranges of family means of 100-seed weight ranged from 6.5 to 29.7g and the family means of controls were 26.03 (G-319), 16.69 (G-97) and 7.41 g (Hyprosola). The ranges of family means were found to be wider in the mutagenized population than in the controls. The estimated ranges of family CV% were from 0.00 to 9.60 in gamma-ray and sodium azide treated population. The highest population CV% was found to be 5.24 in 300 Gy of gamma-ray in the strain G-97. The lowest population CV% was observed in the control G-97 (1.09). The highest percentages of the mutated families were obtained in 300 Gy of gamma-ray in the strain G-97 (16.0%) whereas the lowest percentage was observed in 200 Gy of gamma-ray in the strain G-329 (3.33%).

Mean square values of the analysis of variance, component of variation, heritability and genetic advance (% of mean) for 100-seed weight are presented in

Table 4.17. Means, coefficient of variations and mutation frequencies for 100-seed weight in M_2 generation of chickpea

Strains/ Doses/ Conc.	Ranges of family means	Population CV%	Ranges of families CV%	Frequencies of mutated families No.	%
G-319					
Control	25.9-26.1	1.31	0.46-1.62	0.0	0.00
100 Gy	23.1-27.3	2.99	0.21-3.83	1.0	3.57
200 Gy	23.1-28.4	4.05	0.20-6.75	1.0	3.33
300 Gy	20.1-29.7	2.89	0.00-3.84	3.0	9.67
0.2 mM	24.9-26.4	2.22	0.32-4.40	1.0	3.84
0.3 mM	19.1-26.7	3.62	0.35-5.26	1.0	4.00
0.4 mM	21.2-27.1	2.44	1.19-9.60	2.0	8.33
G-97					
Control	16.1-17.3	1.09	0.41-1.90	0.0	0.00
100 Gy	15.1-19.7	1.98	0.85-5.64	1.0	4.00
200 Gy	15.2-20.2	2.64	0.77-4.16	2.0	8.00
300 Gy	15.8-21.1	5.24	0.61-6.07	4.0	16.00
0.2 mM	16.0-18.0	4.65	0.63-6.66	1.0	3.84
0.3 mM	16.1-20.1	1.77	0.71-2.79	1.0	3.70
0.4 mM	16.7-20.2	1.92	0.51-3.42	1.0	3.84
Hyprosola					
Control	7.3-7.4	1.95	0.73-2.09	0.0	0.00
100 Gy	7.0-7.6	2.45	1.11-7.26	1.0	4.00
200 Gy	6.5-8.4	3.45	0.81-5.76	2.0	8.00
300 Gy	6.6--8.5	4.20	0.00-6.75	3.0	12.00
0.2 mM	7.1-7.5	2.47	1.13-4.31	1.0	4.00
0.3 mM	7.1-8.0	3.57	0.59-5.19	1.0	4.00
0.4 mM	7.1-8.4	2.55	0.59-5.16	2.0	8.00

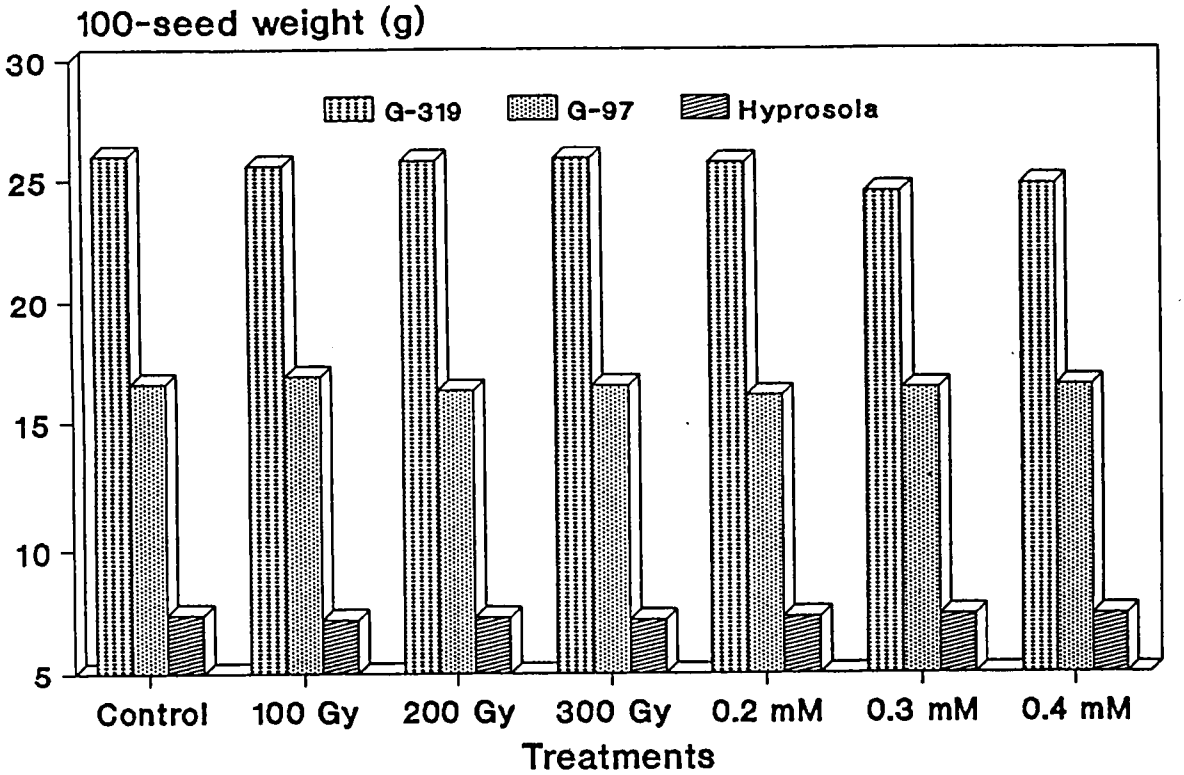


Fig. 9. Effect of different treatments of gamma rays and sodium azide on 100-seed weight in M_2 generation

Table 4.18. The inter-family means squares were found to be highly significant at 1% level. The genotypic coefficients of variation ranging from 0.11 to11.20 in the treated population in the three strains of chickpea were greater than that of the GCV of control families. The highest GCV (11.20) was recorded in 0.4 mM concentration of sodium azide treated population in the strain G-97. Similarly, the ranges of PCV were found to be 0.12-11.40 in the treated populations. Heritability estimates were found to be higher in the mutagenized population (97.91%) of 300 Gy of gamma-ray in the strain G-97 which was much higher than that of the controls. Similarly, genetic advance was higher, ranging from 2.51 to 22.53 in treated population.

(g) Seed yield/plant

The single plant seed yield is obviously a character of higher economic value than the other characters which ultimately contribute to plant productivity. The ranges of family means, population CV%, ranges of family CV% and frequencies of mutated families are shown in Table 4.19. The means of M_2 population for all the treatments showed mutagenic effects (Fig. 10). The highest population mean for seed yield/plant was found in 0.4 mM treatment of sodium azide in the strain G-97 (19.07g), whereas the lowest value (13.90g) was in 100 Gy of gamma-ray treatment of Hyprosola. The range of variation and pattern of frequency distribution in the mutagen treated populations showed that mutagen treatments created wider variability to both positive and negative directions of the controls. The treatments created and increased variability in the form of CV% which was wider in the mutagenized populations (1.98-9.03) than the controls (1.20-2.04). On the other hand, ranges of population CV% (0.00-21.09) were also higher than the controls. The highest percentages of mutated families (12.90%) were observed in 300 Gy of gamma-ray in the strain G-319 and the lowest value (3.57%) was in 100 Gy of gamma-ray in the same variety.

Table 4.18. Analysis of variance, component of variation, heritability and genetic advance for 100-seed weight in M_2 generation in chickpea

Strains/ Doses/ Cone.	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	0.75	0.25	0.00	1.26	0.00	0.00
100 Gy	6.18**	0.11	4.22	4.68	81.94	9.01
200 Gy	7.09**	0.02	4.68	4.69	95.23	9.18
300 Gy	10.62**	0.31	5.58	5.90	89.61	10.88
0.2 mM	3.82**	1.40	3.22	3.92	67.64	5.46
0.3 mM	13.42**	0.06	6.45	7.41	76.13	11.60
0.4 mM	6.54**	2.48	4.46	5.09	76.87	8.07
G-97						
Control	1.28**	1.02	1.33	6.22	4.67	0.59
100 Gy	3.51**	1.09	4.87	5.25	86.07	9.31
200 Gy	9.76**	0.09	1.24	1.27	96.05	2.51
300 Gy	9.37**	1.04	7.77	7.86	97.91	15.85
0.2 mM	0.50**	0.10	1.74	2.36	56.25	2.70
0.3 mM	4.44**	0.14	5.35	5.63	89.69	10.43
0.4 mM	3.98**	0.13	11.20	11.40	97.24	22.82
Hyprosola						
Control	0.04	0.02	1.55	9.75	16.15	1.27
100 Gy	1.39**	0.59	6.88	7.55	90.00	13.96
200 Gy	1.66**	0.27	2.53	5.85	61.11	7.30
300 Gy	1.78**	0.83	7.68	9.92	60.78	12.34
0.2 mM	1.07**	0.07	0.11	0.12	96.20	22.53
0.3 mM	1.28**	0.13	2.76	4.52	38.18	3.51
0.4 mM	1.31**	0.08	0.31	0.40	60.23	5.04

**, significant at 1% level

Table 4.19. Means, coefficients of variation and mutation frequencies for seed yield/plant in M_2 generation of chickpea

Strains/ Doses/ Conc.	Ranges of family means	Population CV%	Ranges of families CV%	Frequencies of mutated families No.	%
G-319					
Control	15.3-16.1	1.20	1.41-3.36	0.0	0.00
100 Gy	14.3-18.9	3.39	0.90-4.37	1.0	3.57
200 Gy	14.6-17.6	8.18	0.87-2.82	2.0	6.66
300 Gy	14.1-19.5	9.03	3.92-10.37	4.0	12.90
0.2 mM	13.6-15.8	4.99	0.91-21.09	1.0	3.84
0.3 mM	11.6-17.6	5.48	0.95-7.91	1.0	4.00
0.4 mM	14.7-17.1	5.93	3.19-9.23	2.0	8.33
G-97					
Control	17.8-18.5	1.55	0.79-2.06	0.0	0.00
100 Gy	14.6-23.3	7.81	2.40-9.29	1.0	4.00
200 Gy	17.1-24.5	5.25	1.72-7.98	2.0	8.00
300 Gy	12.6-25.2	4.58	0.77-5.61	2.0	8.00
0.2 mM	15.2-19.9	5.99	0.88-7.37	1.0	3.84
0.3 mM	15.1-20.5	4.14	0.59-7.32	1.0	3.70
0.4 mM	16.9-25.8	2.46	0.41-2.83	2.0	7.69
Hyprosola					
Control	14.0-14.4	2.04	0.37-3.35	0.0	0.00
100 Gy	12.2-14.5	1.99	0.42-3.84	1.0	4.00
200 Gy	13.9-15.5	4.99	0.26-6.83	3.0	12.00
300 Gy	11.2-17.7	7.39	2.00-9.34	3.0	12.00
0.2 mM	14.1-14.7	3.04	0.23-3.22	1.0	4.00
0.3 mM	13.8-20.1	1.98	0.26-7.90	1.0	4.00
0.4 mM	14.1-21.5	5.59	0.00-7.35	2.0	8.00

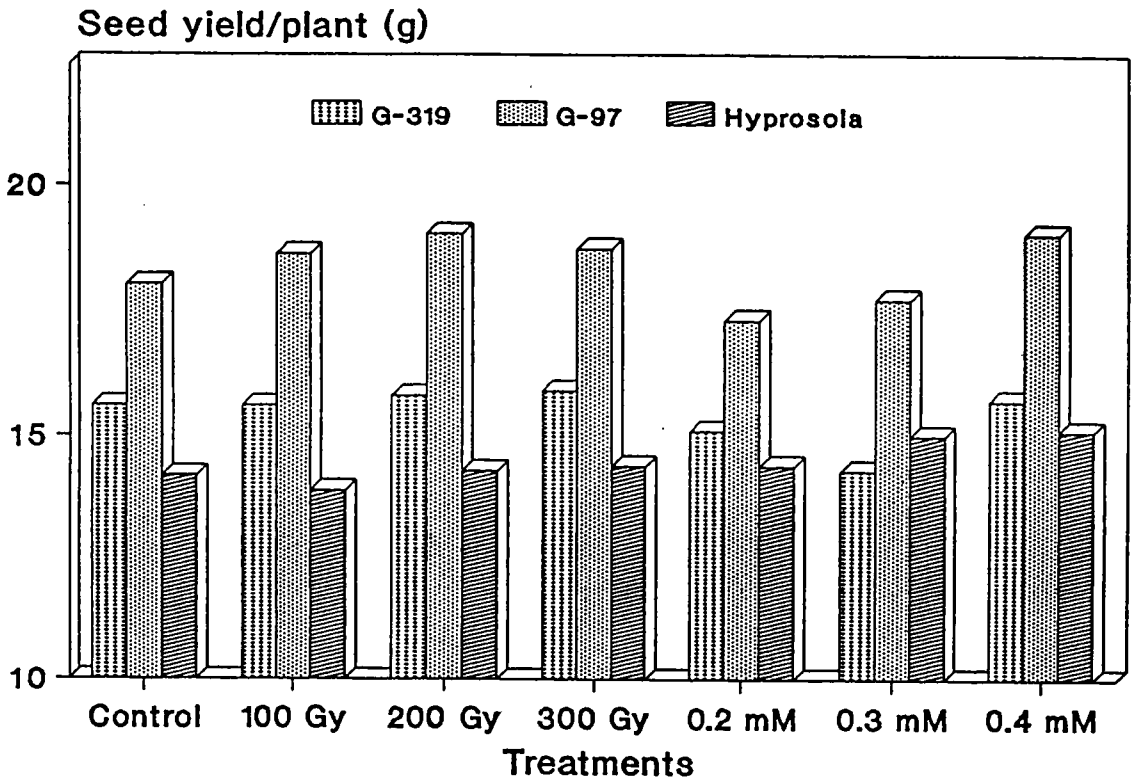


Fig. 10. Effect of different treatments of gamma rays and sodium azide on seed yield/plant in M_2 generation

The mean squares of the analysis of variance showed significant inter-family variances at 1% level (Table 4.20). The changes in the mean values in the treated population were followed by change in the mean square values. The genotypic coefficients of variation showed ranges from 0.98 to 33.92 in the mutagenized population as against the ranges of controls (0.69-1.91). Similarly, wider ranges of phenotypic coefficients of variation was also observed in the population. Estimates of heritability and genetic advances were recorded to be appreciably greater in the mutated generation. High genetic advance value was calculated in the mutagenized populations than the untreated controls.

(iii) Frequency of positive and negative mutations

The frequency of positive and negative mutations for all the characters studied with higher and lower means than controls are shown in Table 4.21.

Shifting of mean values to both positive and negative directions than the control mean were found in all the characters, treated with different doses/concentrations of gamma-ray and sodium azide. The frequencies of negative mutation with lower mean were found to be higher in number of pods/plant (17.77) followed by 100-seed weight (15.46), plant height (13.25), number of seeds/plant (13.00) and seed yield/plant (7.66). On the other hand, days to flowering (11.53) and days to maturity (9.23) showed higher mutation frequencies with higher mean than the controls.

(iv) List of selected families in M_2 generation

A list of the selected families including their pedigree, seed yield and yield contributing characters are presented in Appendix 3. Selection was made from the M_2 generation mainly on the basis of superior performance in respective to seed yield/ plant followed by days to maturity, seed size (100-seed weight), number of pods/plant and number of seeds/plant. Out of 498 families only 124 were selected for growing in the next generation.

Table 4.20. Analysis of variance, component of variation, heritability and genetic advance for seed yield/plant in M_2 generation of chickpea

Strains/ Doses/ Cone	Variance		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	0.51	0.03	1.91	2.31	69.23	2.47
100 Gy	11.75**	0.16	9.65	10.22	89.10	18.77
200 Gy	3.88**	0.67	4.91	9.15	20.19	3.80
300 Gy	10.02**	0.15	8.84	9.08	95.19	17.76
0.2 mM	4.78**	1.51	6.06	7.86	59.57	9.64
0.3 mM	7.32**	1.12	8.08	9.75	68.71	13.81
0.4 mM	6.65**	0.06	7.24	7.37	98.50	14.90
G-97						
Control	0.54	0.08	1.66	2.23	52.94	2.49
100 Gy	40.23**	0.86	33.92	33.97	99.72	69.87
200 Gy	17.77**	0.02	9.87	9.95	98.33	20.16
300 Gy	25.16**	1.02	11.96	12.06	98.43	37.59
0.2 mM	7.10**	1.73	6.66	7.76	73.89	11.80
0.3 mM	12.10**	0.36	8.70	8.96	94.09	17.38
0.4 mM	22.38**	3.91	11.03	11.30	97.15	22.90
Hyprosola						
Control	0.09	0.02	0.69	1.32	33.33	0.83
100 Gy	1.57**	0.10	3.87	4.40	78.37	7.06
200 Gy	1.58**	0.14	2.20	2.48	55.55	3.37
300 Gy	8.48**	0.12	8.96	9.09	97.67	18.24
0.2 mM	2.19**	0.03	0.98	2.30	18.18	0.86
0.3 mM	11.71**	0.06	10.15	10.34	96.26	20.52
0.4 mM	19.46**	0.19	12.96	13.06	98.72	26.54

**, Significant at 1% level

4.21. Direction of mutation frequency for different characters in M_2 generation of chickpea following treatment with gamma-ray and sodium azide

Characters	Mutated families with higher means (%)	Family means similar with controls (%)	Mutated families with lower means (%)
Days to first flowering	11.53	85.89	2.56
Days to maturity	9.23	88.78	1.98
Plant height (cm)	3.01	83.73	13.25
No. of pods/ plant	4.10	78.10	17.77
No. of seeds/ plant	2.79	84.21	13.00
100-seed weight (g)	2.98	81.54	15.46
Seed yield/ plant (g)	1.98	90.34	7.66

C. Observation in M_3 generation

This experiment was conducted with only those families which were selected on the basis of some desirable characters in the M_2 generation. The induced variability in the M_3 population was analyzed in the same manner as in the M_2 generation. The selected plants were grown in plant-progeny rows to observe their breeding behaviour. The number of M_3 families varied from treatment to treatment. Ten plants from each family were taken randomly to study the nature of induced genetic variability with respect to its magnitude, direction, heritable component and response to selection in M_3 generation.

(a) Days to flowering

The means, ranges, inter-family CV% and components of variations for days to first flowering are presented in Table 4.22. The ranges of the family means in all the treatments were higher than the controls. Means of the treated population of different doses/concentrations are shown in Fig. 11. The trend of the population mean in the treated population of the three strains showed reduction in days to first flowering except the 100 Gy treatment of the strain G-319 (70.35) and 0.2 mM treatment of sodium azide in the strain G-97 (73.86). The materials, however, advanced more towards earliness in M_3 than in the M_2 generation. The coefficients of variation were much higher than the CV% of their respective controls. The highest CV% (4.40) was found in 0.4 mM treatment of sodium azide in the strain G-319 which was higher than that of all the treatments and controls.

The analysis of variances and genetic parameters are presented in Table 4.23. It was found that all the families within the treatment showed significant differences at 1% except 0.2 mM treatment of sodium azide in the variety Hyprosola and control of G-97 which were significant at 5%. The remaining two controls, G-319 and Hyprosola had no significant variation. In the treated populations, the genotypic coefficients of variation (GCV) clearly increased over the controls. The highest GCV (36.32%) was

Table 4.22. Mean, coefficient of variation and frequency of mutation to flowering in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	69.80	69.2-71.0	1.39	0.81-1.52
100 Gy	70.35	66.2-74.6	1.84	0.83-3.65
200 Gy	69.63	61.5-73.9	1.49	0.95-3.10
300 Gy	65.94	57.5-72.2	1.80	0.82-3.95
0.2 mM	66.20	61.5-70.0	1.87	0.75-2.75
0.3 mM	65.06	56.1-70.2	1.80	1.16-4.56
0.4 mM	65.06	53.0-69.7	4.40	1.60-10.30
G-97				
Control	73.49	73.0-75.0	1.04	0.00-1.68
100 Gy	69.43	62.1-75.4	2.79	1.60-3.37
200 Gy	69.54	59.9-75.9	2.07	0.00-2.88
300 Gy	73.14	62.7-78.5	2.53	0.60-4.49
0.2 mM	73.86	72.4-75.3	1.36	1.14-1.74
0.3 mM	68.81	63.9-73.3	3.16	1.14-4.93
0.4 mM	70.86	59.1-79.7	1.58	0.88-3.74
Hyprosola				
Control	60.57	60.0-61.1	1.64	0.00-1.33
100 Gy	58.32	51.9-61.3	1.99	0.85-3.64
200 Gy	59.25	53.8-62.5	2.21	0.83-3.33
300 Gy	57.88	48.3-67.0	1.80	0.95-3.77
0.2 mM	60.45	59.0-61.9	1.11	0.88-2.44
0.3 mM	59.20	51.0-61.3	4.31	0.32-3.20
0.4 mM	59.05	54.0-63.9	5.45	1.40-4.41

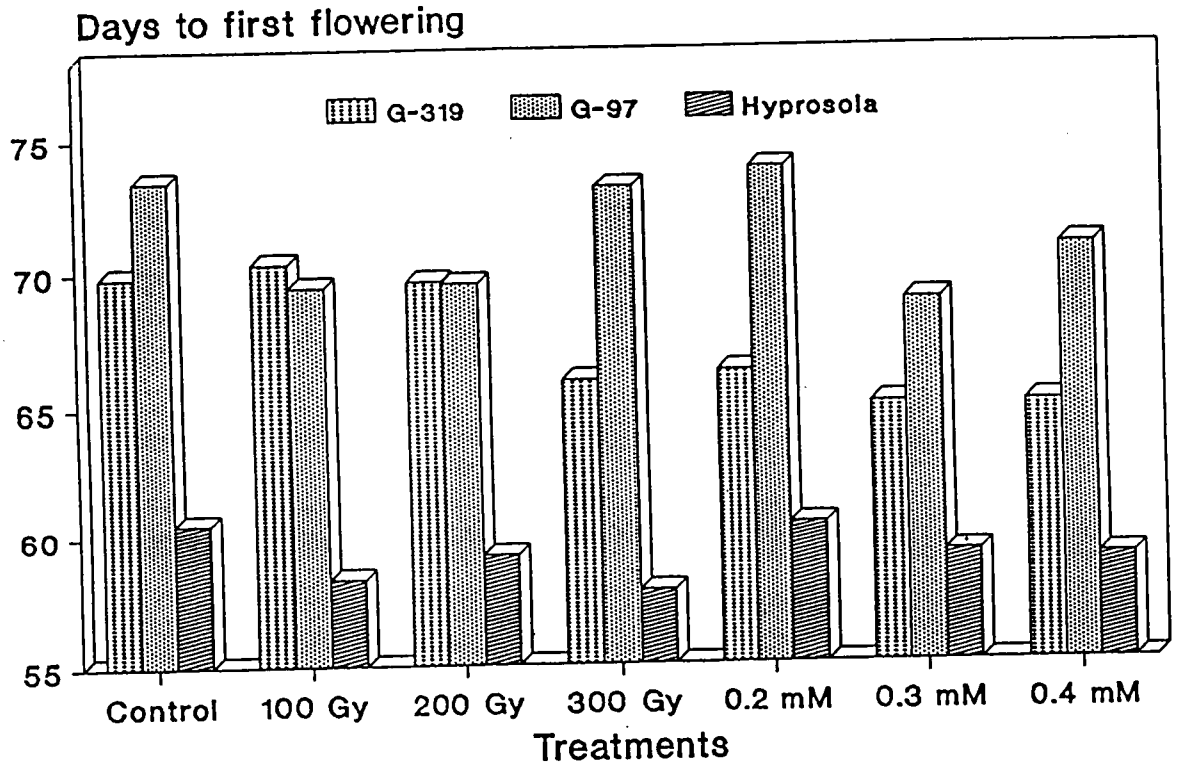


Fig. 11. Effect of different treatments of gamma rays and sodium azide on days to first flowering in M_3 generation

Table 4.23. Analysis of variance, component of variation, heritability and genetic advance for days to flowering in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	1.44	1.59	0.10	1.72	3.36	0.01
100 Gy	49.73**	1.96	3.04	4.16	73.39	4.57
200 Gy	77.34**	5.31	3.83	5.18	94.77	5.84
300 Gy	232.42**	6.70	7.21	8.15	98.23	6.97
0.2 mM	125.93**	5.21	5.13	7.07	82.64	7.67
0.3 mM	333.53**	5.69	8.83	9.26	90.86	17.34
0.4 mM	397.90**	3.45	9.48	11.43	68.71	16.18
G-97						
Control	2.45*	0.36	0.45	1.60	3.97	0.26
100 Gy	308.26**	2.48	7.94	8.42	89.03	15.44
200 Gy	285.29**	3.49	7.61	8.21	96.02	14.55
300 Gy	199.26**	9.13	5.99	6.96	74.23	10.64
0.2 mM	16.53**	2.01	1.68	2.16	60.54	2.70
0.3 mM	146.65**	8.31	5.43	6.60	67.64	9.20
0.4 mM	523.78**	7.32	10.14	10.76	88.91	19.71
Hyprosola						
Control	1.31	0.31	0.28	1.65	2.97	0.10
100 Gy	187.75**	1.28	7.42	8.43	77.62	13.48
200 Gy	151.10**	1.77	6.52	6.88	89.72	12.72
300 Gy	387.71**	3.58	10.64	11.67	83.13	20.00
0.2 mM	42.05*	1.27	3.13	5.16	36.75	2.36
0.3 mM	592.30**	3.42	11.69	12.65	88.37	23.12
0.4 mM	470.45**	7.27	36.32	36.71	97.80	74.00

**, *. Significant at 1% and 5% level respectively

obtained in 0.4 mM treatment of sodium azide in the variety Hyprosola and the lowest value (0.10%) in the control of G-319. Similarly, phenotypic coefficients of variation (PCV) in the treated populations increased even more as compared to controls. The highest PCV value (36.71%) was found in 0.4 mM treatment of sodium azide in the variety Hyprosola and the lowest value (1.60%) was in the control of G-97. The relatively increased GCV over control was also reflected in the higher magnitude of heritability for flowering days in the treated population. The estimated heritability of the families within the treatment was found much higher than the heritability within the control families. The highest heritability (97.80) for days to flowering was observed in 0.4 mM treatment of sodium azide in the variety Hyprosola and the lowest value (3.36) in the control of G-319. The estimates of heritability were higher in M_3 than those obtained in M_2 generation. Similarly, the genetic advance had higher magnitude (2.36-74.00) in the treated population compared to the controls (0.01-0.26).

(b) Days to maturity

The population means, ranges of family means, population CV% and ranges of population CV% for days to maturity in M_3 generation are presented in Table 4.24. The population means of the treated population and untreated controls were found to be 125 to 137 days (Fig. 12). The range of family means varied from 120 to 141 in the treated population and 126 to 138 in the untreated controls. The result revealed that the ranges of family means were higher in the treated populations than the ranges of family means of untreated controls. The population CV% decreased in the M_3 generation over the M_2 and ranged from 0.61-1.50 in the treated population and from 0.49 to1.15 in untreated controls.

Inter-family, intra-family variances (means square values), components of variation (genotypic and phenotypic coefficients of variation) and genetic parameters (heritability and genetic advance) are presented in Table 4.25. The result showed that inter-family variations were significant at 1% level in different treatments. The

Table 4.24. Mean, coefficient of variation and frequency of mutation to days to maturity in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	134	133-135	0.70	0.00-1.15
100 Gy	134	125-138	1.03	0.33-1.37
200 Gy	133	120-138	1.05	0.34-3.03
300 Gy	134	126-140	1.07	0.00-1.75
0.2 mM	133	129-134	1.50	0.32-2.60
0.3 mM	133	125-138	1.30	0.00-2.33
0.4 mM	134	129-136	1.11	0.06-1.70
G-97				
Control	137	137-138	1.15	0.35-1.76
100 Gy	135	120-139	1.11	0.50-2.42
200 Gy	133	120-138	1.10	0.22-2.74
300 Gy	136	128-140	1.17	0.23-2.46
0.2 mM	138	136-141	1.01	0.49-1.14
0.3 mM	134	127-139	1.10	0.00-1.34
0.4 mM	136	126-140	0.76	0.00-1.36
Hyprosola				
Control	127	126-128	0.49	0.00-1.49
100 Gy	126	121-130	0.61	0.43-0.88
200 Gy	128	125-134	1.17	0.37-2.02
300 Gy	125	124-130	1.12	0.51-1.99
0.2 mM	129	127-131	0.93	0.86-2.54
0.3 mM	129	127-131	1.17	0.44-1.52
0.4 mM	126	125-127	0.66	0.41-0.92

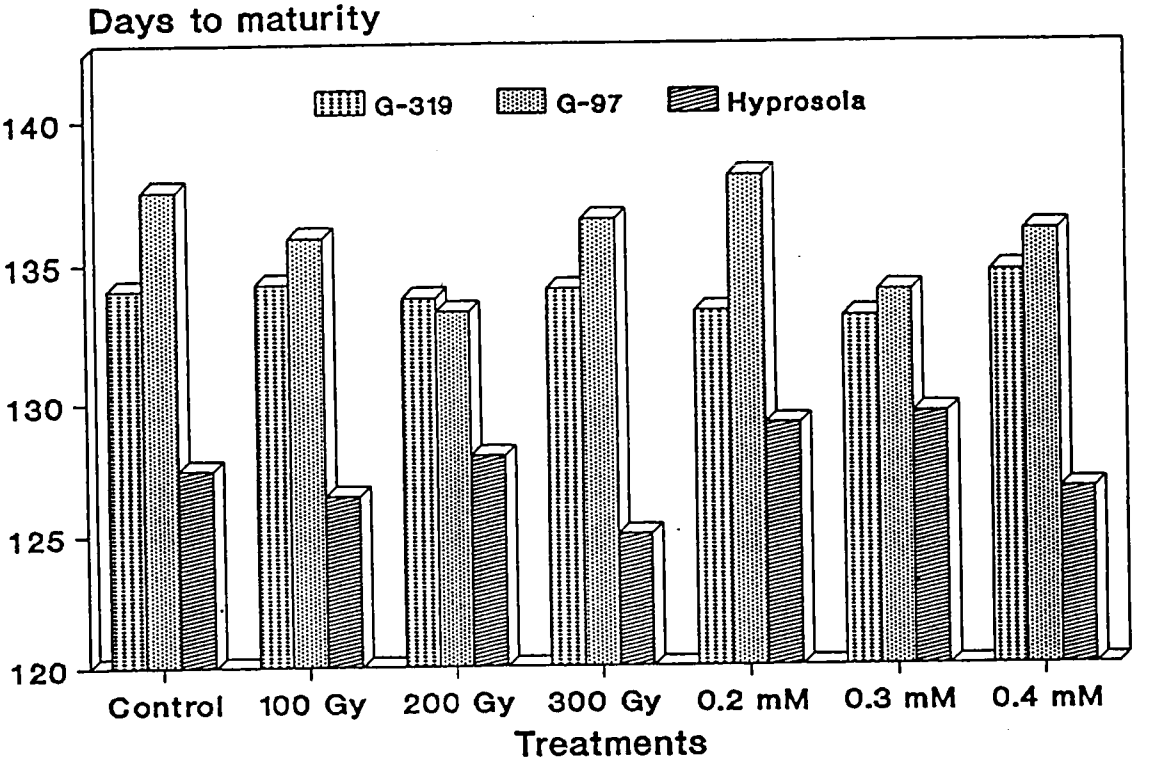


Fig. 12. Effect of different treatments of gamma rays and sodium azide on days to maturity in M_3 generation

Table 4.25. Analysis of variance, component of variation, heritability and genetic advance for days to maturity in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	1.71	0.80	0.21	0.73	8.53	0.12
100 Gy	111.75**	1.72	1.98	2.56	84.93	5.47
200 Gy	224.70**	0.31	3.51	3.84	97.73	13.63
300 Gy	177.03**	4.19	3.11	3.32	87.64	5.99
0.2 mM	57.62**	2.90	1.76	2.29	57.72	6.30
0.3 mM	233.42**	1.35	3.59	3.82	88.44	6.96
0.4 mM	61.90**	1.00	1.81	2.13	72.86	3.19
G-97						
Control	2.44	2.22	0.26	1.17	4.96	0.12
100 Gy	401.61**	2.63	4.61	5.10	82.83	8.70
200 Gy	396.35**	3.47	4.67	5.00	87.02	0.97
300 Gy	85.21**	1.43	2.10	2.40	76.26	3.77
0.2 mM	70.53**	0.81	1.89	2.14	77.84	3.44
0.3 mM	268.02**	4.88	3.82	4.10	86.72	7.33
0.4 mM	238.22**	0.60	3.57	3.65	95.68	7.20
Hyprosola						
Control	0.69	0.24	0.11	0.32	4.76	0.04
100 Gy	147.30**	0.83	3.02	12.03	96.13	6.10
200 Gy	144.06**	9.44	2.91	3.26	79.63	5.36
300 Gy	241.52**	4.31	3.89	4.10	89.63	7.58
0.2 mM	92.45**	6.13	2.27	2.98	58.04	4.60
0.3 mM	25.03**	2.05	1.16	1.65	49.78	1.69
0.4 mM	31.25**	0.91	1.38	1.53	81.55	2.57

**, Significant at 1% level

genotypic coefficients of variation was higher varying from 1.16 to 4.67 in gamma-ray and the sodium azide treated populations than the untreated controls (0.11-0.26). Similar result was also found in case of phenotypic coefficients of variation. Heritability ranged from 49.78 to 97.73 in the treated population which is much higher than that of the control families (4.76-8.53). In case of genetic advance, the treated populations had higher values than that of the controls. The highest genetic advance (13.63) was obtained in 200 Gy of gamma-ray in the strain G-319. On the other hand, the lowest genetic advance (0.04) was in the control of Hyprosola.

(c) Plant height

The population means, ranges of family means, population CV% and ranges of population CV% for plant height in M_3 generation are presented in Table 4.26. Population means of the treated and untreated controls are shown in Fig. 13. The population means in M_3 was found almost similar with means of M_2 generation. The range of variations (41.6-66.9) in the gamma-ray and sodium azide treated populations was higher than the range of the untreated controls (50.5-62.3). Population CV% of the treated population ranged from 0.78 to 2.66 which was much higher than the controls (1.03-1.93). Population CV% in the M_3 generation was lower than the M_2 generation. The ranges of population CV% estimated in the treated population was higher than the controls.

The analysis of variance (mean square value), components of variation (genotypic and phenotypic coefficients of variation) and genetic parameters (heritability and genetic advance) for plant height in M_3 generation are presented in Table 4.27.

The analysis of variance of all the doses/concentrations showed significant inter-family differences at 1% level except in 0.2 mM of sodium azide treatment of Hyprosola while 5% level of significance for inter-family variances were obtained in use of the strain G-319. It also showed that the genotypic coefficients of variation, ranging from 0.00 to 16.01 in treated population which was much higher than the

Table 4.26. Mean, coefficient of variation and frequency of mutation to plant height in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	59.8	59.2-60.8	1.93	1.52-2.06
100 Gy	59.4	55.0-66.6	1.79	0.00-2.94
200 Gy	59.9	54.4-64.4	1.99	0.70-3.09
300 Gy	59.1	50.8-66.7	1.45	0.00-2.58
0.2 mM	60.9	60.0-61.7	2.66	0.78-5.05
0.3 mM	60.6	54.8-66.3	1.52	0.76-1.83
0.4 mM	59.3	56.2-61.0	2.42	0.00-5.00
G-97				
Control	61.2	60.1-62.3	1.03	0.00-2.03
100 Gy	60.6	53.2-66.8	1.01	0.54-1.14
200 Gy	60.4	52.0-65.0	2.38	0.00-2.45
300 Gy	61.9	56.3-65.8	1.54	0.00-1.85
0.2 mM	62.7	61.1-64.9	0.91	0.48-1.20
0.3 mM	60.9	57.0-64.2	1.06	0.00-1.57
0.4 mM	62.5	58.0-66.9	0.78	0.00-1.09
Hyprosola				
Control	51.3	50.5-51.7	1.35	0.92-2.43
100 Gy	50.5	45.2-53.5	1.13	0.81-1.30
200 Gy	50.4	45.3-55.2	2.45	0.76-3.88
300 Gy	49.4	41.6-56.6	1.71	0.00-1.89
0.2 mM	52.5	52.3-52.7	1.30	0.91-1.57
0.3 mM	52.3	50.4-54.0	1.48	0.00-2.23
0.4 mM	46.5	41.9-51.2	1.06	0.75-1.23

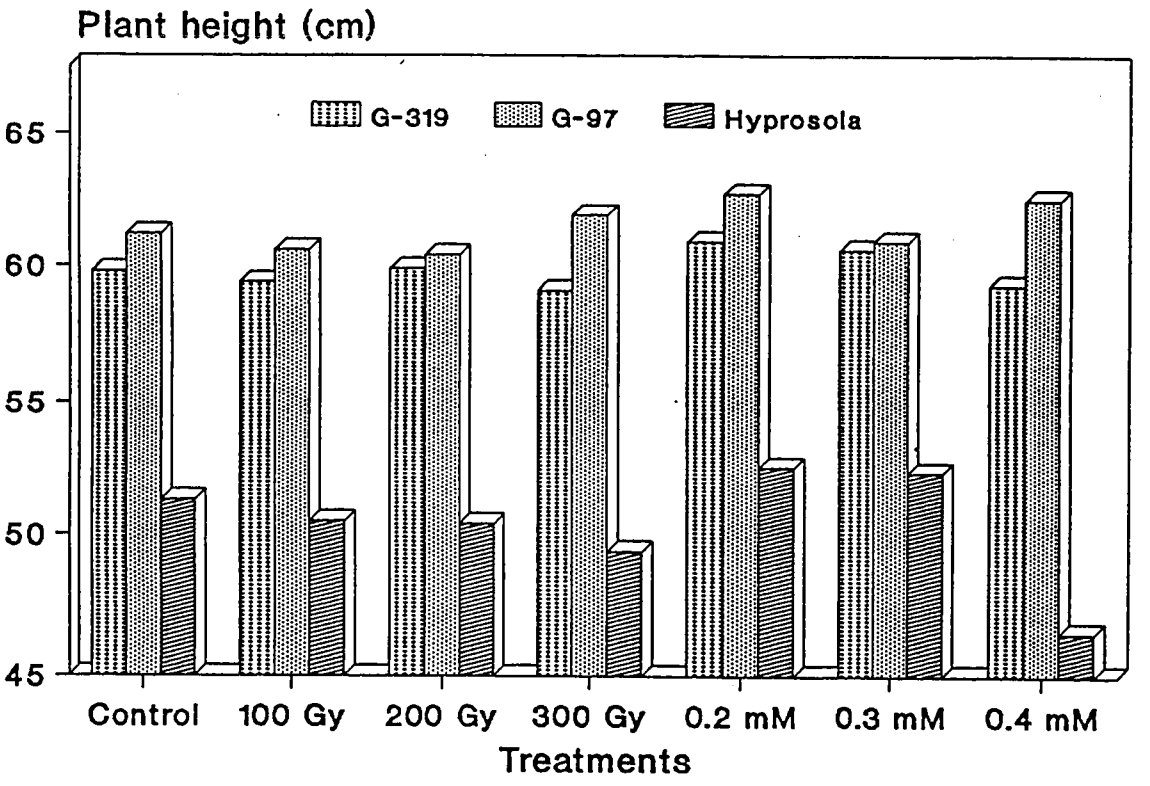


Fig. 13. Effect of different traetments of gamma rays and sodium azide on plant height in M3 generation

Table 4.27. Analysis of variance, component of variation, heritability and genetic advance for plant height in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	2.62*	1.60	0.01	1.85	3.52	0.28
100 Gy	96.16**	5.13	16.01	16.25	97.04	9.40
200 Gy	35.13**	2.13	3.06	3.64	70.35	6.28
300 Gy	215.11**	0.37	7.82	7.96	96.66	15.85
0.2 mM	4.95*	3.89	0.79	2.77	78.15	7.46
0.3 mM	139.53**	2.04	6.13	6.32	94.22	12.22
0.4 mM	31.58**	3.00	2.87	3.76	59.00	6.56
G-97						
Control	0.88	0.44	0.00	0.01	9.30	2.20
100 Gy	137.35**	0.49	6.09	6.23	95.59	12.27
200 Gy	157.24**	3.14	6.51	6.93	88.22	12.59
300 Gy	54.54**	0.91	3.73	4.03	85.62	12.11
0.2 mM	35.10**	0.70	2.97	3.10	91.55	5.99
0.3 mM	65.69**	0.60	4.17	4.45	87.66	8.05
0.4 mM	115.21**	0.23	5.42	5.47	97.95	11.05
Hyprosola						
Control	0.77	1.71	0.27	1.37	4.00	1.50
100 Gy	130.49**	0.16	7.13	7.22	97.52	14.50
200 Gy	160.26**	0.84	7.90	8.27	91.20	15.54
300 Gy	245.29**	3.77	9.93	10.61	87.58	19.15
0.2 mM	0.80	0.44	0.00	0.01	46.00	3.08
0.3 mM	32.93**	0.44	3.43	3.73	84.33	6.49
0.4 mM	432.45**	0.16	14.12	14.17	99.21	28.97

** , * Significant at 1% and 5% level respectively

untreated controls (0.00-0.27). Similar result was obtained in case of phenotypic coefficients of variation. The estimated heritability was higher in the treated population than in the controls. The highest heritability (99.21) was found in 0.4 mM of sodium azide treatment in Hyprosola. On the other hand, the lowest heritability (3.52) was estimated in the control, G-319. Genetic advance of plant height ranged from 3.08 to 28.97 in the treated population which is higher than the ranges in controls (0.28-2.20).

(d) Number of pods/plant

Population means, ranges of family means, population CV% and ranges of population CV% for number of pods/plant in the M_3 generation are presented in Table 4.28. Population means of pods/plant of the treated families and untreated controls showed variation among the different treatments (Fig.14). Number of pods/plants was found to be higher in the variety Hyprosola followed by the strains G-97 and G-319.

The family means ranged from 50.1 to106.0 in the untreated controls while 41.6 to 146.7 in different doses of gamma-ray and sodium azide treated populations. It was found from the result that selection in M_2 generation produced significant effect on the lower range of family means in the treated population of the M_3 generation. Population CV% and ranges of family CV% were found to be lower in the M_3 generation than the M_2 generation.

The result of the analysis of variance (mean square values), component of variation, (genotypic and phenotypic coefficients of variation) and genetic parameters (heritability and genetic advance) are presented in Table 4.29.

The mean square values indicated clearly inter-family differences at 1% level of significance except in 0.2 mM treatment of sodium azide in the variety Hyprosola (at 5% level). In regard to the component of variation, both genotypic and phenotypic coefficients of variation were greater in the treated populations than the untreated controls. Genotypic coefficients of variation (GCV) ranged from 0.72 to 24.35 in the

Table 4.28. Mean, coefficient of variation and frequency of mutation to pods/plant in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	51.0	50.1-54.5	1.88	1.55-5.93
100 Gy	53.3	42.3-77.5	4.67	1.35-8.80
200 Gy	53.6	49.0-65.4	2.92	1.33-4.14
300 Gy	53.1	41.6-61.8	3.33	0.00-3.92
0.2 mM	52.7	50.2-59.7	2.11	0.83-4.13
0.3 mM	52.1	48.9-60.5	3.20	1.29-5.59
0.4 mM	50.5	47.0-54.9	2.80	2.50-5.66
G-97				
Control	73.9	70.7-75.0	2.17	1.26-2.03
100 Gy	83.0	71.5-96.6	2.50	1.61-5.10
200 Gy	75.4	58.9-89.2	2.25	0.96-5.15
300 Gy	74.5	51.5-98.0	2.48	0.94-3.08
0.2 mM	71.5	70.7-72.4	2.69	1.89-3.73
0.3 mM	72.9	62.8-79.1	2.27	2.14-3.69
0.4 mM	82.4	67.0-97.9	1.74	0.32-2.64
Hyprosola				
Control	99.6	97.0-106.0	1.66	1.27-2.16
100 Gy	101.0	98.6-106.5	1.60	1.00-2.95
200 Gy	104.8	99.9-112.8	1.37	0.50-2.97
300 Gy	104.7	79.7-124.1	1.57	1.10-2.40
0.2 mM	104.1	102.1-106.2	2.38	1.83-3.49
0.3 mM	118.0	105.3-134.9	3.37	2.24-4.27
0.4 mM	125.1	103.6-146.7	3.25	2.40-5.72

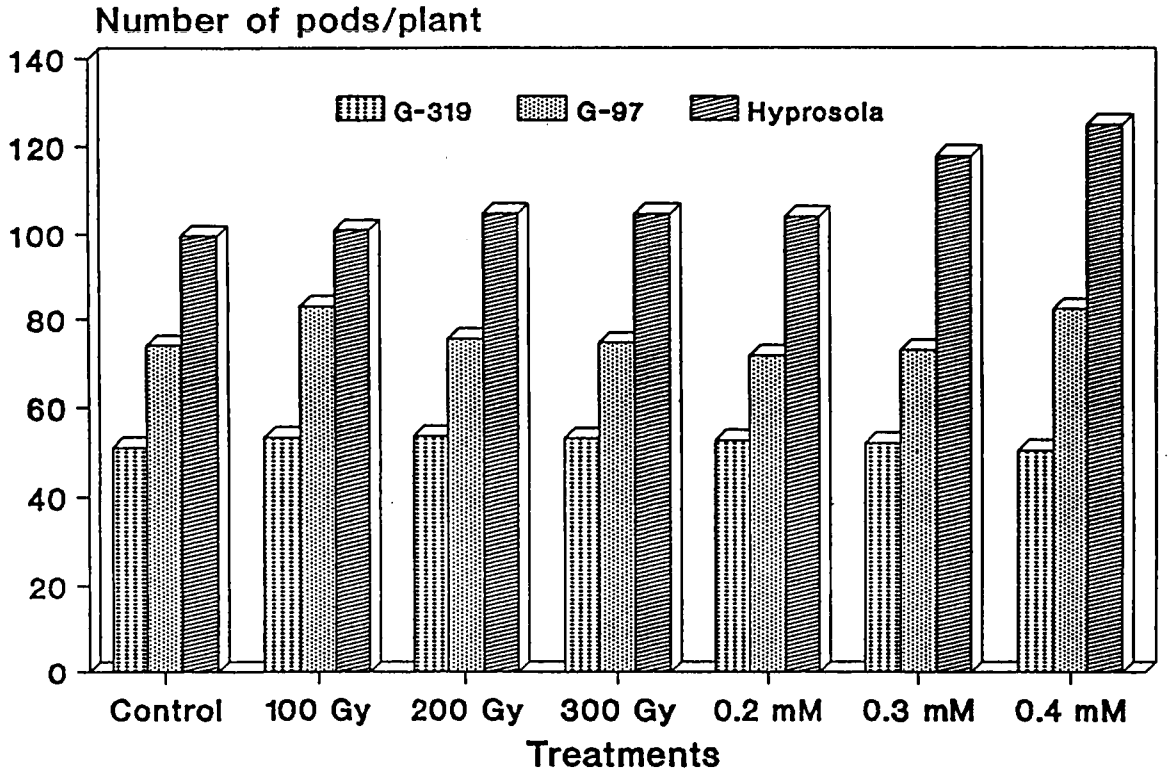


Fig. 14. Effect of different treatments of gamma rays and sodium azide on number of pods/plant in M_3 generation

Table 4.29. Analysis of variance, component of variation, heritability and genetic advance for number of pods/ plant in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	1.07*	5.87	0.23	3.88	0.37	0.02
100 Gy	668.07**	8.49	14.52	15.98	82.57	3.47
200 Gy	331.38**	18.08	10.51	12.58	69.80	9.51
300 Gy	395.49**	5.82	11.60	13.73	71.47	20.21
0.2 mM	214.96**	3.27	8.69	9.61	81.69	16.18
0.3 mM	230.12**	21.34	8.91	11.46	60.53	14.29
0.4 mM	73.38**	14.04	5.04	7.59	44.10	6.90
G-97						
Control	1.35	4.17	0.00	3.19	14.03	0.29
100 Gy	535.69**	7.68	14.88	15.31	94.73	29.55
200 Gy	362.61**	3.17	7.79	9.39	68.79	13.31
300 Gy	461.28**	7.46	15.02	15.42	94.90	30.16
0.2 mM	6.43*	7.38	0.72	2.78	6.81	1.68
0.3 mM	362.84**	3.48	8.19	8.82	86.27	15.68
0.4 mM	607.37**	2.99	16.29	16.95	91.62	31.99
Hyprosola						
Control	1.47*	0.93	0.52	1.74	9.00	0.32
100 Gy	138.42**	5.89	3.58	4.43	65.58	5.98
200 Gy	341.10**	26.78	5.46	6.42	72.49	9.58
300 Gy	7018.32**	22.26	13.44	14.55	85.33	25.58
0.2 mM	. 84.05*	9.45	2.56	4.24	36.64	3.33
0.3 mM	814.43**	4.80	12.81	13.53	89.56	24.97
0.4 mM	931.20**	5.31	24.35	24.95	95.27	48.97

**, * Significant at 1% and 5% level respectively

treated population while it was from 0.00 to 0.52 in the controls. Similar trend was also found in phenotypic coefficients of variation. Phenotypic coefficients of variation was higher than the genotypic coefficients of variation. High heritability ranged from 6.81 to 95.27 in the treated population whereas from 0.37 to 9.00 in the controls. Genetic advance was also found to be higher (1.68-48.97) in gamma-ray and sodium azide treated populations than the controls (0.02-0.32).

(e) Number of seeds/plant

The population means, ranges of family means, population CV% and ranges of population CV% for number of seeds/plant in the M_3 generation are presented in Table 4.30.

Population mean for number of seeds/plant was from 61.1 to 198.1 in the control and from 60.0 to 218.0 in gamma-ray and sodium azide treated populations (Fig. 15).

The result showed that the range of family means in different treatments were greater (50.5-266.6) than that of the controls (59.9-197.0). The range of family means in the M_2 generation was slightly higher than the M_3 generation for this character. Population CV% of the M_3 generation was lower than the CV% of the M_2. Similarly, range of family CV% of the M_3 was lower than the M_2. The ranges of family CV% (0.07-10.80) of gamma-ray and sodium azide treated populations were much higher than the untreated controls (0.25-2.68). The result of analysis of variance (mean square values), component of variations (genotypic and phenotypic coefficients of variation) and genetic parameters (heritability and genetic advance) are presented in Table 4.31.

The present result revealed significant inter-family differences at 1% level in all doses/concentrations except in the 0.2 mM concentration of sodium azide in the strain

Table 4. 30. Mean, coefficient of variation and frequency of mutation to number of seeds plant in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	61.1	59.9-63.9	1.62	0.25-2.68
100 Gy	64.2	51.5-87.5	5.98	0.56-8.55
200 Gy	64.1	55.9-81.4	6.45	1.25-10.15
300 Gy	63.3	50.5-74.8	5.59	1.44-10.66
0.2 mM	62.6	59.0-72.1	4.44	3.08-6.63
0.3 mM	61.4	57.3-70.5	3.43	1.23-10.80
0.4 mM	60.0	56.4-64.6	2.80	2.59-5.93
G-97				
Control	108.3	104.3-112.0	1.21	1.17-2.60
100 Gy	126.3	104.0-154.7	1.94	0.46-4.88
200 Gy	114.7	98.1-144.7	4.09	1.06-5.36
300 Gy	120.3	74.6-147.1	4.69	0.07-6.54
0.2 mM	104.7	103.0-106.5	2.91	1.68-6.13
0.3 mM	108.5	90.6-119.5	3.26	1.68-5.26
0.4 mM	123.6	99.4-146.7	5.45	2.25-6.51
Hyprosola				
Control	198.1	175.0-197.0	1.45	0.54-2.53
100 Gy	188.6	180.0-192.8	6.38	1.97-8.46
200 Gy	196.2	187.6-208.0	3.12	0.53-4.07
300 Gy	199.3	150.2-244.4	5.82	1.52-6.63
0.2 mM	193.3	192.2-194.4	3.00	2.98-3.42
0.3 mM	218.6	188.1-266.6	4.20	2.60-5.55
0.4 mM	197.7	186.5-207.5	4.17	5.26-7.80

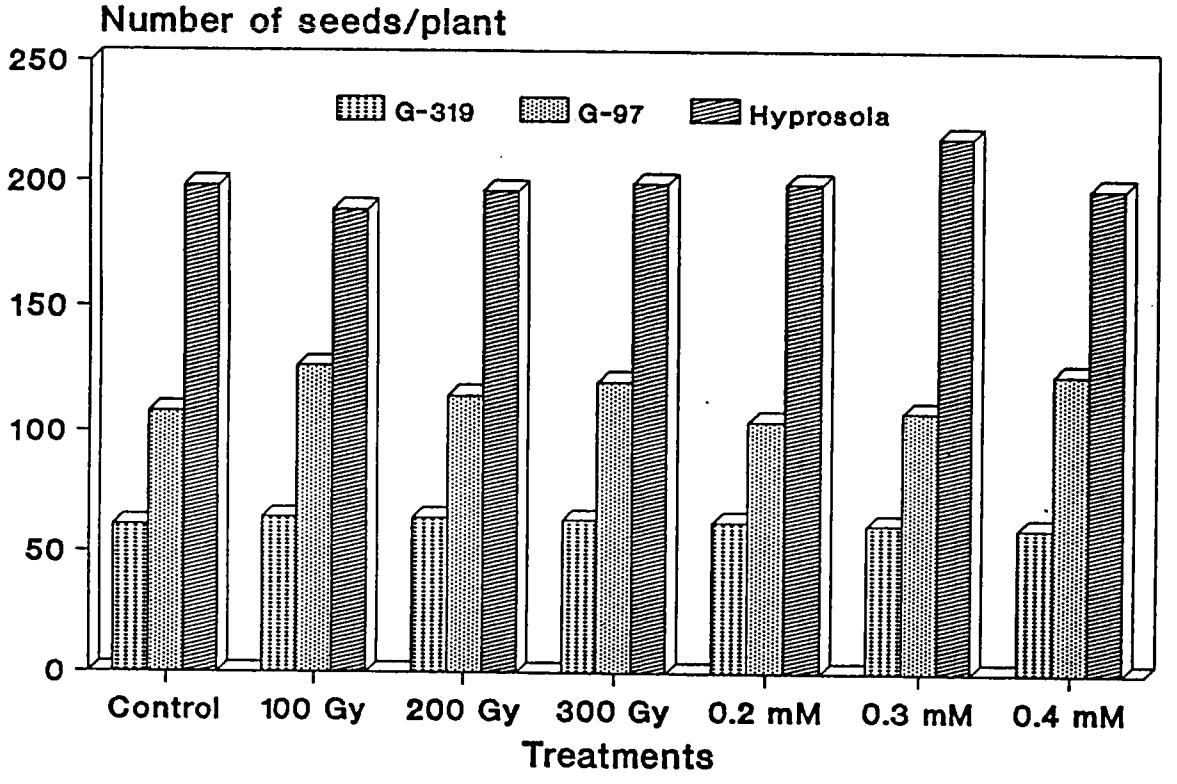


Fig. 15. Effect of different treatments of gamma rays and sodium azide on number of seeds/plant in M_3 generation

Table 4.31. Analysis of variance, component of variation, heritability and genetic advance for number of seeds/plant in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	1.62*	6.44	1.23	4.78	6.61	0.65
100 Gy	931.64**	4.24	14.53	15.71	85.53	10.76
200 Gy	545.96**	9.33	11.33	13.04	75.52	13.01
300 Gy	540.59**	8.85	11.47	12.76	80.83	21.24
0.2 mM	399.50**	6.17	9.99	10.92	83.50	18.80
0.3 mM	272.07**	24.46	8.24	10.45	62.17	13.39
0.4 mM	81.18**	10.06	4.37	7.26	36.27	5.42
G-97						
Control	1.37*	3.57	0.04	0.23	3.56	0.09
100 Gy	957.93**	62.74	17.04	19.24	78.40	31.07
200 Gy	439.78**	14.31	10.37	11.14	86.56	19.83
300 Gy	953.71**	622.12	13.67	10.17	49.20	17.00
0.2 mM	30.63**	46.52	1.14	4.06	7.61	0.63
0.3 mM	61.38NS	14.52	10.29	10.79	90.86	20.21
0.4 mM	641.18**	70.88	15.29	16.60	84.92	29.04
Hyprosola						
Control	1.33	4.88	0.42	1.51	7.79	0.24
100 Gy	701.16**	100.62	6.91	16.11	46.80	9.74
200 Gy	873.42**	31.08	4.57	6.16	55.26	7.01
300 Gy	468.62**	352.48	13.35	16.57	64.87	22.15
0.2 mM	24.20NS	43.24	0.74	1.23	36.14	0.92
0.3 mM	7991.03**	41.02	19.40	19.85	95.52	39.06
0.4 mM	674.45**	247.13	6.40	7.64	70.22	11.06

** , * Significant at 1% and 5% level respectively

G-97 and Hyprosola. The treated population showed high genotypic and phenotypic coefficients of variation over the controls. The genotypic coefficients of variation ranged from 0.74 to 19.40 in the treated population and the lowest coefficient values ranged from 0.04 to 1.23 in the untreated controls. Similar result was found in phenotypic coefficients of variation. Heritability estimated for the treated population were also higher (7.61-95.52) than that of the untreated controls (3.56-7.79) . Similar result was also found in case of genetic advance which ranging from 0.92 to 39.06 in the treated populations and was higher than the untreated controls (0.09-0.65).

(f) 100-seed weight

The population means, ranges of family means, population CV% and ranges of population CV% of their corresponding families for 100-seed weight in M_3 generation are presented in Table 4.32. The population means of different treatments of gamma-ray and sodium azide treated population along with their parents ranging from 7.3 to 26.9 are shown in Fig. 16.

The family means ranged from 6.4 to 29.8 in the treated populations and from 7.2 to 26.3 in the untreated controls. The ranges of family means in the M_3 generation were lower than the ranges of family means in the M_2. The population CV% ranged from 0.87 to 3.90 in the treated populations and 0.30 to 2.18 in the untreated controls. The range of family CV% was from 0.00 to 6.66 in the treated families and 0.00 to 3.81 in control families. The range of population CV% was much lower in the M_3 generation than in the M_2.

The result of analysis of variance (mean square values), components of variation (genotypic and phenotypic coefficients of variation) and genetic parameters (heritability and genetic advance) for 100-seed weight in the M_3 generation are shown in Table 4.33.

Table 4. 32. Mean, coefficient of variation and frequency of mutation to 100-seed weight in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	26.0	25.9-26.3	0.30	0.00-1.50
100 Gy	25.9	24.6-27.2	2.88	0.46-3.49
200 Gy	25.6	23.2-28.4	3.10	0.12-5.72
300 Gy	25.5	20.2-29.8	2.36	0.21-3.32
0.2 mM	26.9	25.9-29.0	0.87	0.46-1.77
0.3 mM	26.6	25.1-28.1	1.43	0.59-2.71
0.4 mM	25.1	22.6-27.0	2.05	0.00-6.66
G-97				
Control	17.1	16.9-17.2	0.94	0.00-1.22
100 Gy	17.5	15.1-19.9	1.70	0.86-2.32
200 Gy	17.9	15.3-20.3	2.09	0.53-3.20
300 Gy	18.1	15.7-21.7	3.37	0.24-5.68
0.2 mM	17.5	17.2-18.1	2.69	0.54-3.22
0.3 mM	18.1	16.0-19.9	1.46	0.24-2.66
0.4 mM	18.2	17.1-20.0	1.48	0.26-2.31
Hyprosola				
Control	7.4	7.2-7.5	2.18	0.00-3.81
100 Gy	7.6	6.4-8.1	2.28	0.64-6.30
200 Gy	7.5	6.5-8.4	3.56	1.47-4.07
300 Gy	7.4	6.7-8.0	3.81	1.18-5.07
0.2 mM	7.3	7.1-7.4	1.38	1.64-3.81
0.3 mM	7.7	7.5-8.0	3.90	0.00-4.42
0.4 mM	7.9	7.6-8.5	1.86	1.92-2.92

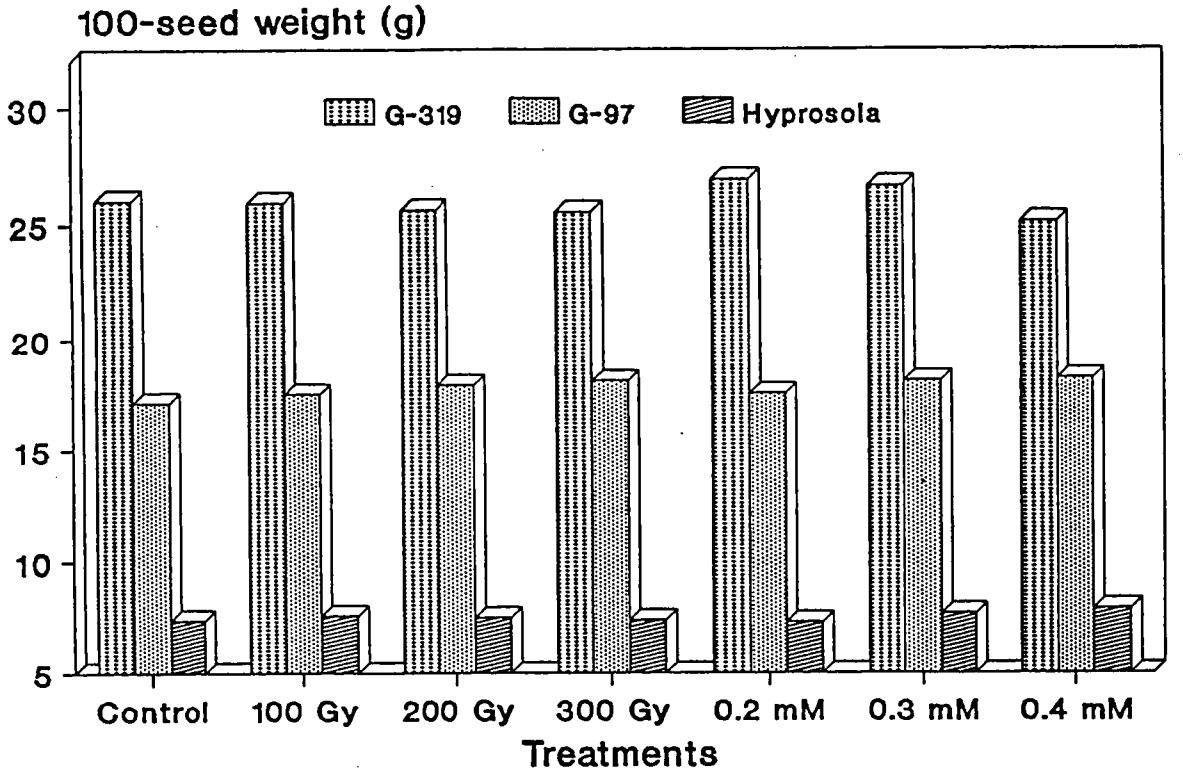


Fig. 16. Effect of different treatments of gamma rays and sodium azide on 100-seed weight in M_3 generation

Table 4.33. Analysis of variance, component of variation, heritability and genetic advance for 100-seed weight in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	0.01	0.01	0.07	0.30	6.25	0.03
100 Gy	4.81**	0.50	2.01	0.02	29.88	3.40
200 Gy	24.61**	0.69	6.03	6.79	78.87	11.03
300 Gy	46.05**	0.35	8.36	8.70	92.49	16.57
0.2 mM	20.67**	0.01	5.32	5.40	97.63	10.84
0.3 mM	13.66**	0.26	4.36	4.59	90.60	8.55
0.4 mM	26.47**	0.57	6.39	7.04	82.74	11.98
G-97						
Control	0.05	0.01	0.29	0.98	8.71	0.17
100 Gy	23.48**	0.08	8.67	8.85	96.28	17.53
200 Gy	21.41**	6.08	8.09	8.36	93.80	16.15
300 Gy	36.44**	0.34	10.46	10.99	90.45	20.49
0.2 mM	2.18*	0.01	0.82	0.88	87.50	10.25
0.3 mM	14.90**	2.12	6.29	9.76	41.53	8.35
0.4 mM	26.72**	4.33	8.17	14.09	33.63	9.76
Hyprosola						
Control	0.03	0.04	0.00	0.00	0.00	0.00
100 Gy	3.05**	0.27	3.91	5.04	60.00	6.24
200 Gy	6.81**	0.04	10.88	11.44	90.54	21.34
300 Gy	1.59**	0.34	4.84	8.28	34.21	5.84
0.2 mM	5.57**	0.02	3.23	3.50	84.84	6.13
0.3 mM	7.90**	0.06	3.36	5.31	47.05	5.14
0.4 mM	6.27**	0.14	9.70	11.34	73.17	17.10

** , * Significant at 1% and 5% level respectively

The means square for families under different treatments estimated was found significant at 1% level except the families of 0.2 mM treatment of sodium azide in the strain G-97 (at 5% level) and the three controls.

In respect of component of variations both genotypic and phenotypic coefficients of variation in the treated population were greater than the untreated controls. The genotypic coefficients of variation ranged from 2.01 to 10.88 in the treated families while it was found to be the lowest in the controls from 0.00 to 0.29. Phenotypic coefficients of variation was slightly higher than the genotypic coefficients of variation. Regarding the genetic parameters, heritability was found range from 29.88 to 97.63% in the treated populations and from 0.00 to 8.71 in untreated controls. Similarly, genetic advance ranged from 3.40 to 21.34 and 0.00 to 0.17 in treated population and controls, respectively.

(g) Seed yield/plant

The population means, ranges of family means, population CV% and ranges of family CV% for seed yield/plant in M_3 generation are presented in Table 4.34. Population means of seed yield/plant ranged from 13.39 to 21.44 in the treated populations and from 13.96 to 18.41 in controls (Fig. 17).

The family means ranged from 11.3 to 27.1 in the gamma-ray and sodium azide treated populations which was much greater than untreated controls (13.1-19.1). It was found that the range of family means in the M_3 generation decreased over the range of family means found in the previous generation (M_2). This was the effect of selection made in the M_2 generation. The population CV% decreased in the M_3 generation than the M_2 generation. The ranges of family CV% of treated population was found to be from 0.09 to 8.64 which was greater than the untreated controls from 0.66 to 3.23. The result of analysis of variance (mean square values), components of variation and genetic parameters are presented in Table 4.35.

Table 4. 34. Mean, coefficient of variation and frequency of mutation to seed yield per plant in M_3 generation

Strains/ Doses/ Conc.	Population means	Ranges of family means	Population CV%	Ranges of family CV%
G-319				
Control	15.66	15.1-16.2	2.46	1.06-2.94
100 Gy	16.58	14.0-23.0	2.77	1.90-4.59
200 Gy	16.54	14.4-21.0	2.57	1.06-2.09
300 Gy	16.38	13.2-19.7	6.09	1.41-8.22
0.2 mM	16.22	15.1-18.8	3.78	1.58-6.83
0.3 mM	15.76	14.6-17.9	4.86	3.71-7.60
0.4 mM	15.38	14.5-16.7	5.68	3.00-8.30
G-97				
Control	18.41	17.9-19.1	1.71	0.83-2.46
100 Gy	21.44	17.6-27.1	4.07	1.17-9.06
200 Gy	19.90	17.0-23.5	2.54	1.06-3.96
300 Gy	18.89	12.7-25.3	2.86	0.09-5.47
0.2 mM	17.90	17.7-18.1	4.47	4.08-6.03
0.3 mM	18.58	15.4-20.6	3.49	2.30-4.77
0.4 mM	20.92	16.9-25.8	1.07	0.08-2.03
Hyprosola				
Control	13.96	13.1-14.6	1.89	0.66-3.23
100 Gy	13.39	12.0-14.4	1.48	0.14-3.01
200 Gy	14.62	13.9-15.0	2.67	1.01-3.28
300 Gy	14.90	11.3-18.3	5.87	1.70-8.64
0.2 mM	14.30	14.3-14.3	1.81	1.61-2.66
0.3 mM	16.44	14.1-20.1	1.83	1.01-2.75
0.4 mM	17.70	14.0-21.5	2.60	1.38-3.14

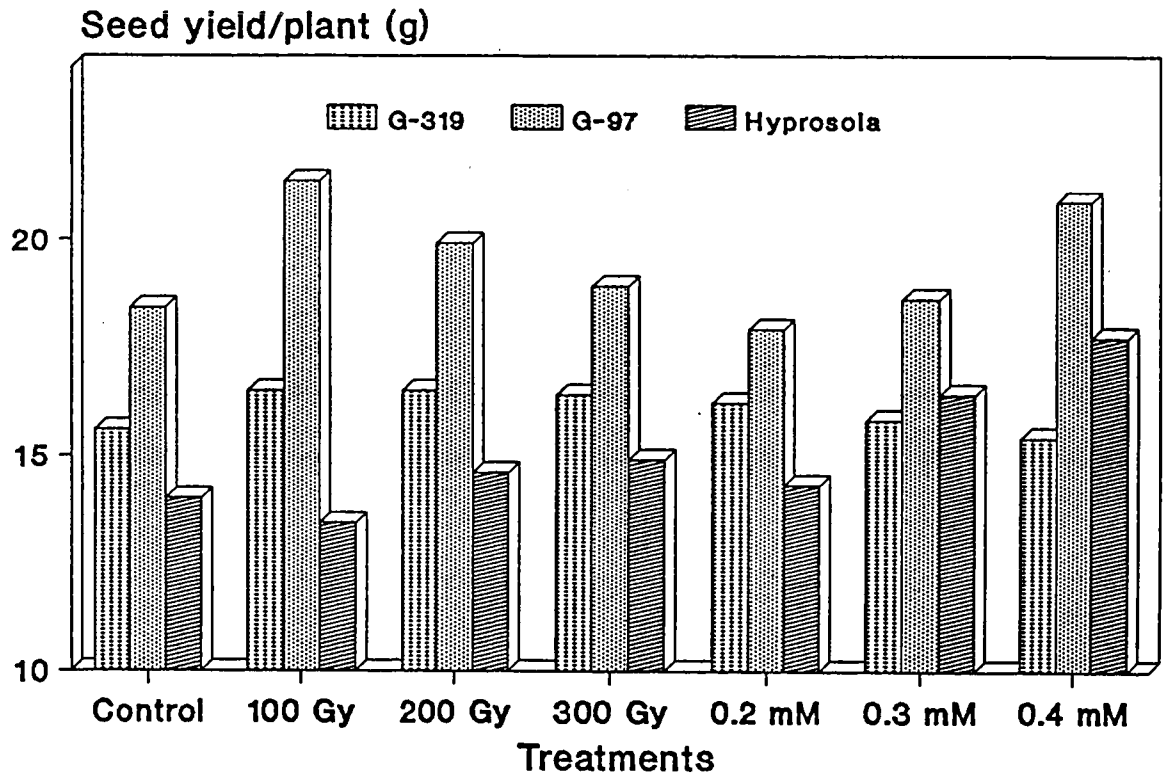


Fig. 17. Effect of different treatments of gamma rays and sodium azide on seed yield/plant in M_3 generation

Table 4.35. Analysis of variance, component of variation, heritability and genetic advance for seed yield/ plant in M_3 generation

Strains/ Doses/ Conc.	Variances		GCV (%)	PCV (%)	Heritability (%)	GA (%) of mean
	Interfamily	Intrafamily				
G-319						
Control	0.84	0.62	0.68	5.49	1.53	0.17
100 Gy	78.10**	0.26	15.74	17.57	80.04	11.13
200 Gy	38.47**	0.90	11.66	13.39	75.76	20.90
300 Gy	42.42**	0.93	12.42	13.84	80.70	22.98
0.2 mM	30.76**	0.65	10.73	11.39	88.85	20.83
0.3 mM	16.21**	0.43	7.77	10.37	56.17	11.99
0.4 mM	5.93**	0.28	4.55	7.85	33.56	5.43
G-97						
Control	0.92	0.52	1.15	3.89	8.78	0.70
100 Gy	172.73**	1.71	19.33	19.75	95.76	8.35
200 Gy	50.37**	0.24	11.30	11.58	95.24	22.72
300 Gy	141.63**	6.28	16.29	16.59	97.03	33.07
0.2 mM	0.46	1.13	0.55	4.50	2.53	0.14
0.3 mM	38.60**	0.13	10.50	11.07	89.85	20.50
0.4 mM	93.72**	4.49	14.27	17.46	66.76	24.02
Hyprosola						
Control	0.60	0.10	1.24	4.05	9.37	0.78
100 Gy	9.55	6.87	4.72	18.11	6.79	2.53
200 Gy	5.26**	0.08	4.88	5.57	77.27	8.84
300 Gy	50.19**	1.60	14.91	16.02	86.66	28.60
0.2 mM	0.30	0.03	0.82	2.85	7.90	0.47
0.3 mM	105.30**	0.04	19.72	19.81	99.15	40.46
0.4 mM	285.01**	1.05	30.11	30.46	97.73	61.32

**, Significant at 1% level

The mean square values of analysis of variance revealed significant inter-family variation in all the treated populations at 1% level except 0.2 mM sodium azide treatment of G-97 and Hyprosola and 100 Gy gamma-ray treatment of Hyprosola. The genotypic coefficients of variation was found to be lower in the controls (0.68-1.24) while it ranged from 0.55 to 30.11 in the treated populations. Similar results were also found in the phenotypic coefficients of variation. The lowest heritability (1.53-9.37) was observed in the untreated controls and higher heritability values were found in treated population (2.53-99.15). The values of the genetic advance for seed yield/plant ranged from 0.14 to 61.32 in the gamma-ray and sodium azide treated populations and in the controls the GA% ranged from 0.17 to 0.78.

(h) List of selected families in M_3 generation

A list of the selected families including their pedigree, seed yield and yield contributing parameters are presented in Appendix 4. Selections were made on the basis of their better performance in respect of seed yield, days to maturity, 100 seed weight, number of pods/plant and number of seeds/plant from the M_3 generation. Some of the selected mutants grown in pots have been shown in Plates 3, 4, 5, 6, 7 and 8. Seeds from the selected mutants in M_3 generation showing their variations in shape, size and colour are presented in Plate 9.

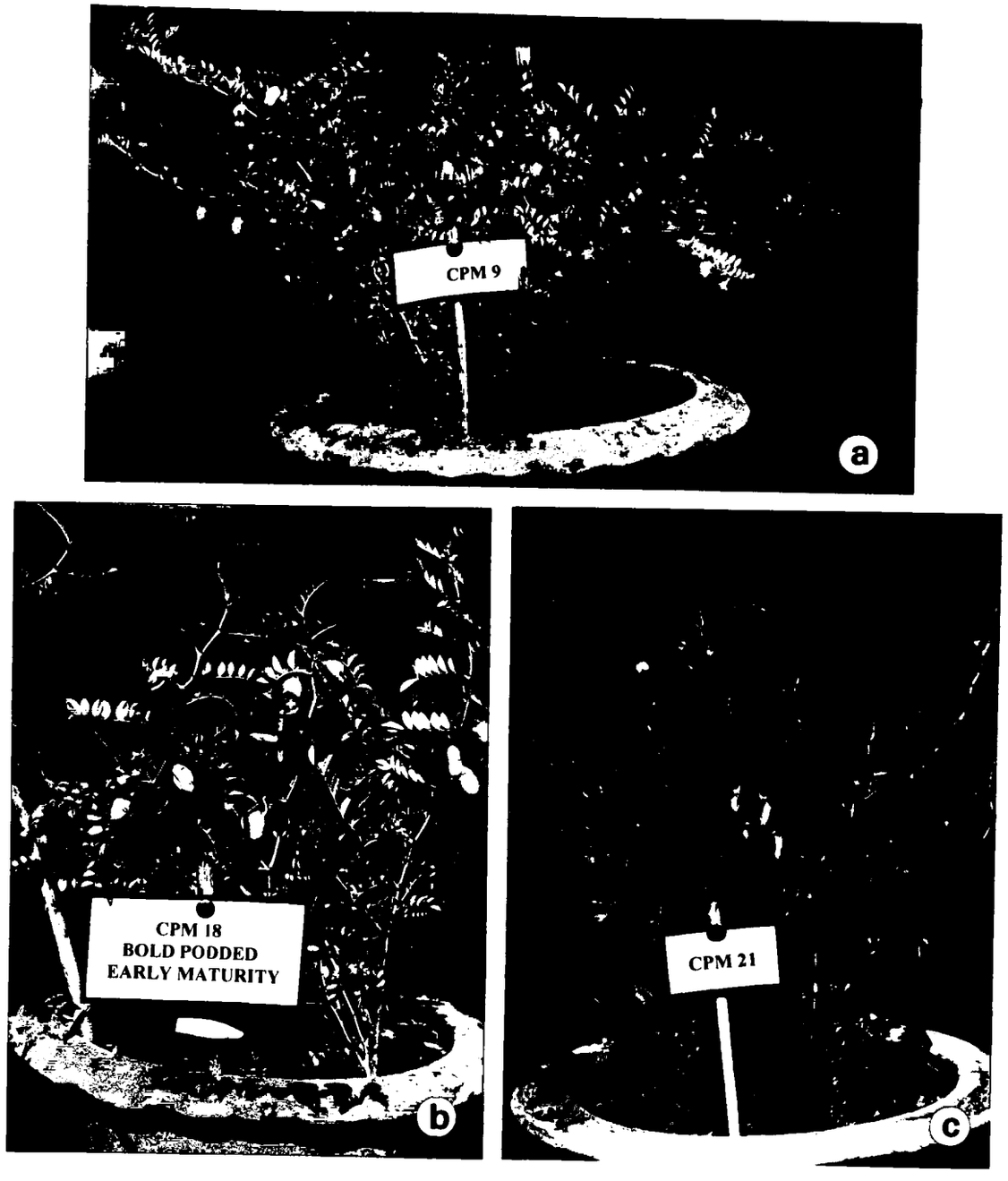
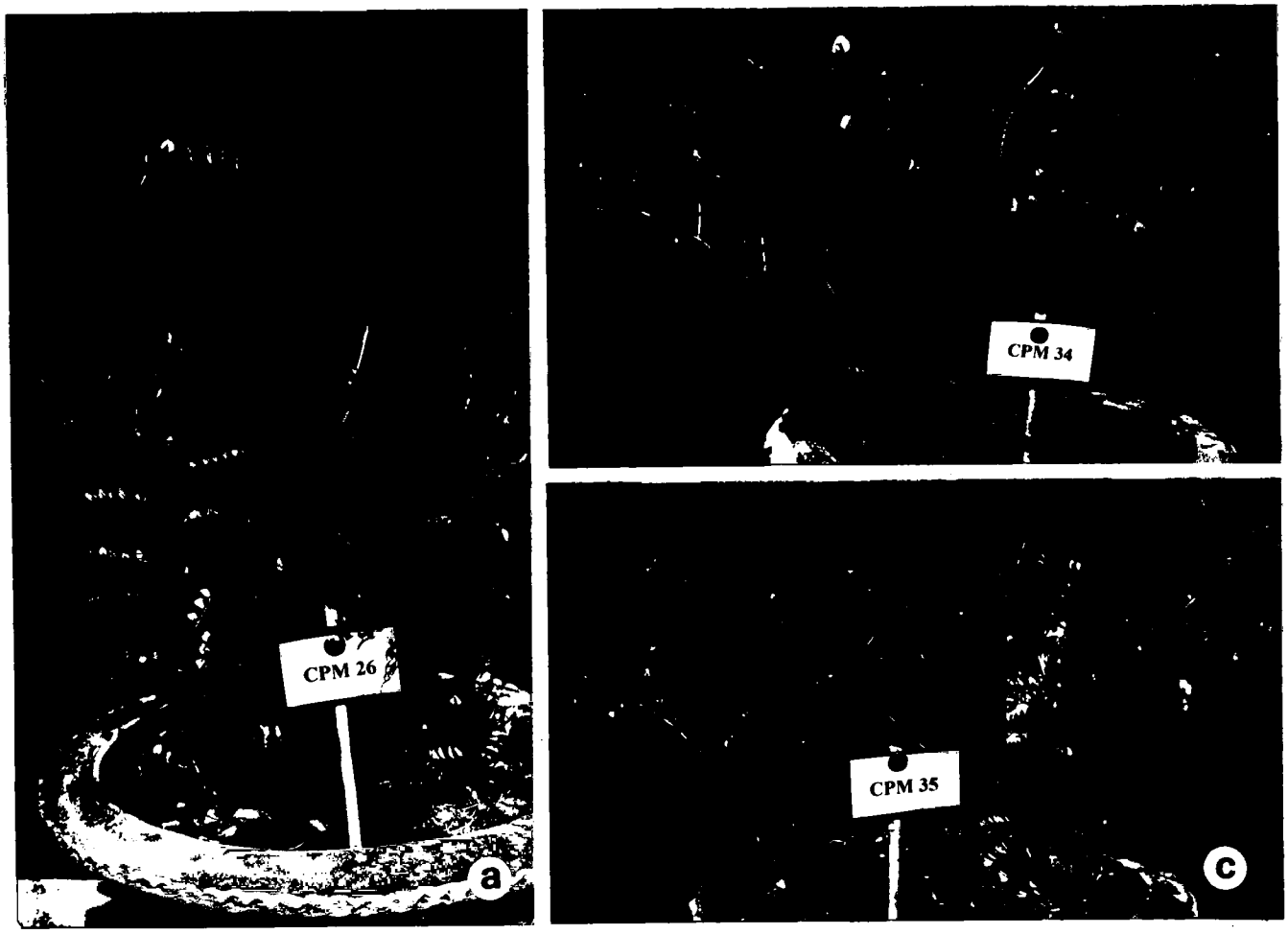


Plate 3. Selected mutants grown in pots: a (CPM-9), b (CPM-18) and c (CPM-21)



Pate 4. Selected mutants grown in pots: a (CPM-26), b (CPM-34) and c (CPM-35)

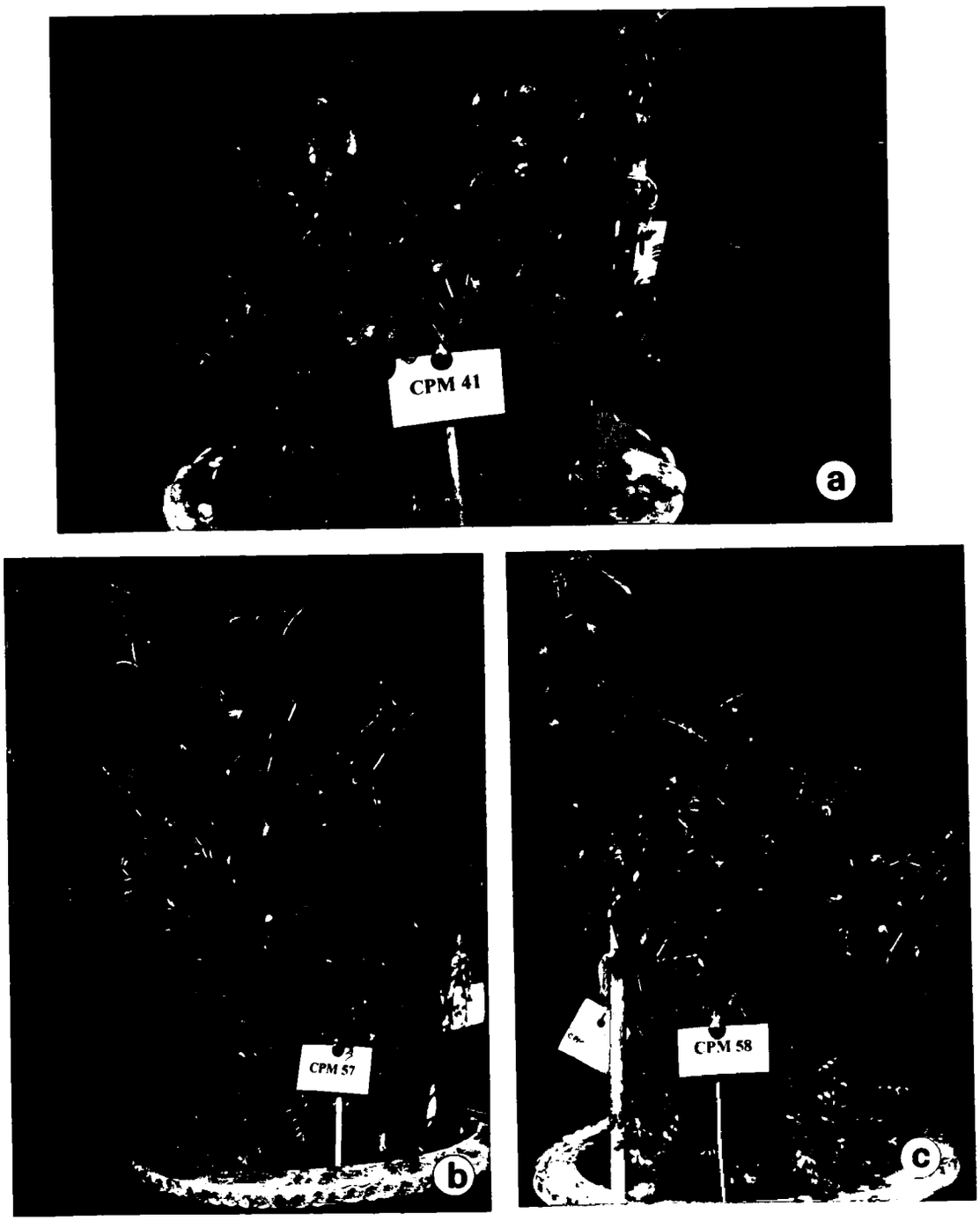
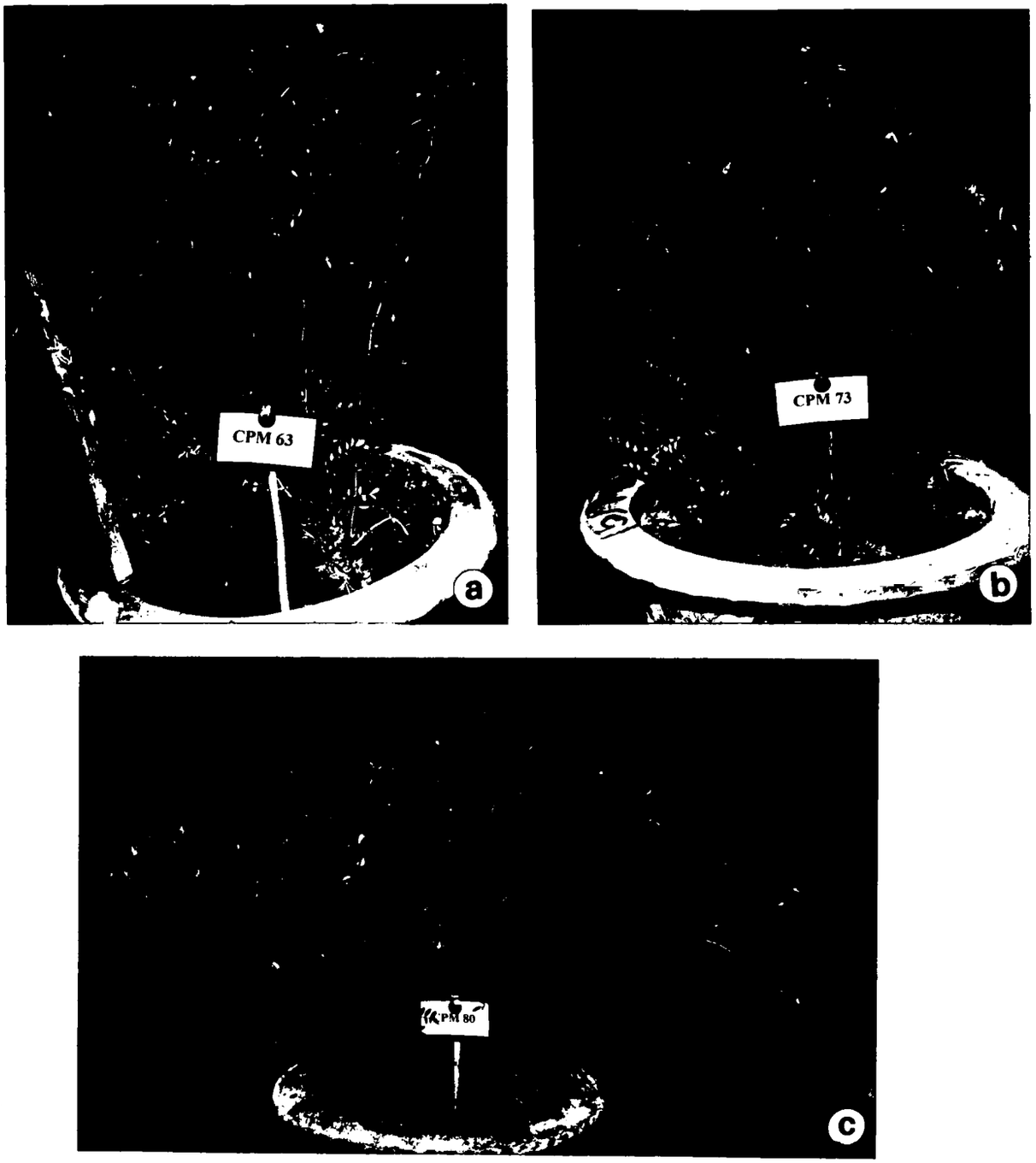


Plate 5. Selected mutants grown in pots: a (CPM- 41), b (CPM-57)and c (CPM-58)



Pate 6. Selected mutants grown in pots: a (CPM-63), b (CPM-73) and c (CPM-80)

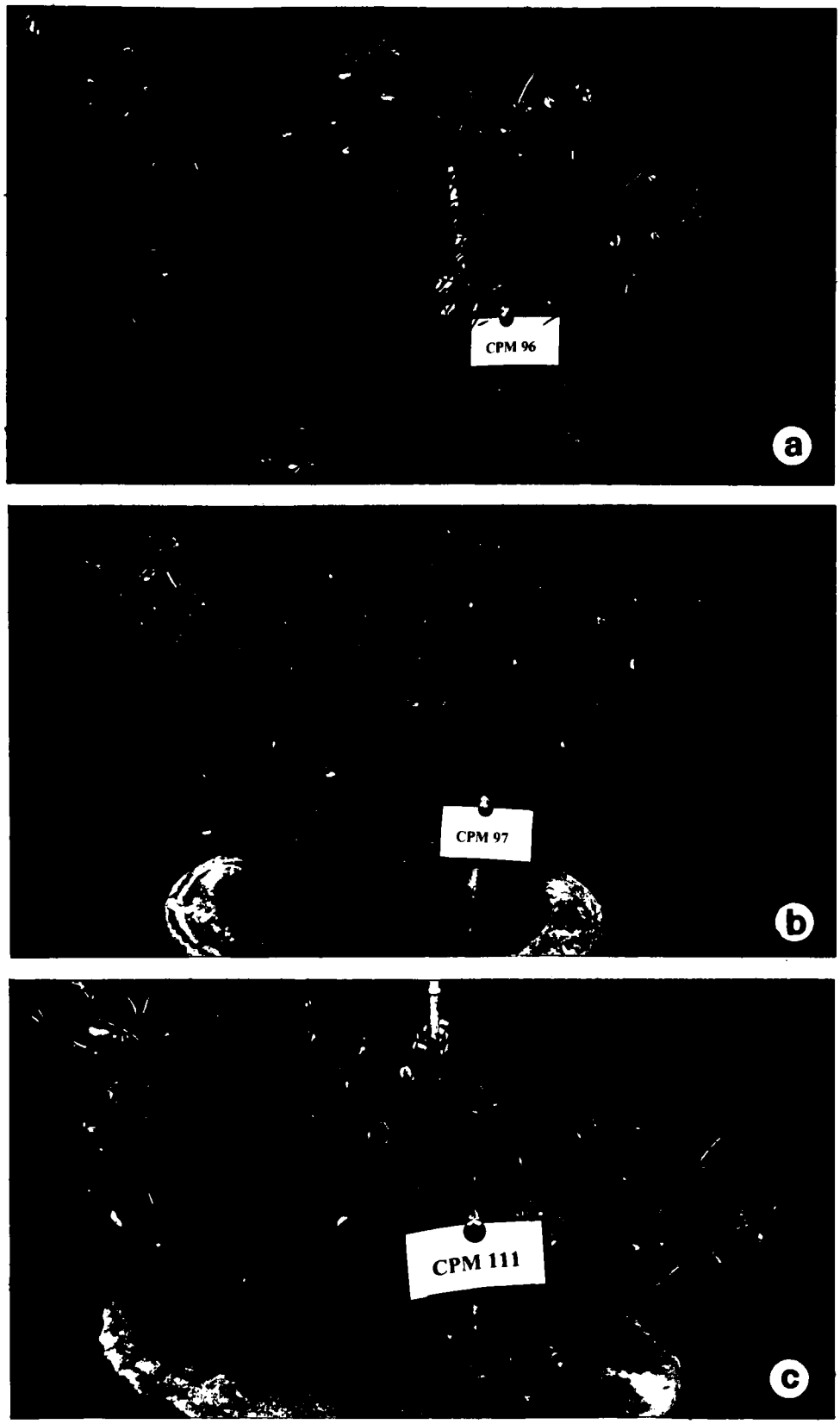


Plate 7. Selected mutants grown in pots: a (CPM-96), b (CPM-97) and c (CPM-111)

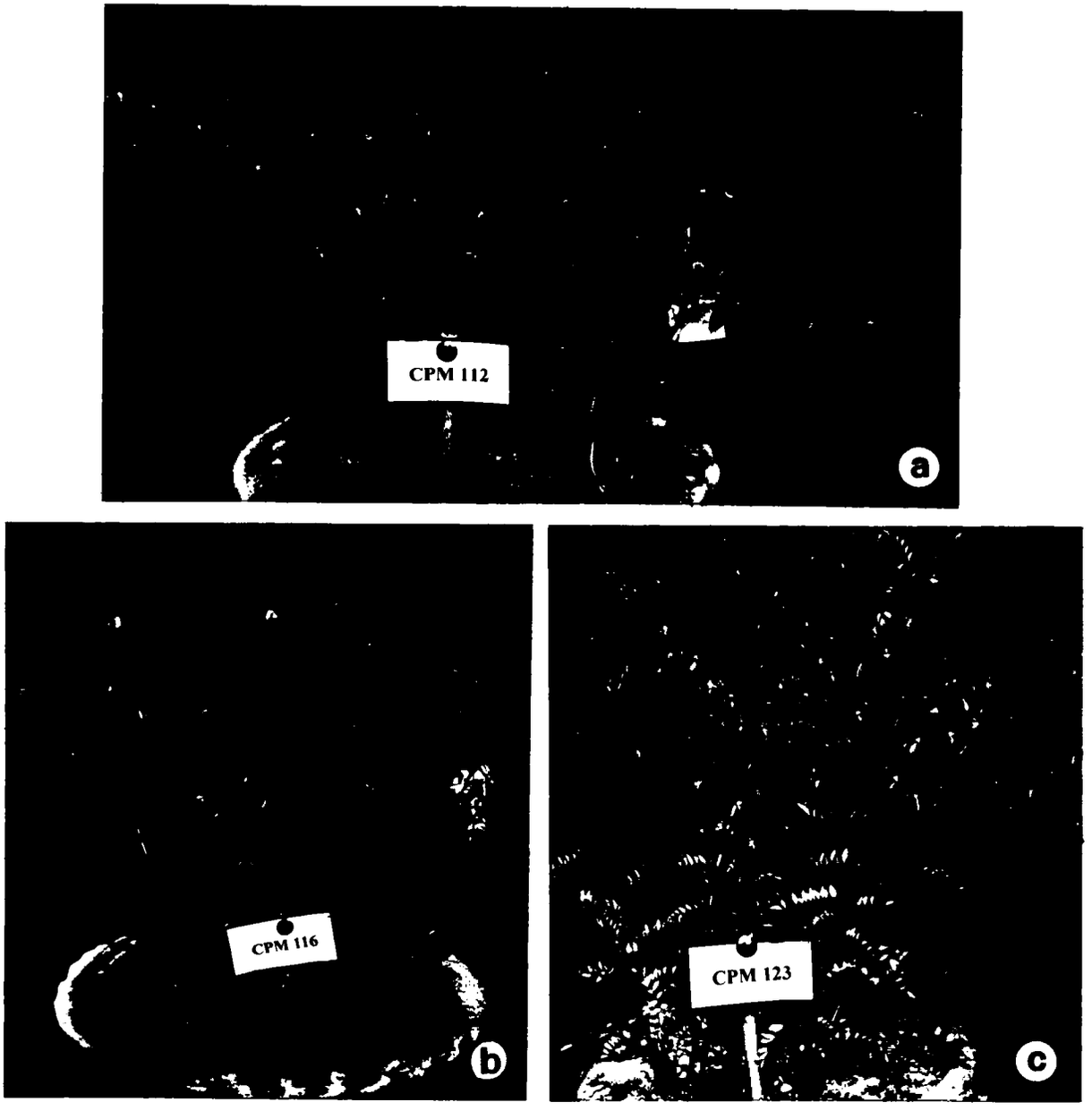


Plate 8. Selected mutants grown in pots: a (CPM-112), b (CPM-116) and c (CPM-123)

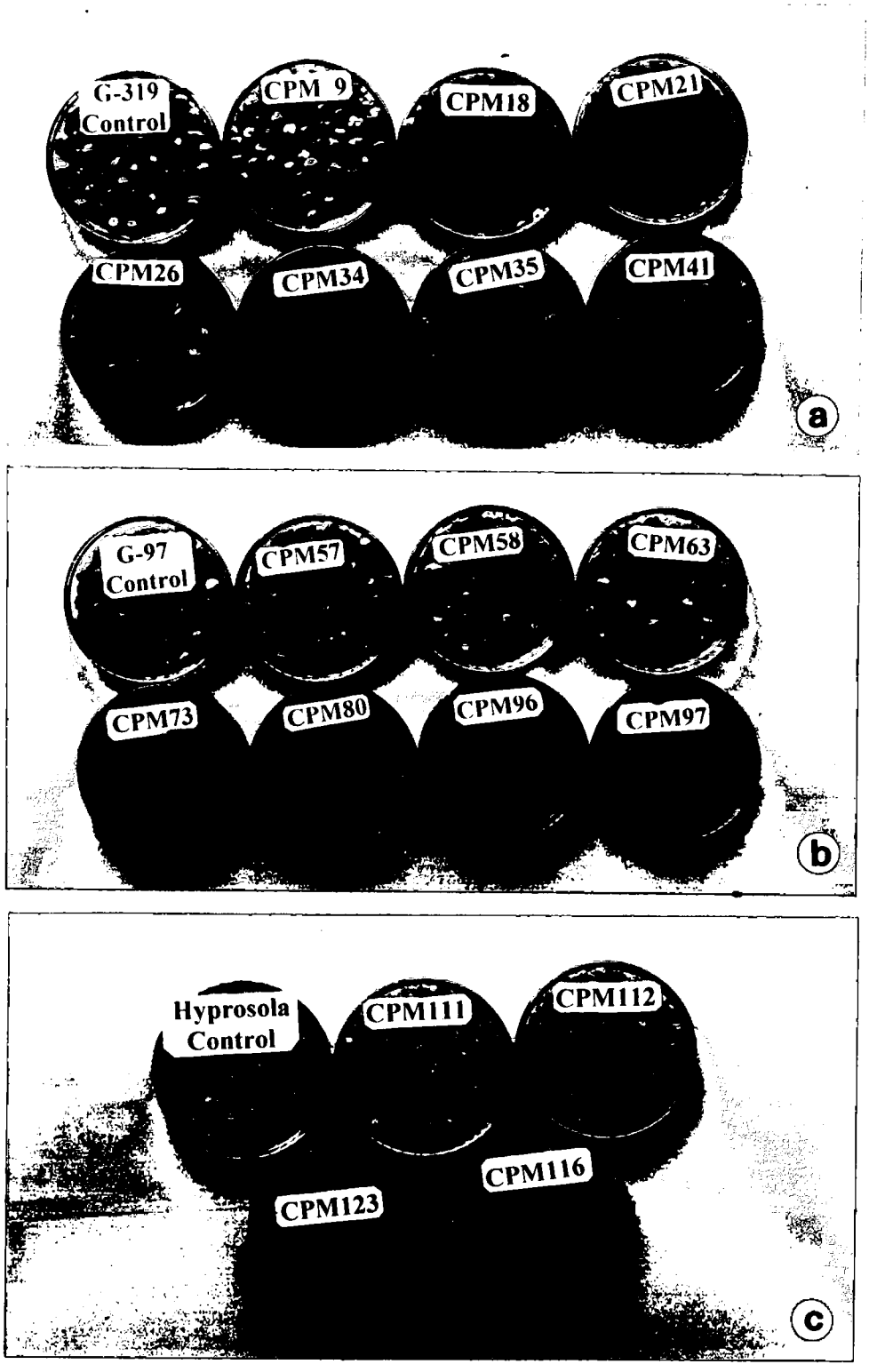


Plate 9. Seeds of the selected mutants from M_3 generation along with their respective parents: a (G-319), b (G-97) and c (Hyprosola)

D. Observation in M_4 generation

Eighteen promising mutants selected from M_3 generation along with their parents G-319, G-97 and Hyprosola were grown at two locations viz., BINA sub-station at Ishurdi and Magura during 1995-96. The experiment was laid out in a randomized complete block design with three replications. Ten plants were taken at random from each plot for recording data on different parameters. All the mean data were subjected to statistical analysis. Comparison among the mean values of the mutants and control were made by using the CD at 5% level.

Analysis of variance of seven characters, viz., days to flowering, days to maturity, plant height, number of pods/plant, number of seeds/plant, 100-seed weight (g) and seed yield/plot (g) were done separately to test the differences within different sources of variation. The results (mean square values) are summarized in Table 4.36.

It is evident from the Table 4.36 that the mean square values of the item, genotypes (G) were highly significant against the experimental errors for all the characters studied in M_4 generation. The significant results suggested the existence of real differences among the mutants and control. Similar results were also found in the item, locations (L). Regarding the interaction between genotypes and locations, the mean square values of the item G × L were highly significant except number of seeds/plant. It revealed the presence of considerable interactions between the mean performances of different characters and locations. The results indicated that the mutants responded differently in different locations. The non-significant mean square values for number of seeds/plant indicated the presence of small variation in the mean performances due to different locations.

Mean performances of the selected mutants and controls for all the seven characters in two locations are presented in Table 4.37, 4.38, 4.39 and 4.40.

Table 4.36. Analysis of variance (mean square) for different characters of selected promising M_4 mutants along with the controls

Source of variation	Degrees of freedom	Days to flowering	Days to maturity	Plant height	No. of pods /plant
Genotypes (G)	20	218.04**	180.23**	181.76**	3241.57**
Locations (L)	1	15.36**	26.69**	32.50**	224.00**
G x L	20	19.29**	7.14**	2.34**	7.20**
Error	82	2.40	2.34	0.95	3.23

Source of variation	Degrees of freedom	No. of seeds/ plant	100-seed weight	Seed yield kg/ha
Genotypes (G)	20	17558.35**	366.47**	822995.72**
Locations (L)	1	311.14**	5.44**	134979.17**
G x L	20	4.42	0.14**	5711.87**
Error	82	6.57	0.03	1848.87

**, significant at 1% level

(a) Days to flowering

There was considerable variation in days to flowering among the mutants and controls (Table 4.37). Out of 18 mutants only six showed early flowering than the earliest control Hyprosola. The mutant CPM123 showed the earliest flowering in 53 days than all the mutants and controls (60-73 days). Most of the mutants flowered earlier than their respective parent except few. As a result, the generation mean of the mutants were found to be lower in both the locations. From the mean results, it can be said that the mutagens gamma-ray and sodium azide, induced genetic changes in this character and selection of early flowering mutants in M_2 and M_3 generations made it possible to develop true breeding mutants which flowered earlier than the controls in M_4 generation.

(b) Days to maturity

It was found in Table 4.37 that there was considerable variation in days to maturity. The maturity period ranged from 116 to 134 days for mutants while from 125 to 136 days in case of controls. The mutant CPM112 showed the earliest maturity (116 days) than all other mutants and controls. Selections were made on the basis of earliness in previous generation. The result also showed that the early maturing mutants selected in the earlier generations bred true in the M_4 generation. The mutagens gamma rays and sodium azide showed potentiality to reduce the maturity period in chickpea.

(c) Plant height

Variation in plant hieight was observed in the means of M_4 mutants (Table 4.38) although the range of variation in this generation was lower than M_2 and M_3 generations. The results showed that out of 18 mutants only two possessed shorter plant height than the semi dwarf variety Hyprosola (50 cm). Only one mutant showed taller

Table 4.37. Mean number of days to flowering and maturity, and the controls of chickpea grown at two locations in the M_4 generation

Genotypes	Doses/ Cone.	Selection no.	Days to flowering			Days to maturity		
			Ishurdi	Magura	Mean	Ishurdi	Magura	Mean
G-319	100 Gy	CPM 9	70	70	70	124	122	123
„	200 Gy	CPM18	61	60	61	119	117	118
„	200 Gy	CPM21	68	65	67	133	129	131
„	300 Gy	CPM26	61	58	60	123	119	121
„	300 Gy	CPM34	57	56	57	126	127	127
„	300 Gy	CPM35	58	57	58	125	125	125
„	0.2 mM	CPM41	61	59	60	127	125	126
G-97	100 Gy	CPM57	67	69	68	119	117	118
„	100 Gy	CPM58	61	61	61	130	137	134
„	200 Gy	CPM63	61	63	62	122	118	120
„	200 Gy	CPM73	68	68	68	120	119	120
„	300 Gy	CPM80	59	57	58	128	127	128
„	0.3 mM	CPM96	61	59	60	129	127	128
„	0.4 mM	CPM97	81	70	76	124	124	124
Hypro.	300 Gy	CPM111	59	59	59	123	122	123
„	300 Gy	CPM112	60	60	60	116	116	116
„	300 Gy	CPM116	67	68	68	119	120	120
„	0.4 mM	CPM123	55	52	54	125	124	125
G-319	Control	-----	69	69	69	134	133	134
G-97	Control	-----	74	73	74	136	136	136
Hyprosola	Control	-----	60	59	60	126	124	125
CD at 5% level					7.76			7.67

plant height (45-49 cm) than the tallest control G-97. As a result, generation mean of mutants was comparatively lower than the control.

(d) Number of pods/plant

The mean result presented in Table 4.38 showed considerable variation in number of pods/plant for the mutants (ranged 53-138) and controls (55-101). Out of 18 mutants only 3 have produced more number of pods than the highest pod bearing control, Hyprosola. The mutants CPM123 produced the highest number of pods/plant (138) among all the mutants and controls. However, the most of the mutants possessed greater number of pods/plant compared to their respective controls. True breeding behaviour of the mutants was also observed in this generation.

(e) Number of seeds/plant

The mean values of number of seeds/plant at two locations showed considerable variation among the mutants (61-235) and their controls (64-191) presented (Table 4.39). Out of 18 mutants studied only three showed greater number of seeds/plant than the highest seed bearing control Hyprosola. The mutant CPM116 have produced the highest number of seeds/plant (235) followed by CPM123 (206) and CPM111 (194). Most of the mutants showed higher number of seeds/plant than their respective controls. True breeding behaviour of the mutants were also observed for this character.

(f) 100-seed weight

Mean values of the mutants grown at the two locations showed significant variation in 100-seed weight (Table 4.39). Out of 18 mutants only four were found to have higher 100-seed weight than the highest 100-seed weight bearing control (G-319). The mutant CPM26 showed the highest 100-seed weight (31.2 g) followed by CPM41 (30.3g), CPM21 (27.1 g) and CPM26 (27.1 g). Beside these, some mutants

Table 4.38. Mean plant height and number of pods/plant of the promising mutants and the controls of chickpea grown at two locations in the M_4 generation

Genotypes	Doses/ Conc.	Selection no.	Plant height (cm)			Number of pods/plant		
			Ishurdi	Magura	Mean	Ishurdi	Magura	Mean
G-319	100 Gy	CPM 9	56	55	56	72	73	73
„	200 Gy	CPM18	56	55	56	63	65	64
„	200 Gy	CPM21	61	61	61	52	54	53
„	300 Gy	CPM26	54	51	53	60	63	62
„	300 Gy	CPM34	51	50	51	55	56	56
„	300 Gy	CPM35	62	59	61	62	65	64
„	0.2 mM	CPM41	62	62	62	59	62	61
G-97	100 Gy	CPM57	55	55	55	89	92	91
„	100 Gy	CPM58	66	65	66	73	76	75
„	200 Gy	CPM63	64	61	62	77	79	78
„	200 Gy	CPM73	60	61	61	76	78	77
„	300 Gy	CPM80	59	58	59	85	91	88
„	0.3 mM	CPM96	59	59	59	75	77	76
„	0.4 mM	CPM97	60	59	60	73	76	75
Hypro.	300 Gy	CPM111	49	49	49	100	103	102
„	300 Gy	CPM112	52	50	51	103	109	106
„	300 Gy	CPM116	45	45	45	120	126	123
„	0.4 mM	CPM123	53	50	52	140	136	138
G-319	Control		61	59	60	53	56	55
G-97	Control		64	63	64	74	75	75
Hyprosola	Control		50	50	50	98	104	101
CD at 5% level					7.67			11.50

Table 4.39. Mean number of seeds/plant and 100-seed weight of the promising mutants and the controls of chickpea grown at two locations in the M_4 generation

Genotypes	Doses/ Conc.	Selection No.	Number of seeds/plant			100-seed weight (g)		
			Ishurdi	Magura	Mean	Ishurdi	Magura	Mean
G-319	100 Gy	CPM 9	83	84	83	26.2	25.6	25.9
„	200 Gy	CPM18	75	77	76	30.3	30.3	30.3
„	200 Gy	CPM21	60	62	61	27.1	27.0	27.1
„	300 Gy	CPM26	68	72	70	27.4	26.8	27.1
„	300 Gy	CPM34	65	67	66	31.5	30.8	31.2
„	300 Gy	CPM35	72	74	73	24.8	24.0	24.4
„	0.2 mM	CPM41	71	73	72	26.4	26.1	26.3
G-97	100 Gy	CPM57	144	145	145	17.2	17.8	17.5
„	100 Gy	CPM58	132	136	134	20.0	19.6	19.8
„	200 Gy	CPM63	119	121	120	20.4	20.0	20.2
„	200 Gy	CPM73	114	117	116	21.5	21.0	21.3
„	300 Gy	CPM80	126	135	131	20.5	20.4	20.5
„	0.3 mM	CPM96	104	107	106	18.9	18.1	18.5
„	0.4 mM	CPM97	114	117	116	20.1	19.7	19.9
Hypro.	300 Gy	CPM111	192	196	194	8.0	7.6	7.8
„	300 Gy	CPM112	188	192	190	8.1	7.7	7.9
„	300 Gy	CPM116	233	236	235	8.4	7.7	8.1
„	0.4 mM	CPM123	204	207	206	8.0	7.5	7.8
G-319	Control		63	65	64	27.2	26.6	26.9
G-97	Control		105	106	106	18.0	17.4	17.7
Hyprosola	Control		188	195	191	8.0	7.8	7.9
CD at 5% level					22.25			1.63

showed higher 100-seed weight than their respective parents. Selections were made on the basis of 100-seed weight. True breeding behaviour of the mutants were also observed in this generation for this character.

(g) Seed yield (kg/ha)

The mean seed yields of locations revealed significant differences among the mutants and controls (Table 4.40). Seed yield range among the mutants was from 1509 to 2647 kg/ha and among the controls it was from 1450 to 1736 kg/ha. Seven mutants out of 18 produced higher seed yield than the highest yielding control, G-97. The highest seed yield was produced by the mutants CPM26 (2647 kg) followed by CPM63 (2553 kg), CPM41 (2494 kg), CPM73 (225.7 kg), CPM34 (2077 kg), CPM9 (2001 kg) and CPM112 (1824 kg). Most of the mutants selected in the previous generation for higher seed yield potential also bred true by producing higher seed yield in this generation. Selections were made in this generation on the basis of seed yield, days to maturity and 100-seed weight. Mutants with excellent performances in respect of the above characters in M_4 generation are listed in Appendix 7.

Table 4.40. Mean seed yield of the promising mutants and the controls of chickpea grown at two locations in the M_4 generation

Genotypes	Doses/ Conc.	Selection no.	Seed yield kg/ha		
			Ishurdi	Magura	Mean
G-319	100 Gy	CPM 9	1927	2075	2001
„	200 Gy	CPM18	1860	1936	1898
„	200 Gy	CPM21	1579	1664	1622
„	300 Gy	CPM26	2604	2689	2647
„	300 Gy	CPM34	2025	2128	2077
„	300 Gy	CPM35	1615	1650	1633
„	0.2 mM	CPM41	2483	2504	2494
G-97	100 Gy	CPM57	1684	1742	1713
„	100 Gy	CPM58	1428	1589	1509
„	200 Gy	CPM63	2508	2598	2553
„	200 Gy	CPM73	2214	2299	2257
„	300 Gy	CPM80	1507	1561	1534
„	0.3 mM	CPM96	1619	1762	1691
„	0.4 mM	CPM97	1656	1652	1654
Hypro.	300 Gy	CPM111	1539	1531	1535
„	300 Gy	CPM112	1881	1767	1824
„	300 Gy	CPM116	1717	1733	1725
„	0.4 mM	CPM123	1620	1685	1653
G-319	Control	-------	1437	1463	1450
G-97	Control	-------	1709	1763	1736
Hyprosola	Control	-------	1574	1672	1623
CD at 5% level					373.3

Chapter V
DISCUSSION

V. DISCUSSION

The method of plant breeding consists of three phases including the creation of genetic variability, selection of useful genotypes and comparative tests to demonstrate the superiority of the selected genotypes. Induced mutations is one of the most useful methods to create variability when natural variations are exhausted. Induced mutation can significantly contribute to increase genetic variability. A number of scientists have been using this technique for creating variability in crop plants. About 70 years have elapsed since Muller (1927) and Stadler (1928; 1930) successfully used induced mutations to mimic the natural ones in living organisms. Variability created by induced mutations is not different from that caused by spontaneous mutations during evolution. Since 1927 a great deal of research has been carried out on the theoretical basis of mutagenesis in plant material which reaching its peak in the mid-1960s (Anonymous,1995). Those investigations led to elaboration of both the theoretical as well as methodological principles for using the various mutagens in creating and selecting desired variability. As a result of intensive research on crop plants, a total of 1790 mutant varieties from 158 plant species have been officially released (Maluszynski *et el.*, 1995).

Yield ability of crop plant is a quantitative character, showing continuous variation and is highly influenced by environmental factors. In spite of the difficulty of detecting yield mutants, a number of mutants/mutant varieties with increased yield have been released (Sigurbjornsson and Micke, 1974; Kawai, 1967; Rutzer, *et al.*, 1976; Thakare *et al.*, 1980; Miah *et al.*, 1981; Shaikh *et al.*, 1982; Rahman *et al.*, 1992). Moreover, there are many reports regarding the improvement of other characters like branching habit, plant type, earliness, adaptability, disease resistance and grain quality by using the induced mutation techniques (Garlicka, 1959; Rai, 1959; Shaikh *et al.*, 1982; Kharkwal, 1983; Ivannikov and Moraru, 1968; Shamsuzzaman and Shaikh, 1991; Langham, 1960; Li *et al.*, 1965 ; Hu, 1973; Halloran *et al.*, 1979).

Large number of physical and chemical mutagens have been used for the last few years to induce mutation for crop improvement. Out of these only two most effective mutagens, e.g. gamma-ray (physical mutagen) and sodium azide (chemical mutagen) were chosen for the present investigation. Many researchers have been successful in creating variabilities in crops and obtained high yielding, high protein and oil content, early maturity as well as large grain size mutants by using these two mutagens (Shaikh *et al.*, 1982; Miah *et al.*, 1981; Konzak *et al.*, 1965; Walbot *et al.*, 1983; Redei *et al.*, 1984; Rahman *et al.*, 1992; Oram *et al.*, 1987; Shamsuzzaman and Shaikh, 1991).

The present study was mainly confined to (a) preliminary selection of superior mutants with higher seed yield, early maturity and larger seed size from the treated population of M_1 to M_4 generations and (b) determination of comparative effectiveness of gamma-ray and sodium azide for creating genetic variability in different quantitative characters of chickpea.

A. Characteristcs in M_1 generation

The effects of mutagens on chickpea were estimated by the germination of seeds, seedling height, survivability of plants and pollen fertility.

(i) Germination of seeds

In the present investigation it was observed that the germination percentage gradually decreases from 88.3 to 64.3 (Table 4.1 and Fig. 1a) with the increase of radiation doses (100 to 300 Gy) and concentrations (0.2-0.4 mM) of sodium azide. At higher doses of gamma rays (300 Gy) some of the embryos showed signs of germination, but they failed to produced shoots. It was found that the sodium azide causes less inhibitory effect on the germination of seeds than gamma-ray. The effect of mutagens in reducing the germination of seeds was reported by Konzak *et al.*(1961).

In legumes, several scientists have also demonstrated that the doses have reduction effect on germination percentage (Santos, 1965; Blixt,1970; Kharkwal, 1978; Tickoo, 1978; Balaravi, 1982; Mahapatra, 1983; Sharma and Sharma, 1983; Sarker, 1985; Shaikh and Begum, 1991). Beside these Swamy Rao (1970), Ananthaswamy *et al.* (1971), Garg *et al.* (1972) and Wonkyi-Appiah (1991) also reported that higher radiation doses caused inhibitory effect on the germination of seeds.

It is clear from the results of the present investigation and also from the earlier reports that the mutagens like gamma-ray and sodium azide could reduced the germination of chickpea seeds.

(ii) Seedling height

In the present study, seedling height (at 30 days old) was influenced by the different treatments of gamma-ray and sodium azide. The seedling height decreased gradually from 23.4 to 12.6 cm with the increase of doses of gamma rays where as sodium azide reduced from 20.7-13.7 cm (Table 4.1 and Fig. lb). Santos (1965) reported the decrease in plant height following treatment with gamma-ray in *Phaseollus aureus.* A number of workers reported earlier that seedling height decreased with the increase of doses/concentrations of physical as well as chemical mutagens in pulses including lentil (Nerkar, 1970; Sharma, 1977), blackgram (Nalampang and Jan-on, 1982), chickpea (Kharkwal, 1978); and soybean (Rajput and Siddiqui, 1982).

(iii) Survival of plants

Plant survival also decreased gradually from 83 to 60% at 30 days and 76 to 55% at maturity with the increase in dose/concentrations of mutagens (Table 4.1 and Figs. 2a and 2b). Similar results were reported by Shaikh *et al.* (1983); Sharma (1977); Sharma and Sharma (1983); Chekalin (1968); Shevtsov (1969) and Bhadra

(1982). Sorour (1989) treated seeds of two peanut cultivars with gamma-ray, EMS and sodium azide and found that all these mutagens caused gradual decrease in the survival of plants in M_1 generation with the gradual increase of doses of mutagens. Wellensiek (1965) reported positive correlation among germination, survival and seed fertility of peas, all of which decreased after treatments with X-rays, gamma-rays and EMS. So, the lethal action of mutagen increases with the increase of doses/concentrations. Results of the present investigation also supported to the findings of the earlier workers.

(iv) Fertility of pollen grains

In the present study, the increase of doses/concentrations of mutagens caused gradual decrease of pollen fertility from 96.3 to 90.6% (Table 4.1 and Fig. 3). Tickoo (1978) reported a decrease in pollen fertility with the increase in dose of Ethyl methanesulphonate (EMS), N-methyl-N-nitroso urethane (MNU), sodium azide (SA) and gamma rays in mungbean. Using gamma rays and MNU, Hussein and Disouki (1976) also observed similar results in *Phaseolus vulgaris*. Germination, survivability, pollen fertility, root and shoot length gradually decrease with the increase of sodium azide concentrations (Shaikh *et al.*, 1983). Nerkar (1970) reported the effect of EMS and MNU on pollen sterility of gamma rays in *Lathyrus sativus*. Chauhan *et al.* (1990) investigated pollen viability in safflower and observed that the percentage of pollen sterility was directly proportional to increase of doses of gamma ray. Which indicated that mutagens could produce deleterious effect on the fertility of pollen grains and increase of doses/concentrations obviously decrease the viability of pollen grains.

In general, mutagenic treatment were quite effective in influencing all these parameters selected for assessing the biological damage caused in the generation of treatment. In this study gamma ray had more drastic effect on pollen fertility, seedling height, germination and survival than sodium azide. Sodium azide displayed minimum toxicity at the concentration used in terms of M_1 damage.

It is clear from the present investigation and from the earlier investigations made by Gaul (1959) that the above four characters are highly sensitive to the two mutagens, gamma-ray and sodium azide.

B. Characteristics in M_2 and M_3 generations

(i) Macro-mutation

The useful macro-mutations in chickpea obtained during this study are discussed below.

(a) Chlorophyll mutation

The frequency of the chlorophyll mutations is the most convenient way to gauge the genetic effect of a mutagen in the M_2 generation (Gustafsson, 1951; Gustafsson and Wettstein, 1958). The result of the present study showed that the highest chlorophyll mutation of 16.12% was found on family and that of 1.09% on plant basis in 300 Gy doses of gamma rays of the strain G-319. The variety Hyprosola and the strain G-97 also showed higher mutation frequencies in the higher doses/concentrations. It indicated that the mutation frequency increased with increase of doses, i e., higher doses could induce more chlorophyll mutation than the lower ones. On the other hand, the highest chlorophyll mutation frequency (8.0%) was found at 0.4 mM concentration of sodium azide in Hyprosola. But lower concentrations of sodium azide did not produced any mutations. In the present study the trend of the mutation frequencies is similar with the results obtained by Rai and Das (1975) in linseed; Sharma and Sharma (1979a) in lentil; Blixt (1964) in peas and Kulshrestha and Singh (1983) in blackgram. On the other hand, Nadarajan *et al.* (1982) reported high chlorophyll mutation in medium dose in red gram. Of course, the dose range for different crops were not the same.

Four types of chlorophyll mutations were observed in M_2 generation (Table 4.3) in this investigation. The relative percentage of these mutants varied with the variation in doses/concentrations. The doses of gamma-ray produced higher chlorophyll mutations (83.1%) that than concentrations of sodium azide treated population

(16.8%). Chlorina (45.4%) showed by xantha (28.5%), viridis (14.2%) and albina (11.6%) in M_2 population. Vandher *et al.* (1981) reported more albina than xantha in barley in M_2 generation of gamma-ray irradiated population.

From the earlier observations as well as from the present investigation it seems that the mutation frequency mainly depend on types of crop, mutagens and doses/concentrations.

(b) Morphological mutation

It was found that out of 498 families and 24,900 plants the total frequency of morphological mutations were found 24 and 82 on family and plant basis respectively (Table 4.4). In this study doses of gamma-ray produced higher number of morphological mutations than the different concentrations of NaN_3. The spectrum of morphological mutations in M_2 generation were 64 and 18 in gamma-ray and sodium azide treated population respectively (Table 4.5).

Similar to chlorophyll mutations many scientists including the above mentioned ones, obtained various morphological changes in the plants treated with mutagens in M_2 population. All these changes in morphological parameters determine the effectiveness of mutagens. In the present investigation, the morphological changes found were recorded to estimate the effectiveness of different doses/concentrations of gamma rays and sodium azide.

The following types of morphological mutants different from the controls were found in M_2 population.

(i) Mutants with fasciated cotyledonery leaves were characterized by having no first pair of true leaves and apical buds. All of them died within 3-4 weeks after germination. Thus they were lethal mutants.

(ii) Mutants were also found which had higher role of elongation of seedling than control. They were designated as tall mutants.

(iii) Morphological changes in respect of flower colour, shape, and size were also found in the M_2 population. Shaikh *et al.*(1983) reported flower colour mutant in chickpea after treatment with sodium azide. Abo-Hegazi (1991) and Datta (1991) also reported mutants with variation in flower colour.

(iv) Particularly higher doses/concentrations of mutagens produced sterile plants without any pod which were clearly different from their controls. Most of the sterile plants were deformed with yellow green leaves. Some times leaves were thick, broad, small and narrow.

(v) In gamma-ray treated population deformed fruits were found.

(vi) Wide ranges of variation in seed colour was found in the treated population.

(vii)Variations in branching habit were also found in the both type of mutagens treated in M_2 population.

There are many examples of useful macro-mutation in crop plants e.g. erectoide mutant in barley. In such mutant the plant height reduced and stiffness of stem, the leaf length and breadth increased/decreased and the higher ear density were affected (Gustafsson, 1954; Halloran *et al.*, 1979). A fully awned mutant of wheat was obtained by Pal and Swaminathan (1960). Athwal *et al.* (1970) reported several albino, sterile and one dwarf mutant in the M_2 population of chickpea. Kharkwal (1981) found dwarf, compact, upright, and gigas mutant of chickpea after gamma irradiation. From the above reports and the present observations it is clear that the gamma-ray and sodium azide could change substantially the morphology of a crop. The desirable changes in morphological characters can be utilized for the improvement of crop variety. Moreover, such marker gene or identifying character for a variety is very much essential to maintain the purity of the variety.

(c) Mutagenic effectiveness and efficiency

The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency (Gupta and Sharma, 1990). Several workers

like Rangaswamy and Ramula (1973), Kaul and Bhan (1977), Nagabhushanam *et al.* (1992), Gumber *et al.* (1995) and many others scientists have studied the effectiveness and efficiency of chemical and physical mutagens.

Mutagenic effectiveness usually refers to the rate of mutation produced by the mutagen in relation to its doses/concentrations. Whereas mutagenic efficiency refers to the mutation rate in relation to the damage (Konzak *et al.*, 1965).

The present result showed that the effectiveness ratio of gamma-ray ranges from 0.4 to 1.0 and sodium azide ranges from 0.0 to 115.3 (Table- 4.6). On the other hand, efficiency of gamma-ray and sodium azide ranges 0.51 to 1.36 and 0.0 to 0.43 respectively. The most effective concentration of sodium azide in chickpea was 0.4 mM. Similarly 300 Gy of gamma-ray was the most efficient dose in the chickpea.

The effectiveness of ionizing radiation as mutagen was first mentioned by Muller (1927) and Stadler (1930). Reasons for predominant use of ionizing radiation include good penetration, precise dosimetry and wide spectrum of mutations (Maluszynski *et al.*, 1986; Micke *et al.*, 1987).

Sodium azide showed the most efficient and effective of all mutagens treated for the chlorophyll-deficient, morphological and certain biochemical mutations in barley (Konzak *et al.*, 1965; Kleinhofs *et al.*, 1974; Konzak *et al.*, 1975; Niknejad, 1976; Nilan *et al.*, 1976; Walther, 1976) and in rice (Awan *et al.*, 1980).

The result of the present investigation and the earlier reports in respect of mutagenic effectiveness and efficiency of the gamma-ray and sodium azide have indicated that they are the most effective and efficient mutagen for creating variability in chickpea.

(ii) Micro-mutation

In the present investigation preliminary selection of micro-mutants was made in M_2 generation for yield and yield contributing characters. The applied value micro-

mutation is high in plant breeding research. This type of mutation can only be detected through appropriate biometrical analysis of the treated population in M_2 and subsequent generations. In a number of experiments various genetic parameters like genotypic and phenotypic coefficients of variation, heritability and genetic advance (as per cent of mean) were estimated in M_2 and M_3 generations for selecting the best materials (Johnson *et al.*, 1955; Allard 1960; Sarker, 1985). Considering the importance of induced mutations for polygenic parameters, an attempt was made in the present investigation to analyze the trend of induced variability and to select the strains with improved characters of economic importance. Regarding the results obtained for the different characters are discussed in the following section.

(a) Days to flowering

The family means showed a wide range of variations in both M_2 and M_3 generations over the controls (Tables 4.7 and 4.22; Figs 4 and 11). The range of family means in M_2 was found higher (48.4-78.6) than the controls (54.8-75.2). Mutagens shifted the means to both positive and negative directions (late and early initiation of flower compared with controls). Thus the results indicated that gamma-ray and sodium azide could induce mutation in days to flowering by shifting the mean values to both directions. Similarly the coefficients of variation (CV%) showed higher ranges in M_2 population (1.51-6.47) than that of the controls (1.11-1.47). From selection point of view, the families having lower mean with higher CV% value for days to flowering are of greater interest. Hence, the mutated families with lower means and higher CV% than the controls were selected to put forward for further evaluation in the next generation.

Many workers reported earlier that micro-mutation could shift the mean values to both positive and negative directions. Gaul (1965) showed that the mean values and genetic variance were highly influenced by different mutagenic treatments in barley . Scossiroli (1965) reported similar observation in the quantitative characters. Oka *et al.*

(1958) and Kao *et al.* (1960) also observed induced variability in both the directions for heading date in rice. Khan (1984) and Nadarajan *et al.* (1983) reported that days to flowering increased in mutated families. Mahla *et al.* (1990) found that mutagenesis could widen variability to both positive and negative direction which resulted sufficient variability in the treated population that could be utilized for selection of early or late flowering plants. Early flowering chickpea varieties are required to minimize the cropping period which will increase the cropping intensity. Saha and Shaikh (1991) found a wide spectrum of variability of flowering characters in the gamma-ray treated population in jute variety D-154. Early flowering and high yielding chickpea mutant were found in M_2 generation (Rao, 1988).

The component of variations in both genotypic and phenotypic level was found to be higher in the gamma-ray and sodium azide treated population than the untreated controls in both M_2 and M_3 generation (Tables 4.8 and 4.23). Similarly heritability and genetic advance estimates were also higher in the treated population than the controls in both the generations.

The results of the present study exhibited that both the mutagens created high heritability and genetic advance in days to flowering. It further indicated that the changed variability was genetic in nature. The present result supported the earlier mutation research in different crops by many researchers. Djelepov (1985) obtained a wide amplitude of variation with high heritability and genetic advance in wheat. Similar results were also reported by Sarker (1985) in lentil; Lather (1989) in *Brassica.*

The present findings offer a good scope for isolation of desirable plants of chickpea with earliness in days to flowering from the gamma-ray and sodium azide treated population from both the M_2 and M_3 generations.

(b) Days to maturity

The range of family means for days to maturity was higher in the treated population (118.8-140.8) than the controls (126.4-137.0) in the M_2 generation (Table 4.9 and Fig .5). Similarly ranges of family means in the treated population (121.0-141.0) was higher than the untreated controls (126.0-138.0) in M_3 generation. Shifting of mean values to both positive and negative direction than the controls were due to mutagenesis which caused wider range of variability in all the treatments (Table 4.23 and Fig. 12). Mutation frequencies were higher in gamma-ray irradiated than sodium azide treated M_2 population. The different treatments of gamma-rays caused desirable mutation for days to maturity by decreasing the population mean. On the other hand, sodium azide appeared to be less effective than gamma rays in both M_2 and M_3 generations. Ivannikov *et al.* (1970) obtained the early ripening, early flowering and high yielding mutants obtained from M_2 generation in chickpea. Nerkar and Mote (1978) found the effects of gamma rays on quantitative characters in the M_2 generation. Tiwari *et al.* (1983) also observed variation in eight quantitative characters in M_3 generation of chickpea.

The genotypic and phenotypic coefficients of variation in M_2 generation were much higher than the untreated controls (Table 4.10). Similarly coefficients of variation in the M_3 generation were higher in the treated population than the controls (Table 4.24). High heritability and genetic advance were found over the controls in the treated population in the both generations. The above results indicated that both gamma rays and sodium azide could bring genetic changes in the treated population in respect of days to maturity. It also indicated that the gamma-ray was more effective for creating desirable mutations than sodium azide of the concentrations used.

The duration for maturity of all the existing varieties of chickpea in Bangladesh is long. Farmers are not much interested to grow long duration crop because which interferes the other crops. Beside this, in Bangladesh chickpea is affected by hailstorm and early monsoon at mature stages which drastically reduces the yield. Here, cropping

intensity can be increased only by developing early maturing varieties through induced mutations. In the present investigation, early maturing mutants were selected. There are a number of similar reports of developing early maturing mutant varieties of chickpea (Ivannikov and Moraru, 1968; Kalia *et al.*, 1981; Shaikh *et al.*, 1982; Shamsuzzaman and Shaikh, 1991) and in rice (Kawai, 1963a and 1963b; Miah *et al.*, 1981 and Hakim *et al.*, 1988).

(c) Plant height

The range of family means for plant height was higher in the treated population (40.8-70.6 cm) than the controls (50.4-63.0 cm). Gamma-ray and sodium azide had little influence on mean plant height in M_2 and M_3 generations (Figs. 6 and 13) but ranges of variation increased in all the treatments of the both generations (Tables 4.11 and 4.26). Number and frequencies of mutations increased in the higher doses/concentrations. Selections were made in M_2 generation and were followed in the next generation. From these facts it is evident that the population means in M_3 generation did not show wide variation, yet the number of dwarf and semi-dwarf plants has increased. The larger number of dwarf or shorter plants increased the seed yield.

Athwal *et al.* (1970) obtained fertile dwarf plant from gamma-irradiated M_2 population in chickpea. Similarly dwarf or shorter plant types in chickpea were found in M_2 and M_3 generation (Nerkar and Mote, 1978; Kharkwal, 1981; Tiwari *et al.*, 1983). Shorter plant height mutant was found in other crops by many researchers e.g., Rutzer *et al.* (1976); Kawai and Amano (1991) in rice; Panda (1981) and Das (1995) in seasame. On the other hand, Nerkar and Mote (1978) reported tall mutant in chickpea; Nayar and George (1969) in seasame and Rahman *et al.* (1992) in mustard. Awan *et al.* (1980) reported that in rice, short culm and dwarf mutant types were frequent in the sodium azide induced mutations.

It was found that the genotypic and phenotypic coefficients of variation were higher in the treated population than the controls in both M_2 and M_3 generations (Tables 4.12 and 4.27). At the same time, high heritability and genetic advance were recorded in those generations. The variations induced were genetic. Kumar and Das (1977) and Das (1995) reported higher mean and coefficients of variation in M_2 and later generations after treating the seeds with gamma-ray.

The results indicated that the mutagens could cause both positive and negative genetic variability in plant height. Selection then could be made for both tall and short mutants. It was also observed that the dwarf mutants mature earlier than the taller mutants. In leguminous crops, indeterminate growth habit is common which causes drastically yield loss. Shorter plants have generally determinate growth habit which is ultimately reflected on yield in positive direction.

(d) Number of pods/plant

The treatment means of this character varied in different doses/concentrations of both M_2 and M_3 generations (Figs. 7 and 14). The range of family means (40.6-145.8) in the M_3 generation were identical to that of the M_2 generation (Tables 4.12 and 4.27). On the other hand, the population mean of the character was higher in M_3 than M_2 generation. Priority was given during selection on increased number of pods/plant. The reduction in mean of the number of pods found in most treatments may be attributed to induction of more mutations with negative effects. Higher or equal mean after mutagenic treatments was recorded by Sharma (1977) and Ravi and Singh(1980) in lentil; Rajput (1974), Shakoor *et al.* (1979) and Verma and Singh (1984) in mungbean; Singh (1981) and Bhadra (1982) in blackgram. The number of pods varied from year to year and from location to locations. It also varied depending on the dates of sowing. The results showed wider range of family means towards both positive and negative directions. The highest mutation frequency was found in 300 Gy doses of gamma-ray in the strain G-319. On the other hand, the lowest frequency was seen in

0.3 mM concentrations of sodium azide in the strain G-97. The treatments of gamma-ray showed more or less similar result with sodium azide. A comparison between the two mutagens indicated that sodium azide was more effective than the gamma-ray in producing higher number of pods/plant. It means that the selection in M_2 generation would be positive for this character.

The variability for number of pods/plant was found to increase in the M_2 and M_3 generations. Increase in variability for yield attributes as a result of mutagen treatments has been frequently reported in many crops (Abo-Hegazi, 1973; Sharma, 1977; Tickoo and Jain, 1980; Rajput and Siddiqui, 1982).

Genotypic and phenotypic coefficients of variation were higher in the mutated population in both M_2 and M_3 the generations (Tables 4.13 and 4.28). Moreover, the heritability and genetic advance were also higher in these treated population than the untreated controls. Thus the selected families with high heritability and genetic advance are expected to retain this character in the subsequent generations.

The increase of heritability and genetic advance in M_2 and M_3 generations were also reported by many workers e.g., Sharma (1977) in lentil; Ramanathan (1983) in groundnut; and Khan (1984) in mungbean. Moreover, mutants with increased number of pods of chickpea were obtained through combined treatments of gamma-ray and sodium azide (Shamsuzzaman *et al.*, 1994).

(e) Number of seeds/plant

The ranges of family means of this character was 41.6 to 260.4 (Table 4.16) in M_2 generation and in M_3, 50.5 to 266.6 (Table 4.31). The population means for number of seeds/plant of different doses/concentrations increased or decreased slightly in M_2 generation as compared to their respective controls (Fig. 8). Similar observations were made in M_3 generation (Fig.15). Shifting of the means to both positive and negative directions was also observed in the treated population which indicated that

both the mutagens produced mutation in number of seeds/plant by widening the range of family means through shifting to the higher or lower directions from the controls. The above result suggests that the selection of desirable mutants for this characters in M_2 generation would be positive effective since the number of seeds/plant directly influences the grain yield.

The genotypic and phenotypic coefficients of variation were much higher in the treated population at both M_2 and M_3 generations over the controls (Tables 4.14 and 4.29). High heritability estimates coupled with high genetic advance in different treated population were also found over the untreated controls indicated that the presence of genetic diversity in number of seeds/plant. Kale *et al.* (1980) found high heritability and expected genetic advance following the treatment with gamma rays in chickpea. Mehdat *et al.* (1989) reported increased range of family means with high coefficients of variation and estimates of heritability and genetic advance for most of the characters by increasing doses of gamma rays. Panda (1981) also isolated some seasame mutants with increased number of seeds from the populations treated with chemical mutagen. He also screened desirable mutants in M_2 generation and tested the breeding behaviour of the selected mutants in M_3 generation.

(f) 100-seed weight

The ranges of family means were wider: (i) in M_2 generation from 6.5 to 29.7 (ii) in M_3, from 6.4 to 29.8 than their respective controls from 7.3 to 26.1 and from 7.2 to 26.3 (Tables 4.18 and 4.32). The treatment means in M_2 and M_3 generations showed slight variation (Figs. 9 and 16). The ranges of CV% in M_2 generation were much higher than the ranges CV% for M_3 generation. It indicated that selections made in M_2 generation were reflected in M_3 generation. It was also observed in this study that 300 Gy treatment showed the highest mutation frequency for 100-seed weight in the three genotypes in chickpea.

Both the genotypic and phenotypic coefficients of variation in M_2 and M_3 generations were higher than those of their respective controls (Tables 4.19 and 4.33). Heritability and genetic advance were also higher in the treated populations than the controls. Abdulomonov and Nigmatullin (1978) reported the increase of 100-seed weight, seed yield, number of pods and seeds/plant through the use of chemical mutagens N-nitroso-N-ethylurea and Ethyleneimine in chickpea. Dhopte *et al.* (1974) irradiated some chickpea cultivars with gamma-ray and observed noticeable changes in 100-seed weight, protein content, seed yield and also in some physiological characters. Therefore, the present investigation also supported the earlier reports regarding this 100-seed character.

Gaul (1965) reported negative population means in 100-seed weight but superior individual seed weight. We also found the same result.

(g) Seed yield/plant

The M_2 and M_3 family means of all the treatments showed wider ranges of seed yield/plant than their respective controls (Figs.10 and 17). The range of family means, coefficients of variation and mutation frequency in M_2 generation varied in both positive and negative directions (Table 4.20) and similar trend was also found in M_3 generation (Table 4.34). This extension of variability in either direction created sufficient number of variants within the treatments or between treatments. The result indicated that in M_2 generation there was a few negative mutations which reduced the population means for seed yield/plant. The dose 300 Gy was found to produce the highest mutation frequency for each of the three genotypes for seed yield/plant. The widest range of family means was also observed at 300 Gy in the strain G-97. Therefore, it may be concluded that there is a better scope to select higher seed yield/plant. In M_3 generation the range of family means decreased but the minimum values in different treatments increased compared to M_2 generation. This may be the

effect of selection in M_2 generation. Moreover, increase of minimum values of family means in M_3 generation was the effect of making selection of mutants with high seed yield performance in M_2 generation. Higher seed yield mutant of chickpea in M_2, M_3 and following generations was reported by a number of researchers (Ivannikov and Moraru, 1968; Dhopte *et al.*, 1974; Chandola *et al.*, 1976; Abdulomonov and Nigmatullin, 1978; Nerkar and Mote, 1978; Kharkwal, 1981; Shaikh *et al.*, 1982 and Haq, 1983).

The statistical analyses showed the highest genotypic and phenotypic coefficients of variation in all the treated populations. High heritability and genetic advance in both the generations were also found. From these it was concluded that the variability caused by mutagens was genetic in nature (Tables 4.20 and 4.35). Gill *et al.* (1974), Sharma (1977), Labana *et al.* (1980), Ravi and Singh (1980), Shaikh *et al.* (1982), Rajput and Siddiqui (1982) Sarker (1985), Pathirana (1992) and Das (1995) reported significant variation in the treated population with high heritability and genetic advance for seed yield. It may be concluded that there is a great possibility for improvement of chickpea genotypes with better yield performance by using gamma-ray and sodium azide.

(iii) Frequency of positive and negative mutations

In respect of days to flowering, days to maturity and plant height, positive mutations (higher means) are undesirable. In case of 100-seed weight, number of pods/plant, number of seeds/plant and seed yield/plant, positive mutations are desirable. It was evident from the Table 4.21 that the positive direction of mutations was lower than that of the negative direction. Those families are considered to be mutated families which showed higher or lower means along with higher CV% than their respective parent.

C. Characteristics in M_4 generation

Eighteen promising mutants along with the three parents G-319, G-97 and Hyprosola were evaluated for yield and other yield contributing characters in M_4 generation. Out of them, 14 mutants were derived from different doses of gamma-ray and only four, following various concentrations of sodium azide treatment. Mean data collected from M_4 generation were subjected to statistical analysis and were compared among the mutants and controls by estimating the CD values at 5 % level.

Significant variation among the mutants was found in the mean performances of the all characters. Significant genotypic and location effects indicated the presence of considerable interaction between genotype and location on all the characters except number of seeds/plant. Thus it can be suggested that the mutants and controls responded differently in different locations.

In respect of mean performances, it was found that most of the mutants started flowering earlier than their respective controls (Table 4.38). Early maturing characteristics was given priority during the time of selection in the M_2 and M_3 generations. The selected mutants also responded to earlier maturity in M_4 generation. It indicated that the selection was done efficiently in the previous generation. This feature is important in Bangladesh. Most of the existing chickpea varieties here require longer maturity period. Farmers are not much interested to grow long duration crops since it interferes with other crops. On the other hand, early maturing varieties can possibly escape early monsoon shower and hailstorm. If the chickpea plant gets rain at mature stage, disease incidence will increase and seed yield will reduce drastically.

Variation in plant height of the mutants was observed in M_4 generation although the range of variation was small compared to the previous generation (Table 4.39). Most of the mutants showed shorter plant height than their respective controls. As a result, generation mean of the mutants was comparatively lower than the controls and as well as the previous generation. Shorter or dwarf plant can be accommodated more in number per unit area which ultimately increases the density of population and

results in higher seed yield. The mutants had more pods/plant than their controls. Similar results were found in case of number of seeds/plant (Table 4.40). The mean of the seed yield (kg/ha) indicated that all the mutants produced higher seed yield than their respective controls (Table 4.41). Generation mean of the selected lines was also observed to be greater than the controls. Thus the main objectives of the present study to development of a variety of chickpea with higher seed yield was achieved. Out of the 18 M_4 mutants only 6 were selected on the basis of higher seed yield, early maturing and larger seed size.

D. Conclusion (M_1- M_4 generation)

1. Different doses and concentrations of gamma rays and sodium azide showed clear influence on germination of seeds, seedling height, plant survival and fertility of pollen grains in M_1 generation.
2. Doses of gamma rays used in this study showed more effectiveness than the used concentrations of sodium azide on those parameters.
3. In M_2 generation both macro and micro mutations were also found in different doses of gamma rays and sodium azide. Four types of chlorophyll mutants (albina, chlorina, xantha and viridis) were observed and the relative percentage of these mutants varied with the variation in doses and concentrations. The result showed the number of chlorophyll mutants and the mutations frequencies in chickpea were largely influenced and depended on strains/varieties, doses/concentrations and type of the mutagen used.
4. As regards mutagenic efficiency, gamma rays found to be the most effective and efficient in creating variability in chickpea than the sodium azide.
5. The different doses and concentrations of the mutagens produced sufficient variability in different quantitative characters including seed yield in M_2 generation. The mean values of all the characters were shifted to both positive and negative directions from the mean values of the controls.

6. High genotypic and phenotypic coefficients of variation with high heritability and genetic advance were found in the treated populations.
7. Number of pods /plant showed the highest mutation frequency with lower mean value than the controls.
8. Seed yield /plant showed the lowest mutation frequency with higher means than that of the controls.
9. A total of 18 promising mutants were selected from M_3 generation towards further evaluation for earliness, higher seed yield and large seed size in M_4 generation.
10. It may be concluded from the entire research work reported in this study that gamma rays and NaN_3 have much potentiality to induce both macro (morphological changes) and micro (change in quantitative characters) mutation of the three genotypes of chickpea.

Chapter VI
SUMMARY

VI. SUMMARY

Dry seeds of three chickpea genotypes viz., G-319, G-97 and Hyprosola were treated with 100, 200 and 300 Gy doses of gamma rays as well as 0.2, 0.3 and 0.4 mM cocentrations of sodium azide in order to induce mutants. In M_1 generation, both gamma rays and sodium azide substantially influenced germination of seeds, seedling height, plant survivals at 30 days and at maturity and also pollen fertility. Gamma rays produced more deleterious effects than sodium azide on these characters. A gradual reduction in germination of seeds, seedling height, survival of seedlings and plants, and pollen fertility were found with increasing doses and concentrations of chemical and physical mutagens.

In M_2 generation, the mutation frequency increased with the increase of doses of gamma rays and concentrations of sodium azide i.e., higher doses/concentrations could induce more chlorophyll mutations than lower ones. Four types of chlorophyll mutants were found viz., chlorina (45.5%), xantha (28.5), viridis (14.2%) and albina (11.6%) in M_2 population. Morphological mutants viz. narrow leaf size, broad leaf size, bushy plant, dwarf plant, sterile plant, large podded, small podded, many podded were also found in this generation. The effectiveness and efficiency of different treatments of the both mutagens in respect of producing variations were determined. Total morphological mutations in the family and the plant basis were 24 and 82, respectively. Gamma rays produced effectively higher number of morphological mutations than sodium azide treatment. In the present investigation sodium azide showed the highest effectiveness (0.0-115.3) than the gamma rays (0.4-1.0). On the other hand, the efficiency of gamma rays (0.51-1.36) was higher than the sodium azide (0.0-0.43). The 0.4 mM concentration of NaN_3 was found as the most effective concentrations and the 300 Gy of gamma rays was the most efficient dose in chickpea. The different doses and concentrations of gamma rays and sodium azide respectively produced sufficient variability in seven different quantitative characters including plant height, days to flowering, days to maturity, number of pods/plant, number of seeds/plant, 100-seed

weight and seed yield/plant in M_2 generation. Most of the characters showed higher negative mutations with lower mean than their respective controls except days to flowering and days to maturity.

Out of 498 M_2 families only 153 families bred true in M_3 generation. Strong selection pressure was given in M_3 generation for selecting early maturity, higher 100-seed weight and higher seed yielding plant. Breeding behaviour of the selected mutants were carefully studied in M_3 generation. Finally, only 18 true breeding mutants were selected from M_3 generation.

In M_4 generation, the 18 selected mutants along with their parents were grown at two different locations during rabi season of 1995-96. Data collected from M_4 generation were subjected to statistical analysis and comparison among the mutants and controls was made by estimating the CD values at 5% level.

The analysis revealed that the item genotype (G) was significantly different for all the seven characters studied in M_4 generation which indicated the existence of real differences among the mutants and the controls. The item location (L) also exhibited similar results. Regarding the interaction between genotypes and locations, the item G×L appeared to be significantly different for all the characters except number of seeds/plant. The results further indicated that the mutants and controls responded differently at different locations. Out of 18 mutants, only six exhibited earliness, only two showed more number of seeds/plant than Hyprosola and only four mutants showed higher 100-seed weight than the large seeded parent G-319. Out of 18 mutants, only 8 produced higher seed yield than the higher seed yielding parent G-97. Finally six mutants were selected on the basis of early maturity, higher 100-seed weight (larger seed size) and seed yield for growing in the next generation.

It can be concluded that the induced mutation technique using gamma rays and sodium azide mutagens was effective tool for creating genetic variability in improving the quantitative characters of chickpea.

Chapter VII
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VII. REFERENCES

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Chapter VIII
APPENDICES

VIII. APPENDICES

Appendix 1. Proximate analyses and chemical composition of chickpea seeds (100g^{-1} sample) dry weight basis (Source: Williams and Singh, 1986)

Constituent	No. of cultivars	Ranges	Mean
Crude protein	373	12.4-31.5	23.0
Nonprotein N*	10	0.16-0.91	0.39
Starch	15	41.0-50.8	47.3
Soluble sugars	16	4.8-8.3	5.8
Total carbohydrate	48	52.4-70.9	63.5
Ash	33	2.5-4.0	3.3
Fat	33	3.8-10.2	5.3
Crude fibre	27	1.7-10.7	6.3
Dietary fibre	17	10.6-27.3	19.0
Calorific value (Cal 100g^{-1})	6	333.8-361.2	347.6
Minerals**			
Phosphorus	32	244.0-429.0	342.9
Calcium	40	103.1-259.0	185.6
Magnesium	27	119.0-167.7	141.0
Iron	47	3.9-9.8	6.6
Copper	21	0.86-1.18	0.96
Zinc	6	2.51-3.51	2.95
Vitamins**			
Vitamin A	1	–	0.19
Vitamin B_1	2	0.28-0.30	0.29
Vitamin B_2	3	0.15-0.30	0.20
Vitamin B_6	1	–	0.55
Vitamin C	42	2.15-6.00	3.87
Niacin	2	1.6-2.9	2.25

*mg 100g^{-1}, **g 100g^{1} N

Appendix 2. Effect (% of control) in germination of seeds, seedling height, plant survivals and pollen grain fertility in M_1 generation following mutagenic treatments in three varieties of chickpea

Strain/ Doses/ Conc.	Germination (%)	Seedling height at 30 days	Plant survival at 30 days (%)	Plant survival at maturity (%)	Pollen grain fertility (%)
G-319					
Control	100.0	100.0	100.0	100.0	100.0
100 Gy	88.1	97.0	89.8	89.5	97.2
200 Gy	78.1	83.4	83.4	81.2	96.9
300 Gy	66.9	68.4	71.2	71.2	93.4
0.2mM	88.8	85.8	88.1	88.7	99.2
0.3mM	82.2	66.8	81.5	81.2	97.5
0.4mM	75.0	61.4	72.4	73.2	94.8
G-97					
Control	100.0	100.0	100.0	100.0	100.0
100 Gy	91.1	93.6	90.1	89.0	96.5
200 Gy	79.0	87.2	81.1	82.0	94.8
300 Gy	72.4	69.0	66.6	64.4	93.4
0.2mM	90.5	83.4	89.2	87.4	99.3
0.3mM	85.0	73.7	77.7	73.8	95.9
0.4mM	79.0	63.1	71.1	70.3	94.4
Hyprosola					
Control	100.0	100.0	100.0	100.0	100.0
100 Gy	90.1	89.3	92.2	90.3	98.9
200 Gy	80.6	82.6	78.4	79.8	96.6
300 Gy	71.8	56.6	72.5	72.2	96.6
0.2mM	92.8	85.3	88.8	86.7	99.7
0.3mM	86.7	72.4	78.8	80.7	98.9
0.4mM	82.4	60.8	72.2	73.4	96.1

Appendix 3. Effects of mutagen on different parameters of chickpea in M_2 generation

Strain/ variety	Doses/ Conc.	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield / plant (g)
G-319	Control	70.0	134.3	60.3	51.8	61.8	26.0	15.6
	100 Gy	69.7	134.5	59.1	53.3	63.6	25.6	15.6
	200 Gy	69.4	133.8	60.7	52.2	62.0	25.8	15.8
	300 Gy	64.8	133.8	59.7	51.3	61.3	25.9	15.9
	0.2mM	67.6	134.0	59.0	48.6	55.6	25.7	15.1
	0.3mM	67.1	132.9	56.0	46.6	54.4	24.5	14.3
	0.4mM	69.5	129.3	57.5	49.3	58.9	24.8	15.7
G-97	Control	74.4	135.9	61.3	72.2	110.9	16.6	18.0
	100 Gy	73.4	134.6	61.5	73.6	110.6	16.9	18.6
	200 Gy	70.6	133.4	61.6	73.5	111.9	16.3	19.0
	300 Gy	73.4	132.4	62.4	73.6	108.1	16.5	18.7
	0.2mM	73.7	134.8	61.3	73.1	110.0	16.1	17.3
	0.3mM	72.5	134.5	61.9	76.3	107.0	16.4	17.7
	0.4mM	70.4	135.5	60.3	74.4	112.1	16.5	19.0
Hyprosola	Control	60.5	126.8	50.8	100.4	191.6	7.4	14.2
	100 Gy	59.3	125.8	50.2	101.2	188.2	7.2	13.9
	200 Gy	61.2	126.0	49.9	103.5	192.7	7.3	14.3
	300 Gy	59.4	124.9	49.5	103.1	193.6	7.2	14.4
	0.2mM	60.6	126.0	51.7	104.2	191.8	7.3	14.4
	0.3mM	58.8	126.1	50.6	113.3	191.9	7.4	15.0
	0.4mM	60.9	126.5	50.9	117.4	192.3	7.4	15.1

Appendix 4. Effects of mutagen on different parameters of chickpea in M_3 generation

Strain/ variety	Doses/ Conc.	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield / plant (g)
G-319	Control	69.8	134.1	59.8	51.0	61.1	26.0	15.6
	100 Gy	70.3	134.3	59.4	53.3	64.2	25.9	16.5
	200 Gy	69.6	133.8	59.9	53.6	64.1	25.6	16.5
	300 Gy	65.9	134.1	59.1	53.1	63.3	25.5	16.4
	0.2mM	66.2	133.3	60.9	52.7	62.6	26.9	16.2
	0.3mM	65.0	133.1	60.6	52.1	61.4	26.6	15.8
	0.4mM	65.0	134.7	59.3	50.5	60.0	25.1	15.4
G-97	Control	73.4	137.5	61.2	73.9	108.3	17.1	18.4
	100 Gy	69.4	135.9	60.6	83.0	126.3	17.5	21.4
	200 Gy	69.5	133.3	60.4	75.4	114.7	17.9	19.9
	300 Gy	73.1	136.5	61.9	74.5	120.3	18.1	18.9
	0.2mM	73.8	138.0	62.7	71.5	104.7	17.5	17.9
	0.3mM	68.8	134.0	60.9	72.9	108.5	18.1	18.6
	0.4mM	70.8	136.1	62.5	82.4	123.6	18.2	20.9
Hyprosola	Control	60.5	127.5	51.3	99.6	198.1	7.4	14.0
	100 Gy	58.3	126.5	50.5	101.0	188.6	7.6	13.4
	200 Gy	59.2	128.0	50.4	104.8	196.2	7.5	14.6
	300 Gy	57.8	125.0	49.4	104.7	199.3	7.4	14.9
	0.2mM	60.4	129.2	52.5	104.1	199.3	7.3	14.3
	0.3mM	59.2	129.6	52.3	118.0	218.6	7.7	16.4
	0.4mM	59.0	126.7	46.5	125.1	197.7	7.9	17.7

Appendix 5. List of families selected for growing in M_3 generation following gamma-ray and NaN_3 treatment of three chickpea genotypes

Strain/ variety	Doses/ Conc.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield /plant (g)
G-319	Control		69	134	59	53	63	25.68	15.67
	100 GY	CPM 1						LS	
		CPM 2	EF		SH				
		CPM 3	EF				HS	LS	HSY
		CPM 4							HSY
		CPM 5			SH				
		CPM 6	EF	EM	SH		HS		HSY
		CPM 7						LS	
		CPM 8						LS	
		CPM 9		EM	SH	HP	HS		HSY
		CPM10						LS	
	200 GY	CPM11				HP	HS	LS	HSY
		CPM12						LS	
		CPM13				HP	HS		HSY
		CPM14				HP	HS	LS	HSY
		CPM15						LS	
		CPM16		EM	SH	HP	HS		HSY
		CPM17	EF						
		CPM18		EM	SH				HSY
		CPM19						LS	
		CPM20		EM					
		CPM21						LS	

EF= Early flowering, EM= Early maturity, SH= Shorter plant height, HP= Higher number of pods, HS= Higher number of seeds, LS= Large seeded and HSY = Higher seed yield/plant

(Continued overleaf)

Appendix 5 (Continued)

Strain/ variety	Doses/ Conc.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield / plant (g)
G-319		CPM22						LS	
		CPM23						LS	
	300 GY	CPM24	EF					LS	
		CPM25	EF		SH				
		CPM26	EF	EM	SH	HP	HS		HSY
		CPM27	EF						
		CPM28	EF						
		CPM29						LS	
		CPM30						LS	
		CPM31	EF						
		CPM32				HP	HS	LS	HSY
		CPM33						LS	
		CPM34	EF	EM	SH	HP	HS	LS	HSY
		CPM35	EF	EM	SH	HP	HS		HSY
		CPM36	EF			HP	HS	LS	HSY
		CPM37	EF						
		CPM38	EF			HP			HSY
	0.2 mM	CPM39			SH				
		CPM40	EF					LS	
		CPM41	EF	EM		HP	HS	LS	HSY
		CPM42	EF					LS	
	0.3 mM	CPM43	EF					LS	
		CPM44	EF						
		CPM45				HP	HS	LS	HSY
		CPM46	EF					LS	

(Continued overleaf)

Appendix 5 (Continued)

Strain/ variety	Doses/ Cone.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield plant (g)
G-319		CPM47	EF	EM	SH			LS	
	0.4 mM	CPM48	EF		SH			LS	
		CPM49	EF						
		CPM50	EF						
		CPM51	EF						
		CPM52				HP		LS	HSY
		CPM53	EF	EM	SH				
G-97	Control	-	94	135	61	72	105	16.69	18.04
	100 GY	CPM54						LS	
		CPM55	EF						
		CPM56						LS	HSY
		CPM57	EF	EM	SH	HP	HS	LS	HSY
		CPM58	EF		SH		HS	LS	HSY
		CPM59	EF		SH	HP	HS	LS	HSY
	200 GY	CPM60	EF					LS	
		CPM61		EM			HS	LS	HSY
		CPM62	EF				HS		HSY
		CPM63	EF	EM	SH	HP	HS	LS	HSY
		CPM64	EF			HP	HS	LS	HSY
		CPM65	EF			HP	HS	LS	HSY
		CPM66					HS		
		CPM67	EF				HS	LS	HSY
		CPM68	EF	EM	SH	HP	HS	LS	HSY
		CPM69	EF	EM		HP	HS	LS	HSY
		CPM70	EF			HP	HS	LS	HSY
		CPM71					HS	LS	
		CPM72					HS		HSY
		CPM73	EF	EM	SH	HP	HS	LS	HSY
		CPM74	EF					LS	

(Continued overleaf)

Appendix 5 (Continued)

Strain/ variety	Doses/ Conc.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield plant (g)
G-97	300 GY	CPM75				HP	HS	LS	HSY
		CPM76	EF			HP	HS	LS	HSY
		CPM77						LS	
		CPM78	EF					LS	
		CPM79					HS		HSY
		CPM80	EF	EM	SH	HP	HS	LS	HSY
		CPM81	EF					LS	
		CPM82			SH		HS		
		CPM83	EF		SH				
		CPM84			SH			LS	
		CPM85			SH			LS	
		CPM86			SH			LS	
		CPM87				HP		LS	HSY
	0.2 mM	CPM88						LS	
		CPM89	EF		SH			LS	
		CPM90						LS	HSY
	0.3 mM	CPM91	EF					LS	
		CPM92	EF			HP	HS	LS	HSY
		CPM93	EF	EM	SH	HP	HS	LS	HSY
		CPM94	EF						
		CPM95	EF					LS	
		CPM96	EF	EM	SH	HP		LS	HSY
	0.4 mM	CPM97		EM	SH		HS	LS	HSY
		CPM98			SH			LS	
		CPM99				HP	HS	LS	HSY
		CPM100	EF		SH	HP	HS	LS	HSY
		CPM101	EF			HP	HS	LS	HSY
		CPM102	EF					LS	HSY

(Continued overleaf)

Appendix 5 (Continued)

Strain/ variety	Doses/ Conc.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield /plant (g)
Hyprosola	Control	-	60	126	50	100	191	7.41	14.28
	100 Gy	CPM103		EM	SH	HP		LS	
		CPM104						LS	
		CPM105	EF						
		CPM106	EF			HP		LS	
	200 GY	CPM107	EF		SH	HP	HS	LS	HSY
		CPM108	EF			HP	HS	LS	HSY
		CPM109				HP	HS	LS	HSY
		CPM110					HS	LS	
	300 GY	CPM111		EM	SH	HP			
		CPM112		EM	SH	HP			
		CPM113				HP	HS	LS	HSY
		CPM114	EF		SH			LS	
		CPM115				HP			
		CPM116		EM	SH	HP	HS		HSY
		CPM117		EM	SH	HP	HS		HSY
	0.2 mM	CPM118				HP	HS		
		CPM119				HP	HS		
	0.3 mM	CPM120	EF			HP	HS	LS	HSY
		CPM121				HP			
		CPM122				HP		LS	HSY
	0.4 mM	CPM123	EF		SH	HP	HS		HSY
		CPM124			SH	HP		LS	

Appendix 6. List of families selected for growing in M_4 generation

Strain/ variety	Doses/ Conc.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield / plant (g)
G-319	Control	-	69	134	59	51	61	25.9	15.66
	100 GY	CPM 9		EM	SH	HP	HS	LS	HSY
	200 GY	CPM18		EM	SH	HP	HS	LS	HSY
		CPM21	EF	EM				LS	HSY
	300 GY	CPM26	EF	EM	SH	HP	HS	LS	HSY
	300GY	CPM34	EF	EM	SH	HP	HS	LS	HSY
		CPM35	EF	EM	SH	HP	HS		HSY
	0.2 mM	CPM41	EF	EM		HP	HS	LS	HSY
G-97	Control								
	100 GY	CPM57	EF	EM	SH	HP	HS	LS	HSY
		CPM58	EF	EM		HP	HS	LS	HSY
	200 GY	CPM63	EF	EM	SH	HP	HS	LS	HSY
		CPM73	EF	EM	SH	HP	HS	LS	HSY
	300 GY	CPM80	EF	EM	SH	HP			HSY
	0.3 mM	CPM96		EM	SH		HS	LS	HSY
	0.4 mM	CPM97		EM	SH	HP	HS	LS	HSY
Hyprosola	Control								
	300 GY	CPM111		EM	SH	HP			HSY
		CPM112		EM	SH	HP			HSY
		CPM116		EM	SH	HP	HS		HSY
	0.4 mM	CPM123	EF	EM	SH	HP	HS	LS	HSY

Appendix 7. List of families selected for growing in M_5 generation

Strain/ variety	Doses/ Conc.	Selection number	Days to first flowering	Days to maturity	Plant height (cm)	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Seed yield kg/ha
G-319	100 GY	CPM 9	70	123	56	72	83	25.9	2001
	300 GY	CPM26	59	121	52	61	70	26.8	2647
	300GY	CPM34	57	126	51	56	66	31.1	2077
G-97	200 GY	CPM63	62	120	62	78	120	20.2	2553
	200 GY	CPM73	68	119	60	77	115	21.3	2256
Hyprosolsa	300 GY	CPM112	60	116	51	106	190	7.9	1824
G-319	00 GY	Control	69	134	60	55	64	26.9	1450
G-97	00 GY	Control	73	136	63	74	105	17.7	1736
Hyprosola	00 GY	Control	60	125	50	101	191	7.9	1623