MODULATION OF RADIOSENSITIVITY WITH GIBBERELLIC ACID FOR CYTOGENETICAL, BIOCHEMICAL AND GENETIC SPECTRUM IN CHICKPEA (*CICER ARIETINUM* L.)

BY

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In the name of Allah, the Compassionate,

the Merciful

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DECLARATION

This is to certify that this dissertation submitted by Muhammad Rashid Khan, is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the dissertation requirements for the degree of Ph.D. in Biological Sciences.

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ABSTRACT

Modulation of radiosensitivity with gibberellic acid (GA_3) for cytogenetical, biochemical, seedling physiology and genetic variation was carried out in three chickpea (*Cicer arietinum L*) genotypes having different seed coat colours. Dry seeds of three genotypes namely Noor 91, Punjab 91, and C 141, were irradiated at dose level of 10, 20, 30, 40, 50, 60, 70, 90, and 110 Kr. A part of the irradiated seeds were treated with 0.5 mM solution of GA_3 for 16 hours prior of various experiments. Mitotic index decreased, while chromosomal anomalies increased with an increase of irradiation level. The chromosomal aberrations recorded were fragments, bridges, and laggards at anaphase. However, these anomalies were not observed uptil 30 Kr in the three varieties. Post mutagenic application of GA_3 increased the mitotic index and decreased the chromosomal anomalies and it was more pronounced at higher doses. Irradiation level of 10, 60 and 110 Kr was used to determine the effect on nucleolar volume and structure. Frequency of cells having greater nucleolar volume increased with an increase of irradiation, while the effect was reduced with the application of GA_3.

Gamma irradiation decreased the fresh weight, protein, RNA and DNA contents with an increase of irradiation dosages except at lower doses where simulation as compared to control was observed. Application of GA_3 modulated the radiosensitivity and fresh weight, protein, RNA and DNA contents increased at various irradiation dosages. Peroxidase and catalase activity increased over the control uptil 3^{rd} and 5^{th} day, respectively and then decreased for the following days at various doses of irradiation. Exogenous application of GA_3 increased the catalase and peroxidase activity at various doses throughout the developmental period. IAA oxidase activity stimulated at lower doses of irradiation, while at higher doses it was decreased regularly with irradiation intensity. GA_3 treatment increased this activity across various doses. Germination was inhibited regularly with an increase of gamma irradiation except at lower doses, while with GA_3 treatment inhibitory effect was recorded only at higher doses of 60, 70, 90 and 110 Kr. Shoot length, root length and number of roots decreased consistently with an increase of gamma irradiation. Application of GA_3 restored the growth and an increase was observed at various doses. From this study doses of 40, 50 and 60 Kr were considered as appropriate for induction of genetic variability.

Irradiated seeds at 40, 50 and 60 Kr of three genotypes along with the application of GA_3 were sown at Barani Agriculture Research Institute (BARI), Chakwal. A wide range of genotypic

variation was induced with gamma irradiation for all the characters like plant height, number of primary and secondary branches, pods per plant, seeds per pod, 100-seed weight, biological yield, grain yield, harvest index, days to 50% flowering and days to maturity. Application of GA_3 changed the spectrum of induced variation either in positive or negative direction at various intensities of irradiation. Genotype-treatment revealed significant and highly significant interaction for all the characters under study in one or the other generation. Stimulating effect with GA_3 were observed for seeds per pod, 100-seed weight, grain yield and harvest index in M_2 and M_3 population. It was, therefore, suggested that post mutagenic application of GA_3 might be beneficial if utilized for grain yield enhancement.

Albina, xantha, chlorina and *viridis* chlorophyll mutants along with other morphological i.e., stem, branching, leaf, pod, seed, flowering and maturity mutants were obtained in M_2 generation. Chlorophyll mutants decreased, while morphological increased with application of GA_3

The results regarding correlation coefficients revealed a high magnitude of genotypic than phenotypic for most of the characters in M_1, M_2 and M_3 generation. However, the association of grain yield with other characters changed in succeeding generations independently among the genotypes. Grain yield was positively correlated with pods per plant, seed per pod, and 100-seed weight in different populations of the three genotypes, while the association of other characters with grain yield was inconsistent.

Harvest index and biological yield in variety Noor 91 had positive direct effect on grain yield in M_1, M_2 and M_3 population. In Punjab 91, harvest index and biological yield in M_1 generation, while in M_2 and M_3 generation pods per plant, seeds per pod and 100-seed weight had strong positive direct effect on grain yield. In case of variety C141, 100-seed weight and pods per plant had direct positive effect on grain yield in M_1, M_2 and M_3 generation. So harvest index and biological yield would be a reliable criterion in Noor 91 and pods per plant and 100-seed weight in variety Punjab 91 and C141, in the selection of best genotypes of chickpea.

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GLOSSARY OF TERMS AND ABBREVIATIONS

anaphase	stage of mitosis or meiosis at which chromosomes of a homologous pair or chromatids of a chromosome separate and move towards opposite poles of a dividing cell
breeding	genetic modifications of living organisms
cleistogamy	pollination and fertilization occur in an unopened flower
chromatid	one of two identical sister strands of a replicated chromosome held together by a centromere
chromosome	structural unit in the nucleus that carries genes in a linear order
DMRT	Duncan's new multiple range test, used for specific treatment comparisons; it permits to make decision as to which differences are significant and which are not
enzyme	one of a group of proteins produced by living cells which act as a catalyst in specific biochemical reactions
family	group of individuals directly related to a common ancestor
genotype	the sum-total of the genes affecting the expression of a character; also employ for a variety
heritability	portion of the phenotypic variation among individuals that is due to genetic differences among them
metaphase	stage of mitosis or meiosis at which the chromosomes or homologous pair are arranged in a linear manner at the centre of the cell, immediately before the chromatids or homologous chromosomes separate and pass to the two poles during anaphase
mitosis	process by which the nucleus of a cell is divided into two genetically identical nuclei
mutation	heritable variation in a gene or in chromosome structure
pH	a term used to describe the acidity or alkalinity of a system
phenotype	a sum-total of the expression of a character
population	community of individuals with a common origin
polygenic	controlled by a number of genes

probability	likelihood that an event will or will not occur
protein	a high molecular weight compound composed of a range of amino acids; they are the product of genes
variation	differences among individuals due to differences in their genetic constitution, their response to the environment, genotypic-environment interaction and experimental error

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INTRODUCTION

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Mutation breeding is now widely used for inducing genetic changes and creation of new genetic resources, particularly in crops that are not easily amenable to improvement through conventional techniques (Awan, 1991). It is well established that mutagenic agents are effective for inducing genetical changes in treated population (Kasim et al., 1977; Shakoor et al., 1978 a and b; Kalia and Gupta, 1988; 1989). Radio sensitivity of a plant is influenced by biological, environmental and radiological factors (Gunckel and Sparrow, 1961).

The extent of genetic variability is more important than the total variability. Since, greater the genetic diversity in the base population, wider would be the scope of selection. However, owing to the cleistogamic nature of the flower in chickpea (*Cicer arietinum* L), there is a little availability of the basic germ plasm for crop improvement.

The frequency and severity of induced genetic changes depend on the dose applied as well as age and type of tissues irradiated. On exposure to ionizing radiation a plant may die, or may show inhibited or stimulated growth pattern, which may exhibit morphogenetic abnormalities or accelerate differentiation of cells.

Inheritance is a conservative process and is responsible for continuity between generations through a remarkably precise replication of chromosomes and genes. The inheritance of important economic traits such as yield, quality, adaptation, pest and stress resistance, upon which rests much of the future of plant improvement can be understood through the analysis of a wide range of induced mutations. These manifestations are usually due to a) chromosomal rearrangements or b) gene mutation or c) both (Gustaffson, 1947 and Gaul, 1965).

Certain features are commonly seen in irradiated plants, which in themselves may not be useful unless combined with other useful traits. Notwithstanding, little natural variability in chickpea for conspicuous morphological and physiological characters, several workers have attempted for induction of mutation using either physical or chemical mutagens for evolving new genotypes. (Bravo, 1988; Hassan and Khan, 1991 a and b; Shamsuzzaman and Shaikh, 1991; Kharkwal, 1983; Kharkwal et al., 1988; Haq et al., 1988; Haq et al., 1989).

Radiation, therefore, appears to be a useful tool in plant breeding and genetics. The primary objectives of mutation are to enhance mutation frequency, widen the mutation spectrum and realize directed mutagenesis. Practical application of mutagenesis rests largely on the efficiency and effectiveness of the mutagenic agent (Singh, 1988 a). The frequency of mutant recovery depends on the methods both induction and selection. To obtain desirable mutations at maximal rates, choice of the best mutagen and optimal dose are evidently of prime consideration.

Mutation breeding has been used in recent years as a valuable supplement to the method of plant breeding in the development of better crop cultivars. Seedling studies are considered as early manifestation of various mutagenic treatments. These early studies are used to manipulate the particular dose of gamma irradiation used to enhance the breeding program.

Induced mutations have been recognized as an important tool for crop improvement and are believed to have sufficient scope in pulses. Several types of chlorophyll deficiencies and morphological mutants occur in mutagenically treated plant material. These are considered an appropriate measure for determining the effectiveness of a mutagen.

Grain yield is the product of several contributing traits. However, yield could be estimated on the basis of direct and indirect expression of yield components. For any varietal improvement, it is necessary to have a thorough comprehension of the information regarding the inter-relationship of various yield components. Correlation analysis provides the information on the interdependent response either on positive or negative direction of the important plant characters and hence, leads to directional model for the yield.

Gamma radiation in combination with other chemical mutagens is applied for widening the frequency and mutation spectrum for extra genetic variability. In experimental mutagenesis, mutagenic efficiency or the ratio of gene mutation to physiological damage is of both theoretical and practical importance (Gaul et al., 1972). The radiation interferes with a number of biochemical processes especially those concerned with protein and nucleic acid synthesis. Gamma radiation damages the biological system by producing H_2O_2 and organic peroxy radicals. Catalase and peroxidase are internal mechanisms for the removal of these radicals. Effect of gamma radiation is changed with the radio protective effect of gibberellic

acid. It has been established that the impaired growth due to gamma irradiation can be restored by exogenous application of gibberellic acid. Gibberellic acid serves manifold growth related functions in plants by enhancing replication, transcription and different enzymatic systems. (Callebaut et al., 1980; Uppal and Maherchandani, 1988; Zhebrak, 1989; Ali and Ansari, 1989; Arora et al., 1989).

Van-Rheenan et al. (1993) determined that chickpea has shown little polymorphism in isozyme and restriction fragment length polymorphism (RFLP), which may be an indication of limited genetic variability. For obtaining a wide genetic spectrum in chickpea, studies for a suitable radiation dose is of primary importance. High doses produce drastic effects, which may lead to death of the organism. For breeding purposes, mutagenic treatments with low physiological and strong genetic effects are desirable. Gibberellic acid treatment, does not interfere with gene mutation and restore growth by reducing physiological damage caused by gamma irradiation. It brightens the scope for increasing both the frequency and spectrum of mutation. But how far a specific genotype modulates radio sensitivity is a matter of systematic research. It was, therefore, planned to manipulate the mutation response along with cytogenetical and biochemical studies in three commercial genotypes of chickpea (*Cicer arietinum* L.). The main objective of the studies were to determine the effectiveness of gamma irradiation and efficiency of gibberellic acid to modulate the radio sensitivity for:

i. Cytogenetical effects like:
   a. Mitotic index.
   b. Chromosomal abnormalities.
   c. Nucleolar size and structure.
ii. Modulate the biological effects of gamma irradiation with gibberellic acid for:
   a. Germination
   b. Seedling growth
   c. Determination of protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents in chickpea seedlings.
   d. Changes induced in the activities of catalase, peroxidase and indole-3-acetic acid (IAA) oxidase enzymes.
iii. Evaluate optimal dose of gamma irradiation for inducing useful mutations.
iv. Study the effects of treatments on biological responses in M_1 generation.
v. Study the effects of treatments on mutation frequency and spectrum in M_2 generation.

vi. Manipulate the genetic variations in M_2 and M_3 generations.

vii. Estimation of heritability and genetic advance.

viii. Unidirectional and alternate pathway influences on grain yield and yield components.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The information on the subject is not lacking but the inferences of various investigations are not consistent and differ greatly according to the materials used. However, the results of studies having some relevance to the subject are reviewed here briefly.

I: GENETIC EFFECTS OF IONIZING RADIATION

The chromosomes are composed of deoxyribonucleic acid and basic proteins known as 'histones' which are rich in lysine and arginine. Growth and differentiation of a plant depend upon their precise replication and transcription, respectively. The integrity of the chromosomes is very essential for their proper function and ultimately the survival of the cell or organism. Gamma irradiation is known to interfere with a number of biochemical processes, especially those concerned with protein and nucleic acid synthesis.

The genetic effects of ionizing radiation have been established primarily through the work of H.J. Muller (1928), Altenberg (1928) and Stadler (1928). Genetic effects of gamma irradiation had been studied by Haber et al. (1961), Haber and Foard (1964) and Kuzin et al. (1981). They found that gamma irradiation inhibited the synthesis of DNA and consequently cell division. The inhibition of DNA synthesis resulted in the delay or altogether inhibition of mitotic cycle. This delay in mitosis as a result of gamma irradiation could be attributed to two distinct pathways (Rowley et al., 1992). They determined that gamma irradiation induced mitotic delay requires functional weel protein kinase but does not seem to involve cdc25 pathway. Mitotic delay in respect to DNA damage is thus distinct from the delay induced by inhibition of DNA synthesis, which involves cdc25 but is not dependent on weel.

Gamma irradiation is also known to cause breakage and depolymerization of DNA (Oleinick et al., 1994). DNA protein cross link (dpc) formation and induction of double stranded breaks occur at specific sites in the chromatin by gamma irradiation. Xue et al. (1994) investigated the influence of chromatin proteins on the induction of DNA double stranded breaks (dsb) and DNA protein crosslinks (dpc) by gamma irradiation. By gradual removal of low molecular weight nonhistone proteins and classes of histone proteins with

increasing concentration of NaCl resulted in gradual increase of double stranded breaks. Datta et al. (1993) studied that ionizing radiation inhibited growth and DNA repair. They demonstrated that exposure to ionizing radiation is associated with a dose dependant decrease in histone H1 gene expression.

Bagi and Hidvegi (1983) noticed decrease of H1 histone and changes of chromatin structure and transcription in pea seedlings after gamma irradiation. Their data suggested that irradiation of either seeds or seedlings resulted in loosening of the seedling chromatin structure. This specific degradation or dissociation of histone H1 localized in the internucleosomal region may be responsible for the easier accessibility of chromatin to DNAase II after irradiation.

Now it is becoming quite clear that gamma irradiation not only affect the synthesis and structure of DNA, however its effects on biological system are manifold. Synthesis of adenine-7-oxide, a radiolytic product was determined by an aqueous irradiation of adenine. The noncovalent binding of this radiolytic product with cysteine has been confirmed (Yamamoto, 1980). These results support an interaction structure greater than or equal to N-O...H-S-, for non-covalent binding, which may be applied to biological system as a radiation induced damage. Izvorska and Bak "Rdzhieva (1975) as a consequence to gamma irradiation determined depletion in protein and RNA content. There was a delay of mitotic cycle, ribonuclease activity, DNA content of embryo axis and cotyledons and RNA content of cotyledons as an effect of gamma irradiation in chickpea seedlings (Khanna, 1988). Damayanti and Sharma (1990) studied that depletion of protein increased with an increase of gamma irradiation dose in pea.

I: CHROMOSOMAL ABERRATIONS

Chromosomal changes resulting from radiation have been of particular interest to cytologists. Differences in both quantity and kind of chromosomal aberrations provide excellent data for the study of differential sensitivity and variation in chromosome organization. These chromosomal aberrations give direct evidence of the mutagenic efficacy of radiation.

Rahman and Mia (1970) studied various types of chromosomal abnormalities in *Corchorus capsularis* due to gamma irradiation. The abnormalities observed in mitosis were bridges and breakages at anaphase. Chromosomal aberrations in mitotic system as influenced by gamma rays in *Pisum* were determined by Kalloo (1972). Analysis of root tip cells revealed that at anaphase, single bridge, double bridge, bridges with fragments and lagging of chromosomes were observed.

Mujeeb and Greig (1973) developed seedling performances and cytological criteria for determining radio sensitivity in *Pisum*. They studied that in root tip cells, the proportion of abnormal anaphases increased significantly with higher doses of gamma irradiation. Fragments decreased with increasing doses, while bridges and bridges plus fragments increased.

A higher rate of chromosomal aberration was also observed in mitosis with gamma irradiation in *Pisum sativum* L. by Vo-Hung (1989). Khanna (1990) observed various types of chromosomal abnormalities during mitosis in bread wheat and durum wheat due to gamma irradiation. Lagging of chromosomes, bridges and chromatin stickiness was observed in mitosis.

Chromosomal aberrations in root tip cells of pea as an effect of gamma irradiation were determined by Tan et al. (1990 and 1991). Rukmanski and Rodridges (1990) reported a parallel response between the dose rate of gamma irradiation and structural rearrangement of chromosomes during the first mitosis in root tip cells.

Chromosome breakage, dicentric chromosomes and ring chromosomes in root tip cells were observed with higher doses of gamma irradiation in *Secale cereale* L. by Savaskan and Toker (1991). Vandana (1993) studied mitotic anomalies in *Vicia faba* with the mutagenic treatment of EMS and DES. The anomalies were chromosomal fragmentation, chromatic bridges, laggards, fragments and unequal distribution at anaphase. He observed that the percentage of mitotic anomalies at various stages was directly correlated to the amount of mutagen used.

II: NUCLEOLUS STRUCTURE

The nucleolus structure is an intranuclear organelle which has the important function of producing the vast bulk (80-90%) of cellular RNA and probably serves as a site of organization of ribonucleo-proteins which are subsequently transported to the remainder of the nucleus and to the cytoplasm (Busch and Smetana, 1970). Swift (1963) by using enzymatic digestion and radioautography has shown the presence of ribonucleoproteins and deoxy ribonucleo-proteins in the nucleolus. Under the electron microscope it appears to be a large granular mass like structure, containing DNA chromatin and ribonucleo-protein particles as well as other structural components (Ashraf and Sharif, 1990; Ashraf et.al., 1992). These constitute three components generally in every nucleolus, dark fibrillar region, pale fibrillar region and granular regions (Bernhard, 1969; Chouinard, 1971; Lord and Lafontaine, 1973; Lafotaine and Lord, 1974; Chouinard, 1975).

The nucleolus is believed to serve as a cytological indicator of structural and molecular changes that take place normally in response to various stresses. Nucleolar volume appears to be regulated by many factors. The fine structure and size of the nucleolus are very sensitive to changes in ribosomal synthesis (Knowland and Miller, 1970).

II: PEROXIDASE ENZYMES SYSTEM

Plant exposure to gamma irradiation bring about changes in biochemical activity through various metabolites produced as a function of gamma irradiation. The most important of these metabolites are peroxy radicals. The accumulation of organic peroxides and oxidation of membrane lipid place a stress on cellular activity (Mead, 1976). In plants the physiological effects of gamma irradiation were recognized by Motoji and Sergio (1989) and Voisine et al. (1991) caused the production of free peroxy radicals through the peroxidation of unsaturated fatty acids. Mutagenic potential of thymine hydroperoxides was reported in 1976 by Thomas et al., when it was found that addition of ThyOOH produced mutations in transforming DNA. Previously in 1974 Teole et al. reported that gamma irradiation of DNA in aqueous aerated solution gave rise to pyrimidine hydroperoxide and thymine hydroperoxides. Formation of hydroperoxide has been observed in *Micrococcus radiodurns*, after exposure to gamma irradiation (Harihorn and Cerutti, 1972).

These hydroperoxides interact with transition metal ions forming free radicals, which react with and modify neighboring or other bases of cell genome. These base damages were being introduced by the combine action of hydroxyl radicals and superoxide anion radicals. In addition to this the nucleotide damage was introduced into DNA by an enzymatic super oxide generating system (Feldberg and Carew, 1981). In 1981 Sokolov et al. confirmed that gamma radiation were effective in producing stable macroradicals in DNA.

Enzymes which are reported to counteract the peroxidation damage to lipid or DNA include catalase and peroxidases. Peroxidase is also known to exhibit indole-3-acetic acid (IAA) oxidase activity (Shinshi and Noguchi, 1975).

I: CATALASE (1.11.1.6)

Catalase is widely distributed in nature. It is found in all aerobic organisms, in plant and animal cells. It has long been considered to be the major enzyme responsible for reducing the H_2O_2 (Donny et al., 1976). Low levels of gamma irradiation have been shown to influence the composition of multiple forms of catalase (Romodonova and Lvova, 1993) and specific changes in catalase activity (Stajkov et al., 1985). Catalase is enzyme showing unusual features and has received increased attention owing to its role in oxidative metabolism (e.g. peroxidation of methonol, formic acid and phenol: $ROOH + AH_2 \xrightarrow{\text{Catalase}} H_2O + ROH + A$) as well as its protective function by acting as a H_2O_2 scavenger = $2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O+O_2$.

II: PEROXIDASE (1.11.1.7)

Peroxidase (donor:H_2O_2 oxidoreductase) is a nonspecific enzyme known to oxidize substrates as phenols, aromatic amines, cytochrome C and indole-3-acetic acid (IAA). The general equation for peroxidase activity is:

$$H_2O_2 + DH_2 \xrightarrow{\text{Peroxidase}} 2H_2O + D$$

Increase in peroxidase activity was determined to be one of the biochemical changes involved with low doses of gamma irradiation (Croci et al., 1987 and 1991) and specific changes in peroxidase activity (Stajkov et al., 1985). Peroxidase is known to be involved in growth and cellular differentiation (Arnison and Boll, 1976, Huystee and Cairns, 1982) and an inverse relationship had been established between the growth rate and peroxidase activity in dwarf plants (Galston and Davies, 1969; Birecka and Galston, 1970; Gardiner and Cleland,

1974). It was suggested that H_2O_2 is probably the most important of the peroxide involved in radiation induced lethality. The radiation protective effect of peroxidase is due to the removal of H_2O_2, other peroxides and especially lipid hydroperoxides. This accounts for the greater effectiveness of peroxidase than catalase (Croute et al., 1982). Khanna and Maherchandani (1981) observed stimulated growth and an increase in peroxidase activity at low doses of gamma irradiation in chickpea. Since gamma radiation can influence the isozymatic composition of peroxidase, as demonstrated by various authors in other species (Endo, 1976; Ogawa and Uritani, 1970; Shen et al., 1991). The study of peroxidase activity will contribute to as understanding of the mechanism involved in radiation induced inhibition of plant growth. The peroxidase isozyme pattern was related to the damage caused by irradiation (Shen et al., 1991). Gamma irradiation of the callus cultures of *Datura innoxia* resulted in stimulation of peroxidase activity and particularly increased the peroxidase enzymes with high electrophoretic mobilities (Jain et al., 1990).

III: INDOLE-3-ACETIC ACID (IAA) OXIDASE

The ability of peroxidase to catalyse the oxidation of indole-3-acetic acid (IAA) has been of particular interest. It is generally accepted that peroxidase is responsible for some IAA oxidase activity, but it has not yet been clearly established whether all such activity can be ascribed to peroxidase (Shinshi and Noguchi, 1975). According to the litreature some isoperoxidases are able to oxidize IAA in the presence or in the absence of H_2O_2 (Lee, 1974; Stonier et al., 1979; Klisurska and Dencheva, 1983; Rokicka, 1980; and 1985). IAA oxidase activity is thought to be connected with IAA metabolism and regulation of plant growth. A relatively simple action to explain the inhibitory effects of various phenolics on plant growth, is the activation of IAA oxidase. The oxidative decarboxylation of indole-3-acetic acid by the enzyme require a phenolic cofactor as an oxidant of the metallic oxidizer Mn_2+; the net effect of these phenolic would presumably a depression of growth. If the action of a growth inhibitor were simply this, one would hope that its addition would restore growth when auxin concentration were excessive but it does not indicate a simple relationship (Machackova et al., 1975).

III: SEEDLING STUDIES

Important parameters of mutagen sensitivity are their effects on germination and seedling growth because these are the direct manifestations of biological effects of the mutagen used. The biological effects on seedling growth provide the criteria of optimal doses for inducing useful mutations. The determination of an efficient radiation dose for obtaining maximum number of useful mutations is of utmost importance in a breeding program. The differences in radio sensitivity among different varieties and various crops have been reported in different studies.

Effect of different doses of gamma radiation on two species of *Corchorus* was reported by Rahman and Mia (1970). A differential response of two species towards radio sensitivity for germination and seedling height was studied. Germination and seedling height were decreased with an increase of radiation dose. In soybean effect on germination was irregular, while at lower dose stimulation for seedling height was observed (Vasti and Keerio, 1974).

To assess the effects of ionizing radiation on *Cicer arietinum*, Rajput (1977) irradiated the seeds at various levels of gamma rays. Trends towards seedling emergence were erratic, but generally the emergence was depressed by radiation treatment. Aslam and Siddiqui (1979) found a significant differential varietal response towards gamma irradiation for germination in four varieties of *Pennisetum americanum*. The radiation doses showed significant negative correlation with germination in all the four varieties.

Khanna (1981) reported radiation effect on morphological characters in three varieties of chickpea with different seed coat colors. He studied that there was a progressive reduction in germination percentage with an increase of radiation dose. Bhatnagar (1984) studied the combined effect of physical and chemical mutagens in kabuli chickpea. Seed germination was reduced and the negative effects were more pronounced at higher doses.

Effect of physical mutagens, gamma radiation and fast neutrons were studied in three varieties of soyabean by Hassan et al. (1985). Seedling growth was found to decrease with increasing radiation doses. The effects on germination were observed only at higher doses.

Among early assessable M_1 parents for radio sensitivity, epicotyl length proved to be most sensitive, and hence most useful.

Stajkov et al. (1985) investigated the mechanism of stimulating effects of gamma irradiation on peas. Results showed that gamma irradiation enhanced the level and activity of indolyl auxines and gibberellins and also raise the contents of crude proteins and essential amino acids. Sinha and Chaudhry (1987) determine the proper dose of gamma irradiation, and its effect on M_1 generation in three lentil genotypes. Genotypic variation in sensitivity was detected. Plant characteristics like germination, seedling height, root and shoot length were significantly decreased with an increase of radiation dose.

Rao (1988) studied that low doses of gamma radiation increased, while higher doses decreased the seedling height in chickpea. Asghar and Khan (1988) studied the effect of irradiation on germination and growth of *Sorghum bicolor*. They found that seedling germinability in the laboratory was not generally affected by irradiation treatments. However, seedling height decreased measurably with increasing treatments of irradiation.

Sarkar and Sharma (1989) reported effects of gamma radiation on lentil. The reduction in germination percentage was positively correlated with dose. Mahto et al. (1989) evaluated the biological effects of gamma rays on chickpea. They found that germination percentage was not affected with irradiation at I5 Kr, but decreased progressively with further increase in irradiation dose.

Radio sensitivity in common bean was evaluated by Colaco et al. (1995). They found that shoot and root length was significantly reduced at higher doses of gamma irradiation in relation to control. Ahmad and Godward (1990) studied the radio sensitivity in *Cicer arietinum*. They determined that at lower doses up to 20 Kr there was no reduction in germination. However, at higher doses there was a consistent reduction in germination with increasing dose.

Khan et el. (1990) evaluated the effects of irradiation on four pea varieties. Germination was decreased with an increase of radiation dose and maximum reduction in germination was noted at the highest dose. Mehetre et al. (1990) reported continuous decrease in germination in two cultivars of mungbean after gamma irradiation.

Savaskan and Toker (1991) studied that gamma irradiation had no effect on germination in rye. But seedling height and root length was reduced as dose increased. A continuous reduction in germination with increasing dose in lentil was determined by Shaikh and Begum (1990). Eser et al. (1991) investigated the effects of gamma rays in lentil. They found that seedling height and root length decreased with increasing radiation dose and the varietal response to gamma irradiation was also varied.

Hassan and Javed (1991) reported radio sensitivity in six varieties of kabuli chickpea. A differential response of varieties at lower dose of gamma radiation for germination was observed, while at higher doses a significant reduction in germination was noticed. Gamma radiation caused a significant reduction in seedling height and root length at all the treatments. Gamma irradiation had no appreciable reduction in the number of roots at lower doses whereas a marked reduction occurred with an increase in radiation dose.

Tripathi and Dubey (1992 b) studied the effect of gamma radiation in two lentil varieties. The varieties differed in response regarding germination and seedling growth. Germination was decreased while seedling height was promoted by the application of gamma radiation. Svetleva and Petkova (1992) studied that germination was reduced with gamma irradiation in french bean.

Haq et al. (1992) studied the mutagenic sensitivity in three kabuli chickpea genotypes. At lower doses of gamma irradiation, stimulatory effect on shoot and root length was observed in all the genotypes, but adversely affected at higher doses of gamma irradiation. Germination was not affected up to 60 Kr but from 70-110 Kr germination decreased with increasing dose. They suggested that three gamma irradiation doses (40, 50, 60 Kr) which cause reduction in shoot and root length around 20-40% could be chosen for larger scale mutagenic treatments.

Veeresh et al. (1995) demonstrated the effects of gamma radiation in winged bean. The results showed that there was more reduction in germination, shoot length and root length at higher doses as compared to lower doses. Mehetre and Mahajan (1996) reported the effects of different doses of gamma rays on germination in soyabean. Seed germination was reduced with increasing the irradiation dosage. They also found that the varieties varied in their response to irradiation.

Rabie et al. (1996) studied the effect of gamma radiation on germination and seedling height in faba bean. They reported that gamma irradiation at lower doses increased seed germination and seedling height as compared with control. Mallick et al. (1997) found the effect of gamma radiation on germination in green gram. They studied that the seed germination was decreased with an increase in radiation dose. A differential response of varieties towards irradiation was also reported.

IV: MUTATION STUDIES IN M_1 GENERATION

Chickpea (*Cicer arietinum* L.) is an autogamous plant, which result in lack of sufficient genetic variability in germplasm. Induced mutation can be employed as an additional tool for creation of additional genetic variability. Gamma radiation is intensively used in various crops to widen their genetic spectrum. Treatment with gamma irradiation alters the physiology of plants. Plants show differential response towards different gamma irradiation doses in different characters. Various studies with gamma irradiation had been conducted on different crops to indicate their response to gamma radiation. Some of these reports are reviewed here:

Rajput (1977) studied the effects of gamma radiation on yield attributes in five varieties of *Cicer arietinum* in M_1 generation. Pods and yield per plant were significantly reduced at higher doses. Irregular response to radiation treatments was observed for 100-grain weight. However, mostly seed size was reduced in treated populations. In addition numerous morphological abnormalities and chlorophyll deficient chimeras were also observed.

Kasim et al. (1977) reported the effectiveness of gamma radiation for the induction of quantitative variation in three broad bean varieties. They found that all varieties exhibited earliness of flowering and increase in stem length in M_1 population, although response to different doses was different in the three varieties.

Khanna (1981) reported radiation effects on morphological characters and yield parameters in three chickpea varieties having different seed coat colors. Number of pods per plant, 100-seed weight and grain yield per plant was reduced at higher doses. For number of fruiting branches the cultivars responded differently and plant height was reduced only by the

highest dose. Only the 100-seed weight gave consistent positive response to the lower rates of irradiation (5 and 10 Kr). The number of primary branches and the number of seeds per pod were remained unaffected.

Aslam et al. (1985) studied the effect of gamma rays on growth and seed index in four varieties of *Pennisetum americanum*. Varieties responded significantly different for plant height to gamma radiation. Significant, decrease in plant height was observed in Cv. Awn selected, where as in Cv. Ex-Bornu plant height significantly increases in the irradiated population. Genotypic differences as well as the differences due to various radiation treatments were highly significant with respect to seed index. Hassan (1986) studied highly significant effects of radiation doses on two mungbean varieties. Magnitude of variation was different in both the varieties. Highest treatment of gamma radiation induced wide variations towards negative direction for pods per plant and seeds per pod, except for 100 seed weight. Increased number of pods per plant and seeds per pod were generally obtained in the dose range of 10 to 40 Kr.

Sinha and Chaudhry (1987) determined that yield characters, number of branches per plant, number of pods per plant and seed yield per plant were significantly decreased in M_1 population of lentil following gamma irradiation. Hassan et al. (1988) reported that higher doses of gamma radiation in wheat decreased plant height, tillers per plant, number of spikelets per spike and delay in time to maturity. Rao (1988) investigated that low doses of gamma radiation increased, while higher doses decreased the plant height and yield attributes in chickpea in M_1 and M_2 generation.

Din et al. (1988) observed significant inhibitory effects on yield characters in three varieties of sunflower following gamma irradiation. Significant decrease for 1000-grain weight and grain yield per plant was noticed. Maximum inhibitory effects of radiation were noticed at highest dose. Mahto et al. (1989) observed in two varieties of chickpea that irradiation delayed flowering, maturity and reduce growth. Number of pods, seed yield and seeds per plant were also decreased.

Kumar and Sinha (1989) reported the differential response of two varieties of *Canjanus cajan* following gamma irradiation. It was found that plant attributes like plant height, number of primary branches, tillers per plant, pods and seeds per plant showed

decreasing trend with an increase in dosage in M_1 generation. Khan et al. (1990) evaluated the effect of gamma irradiation on morphological characters in pea. The radiation adversely affected plant height, number of pods and flowering. The highest dose of 25 Kr had the maximum effect on the above characters. Mehetre et al. (1990) studied the morphological attributes in M_1 generation following gamma irradiation in mungbean. Plant height and number of pods per plant decreased significantly in both varieties, while the number of branches per plant behave differently to gamma radiation in two varieties. Hassan et al. (1990) studied variability induced through gamma radiation in two varieties of mungbean. All the doses of radiation caused a significant reduction in grains per pod. The number of pods per plant had shown an obvious increase at lower doses, while the higher doses had produced categorically inhibitory effects.

Svetleva and Dimeva (1991) reported adverse effect of gamma radiation in *Phaseolus vulgaris*. Number of pods per plant, grain per plant were decreased as compared to control. Eser et al. (1991) reported a significant decrease in yield characters following gamma irradiation in lentil. Varying response of varieties was noted towards the treatment. Number of pods and seeds and plant height were decreased with increasing radiation dose. Statistical analysis showed that differences due to doses were significant.

Yousaf et al. (1991) investigated that gamma irradiation in lentil produced highly significant differences for maturity and number of seeds per pod. While non-significant inhibitory effects on flowering, plant height, secondary branches per plant, pods per plant, seed yield per plant, harvest index and 1000 grain weight were noticed. Svetleva and Petkova (1992) reported adverse effects of gamma irradiation in French bean. Gamma irradiation prolonged the growth period and retarded the plant height.

Tripathi and Dubey (1992 b) studied effect of gamma irradiation in two lentil varieties. The varieties differed in response regarding plant height and seeds per pod, but had a similar depressive response for branching, maturity, pods per plant, seeds per plant and seed yield. Amjad et al. (1993) studied the variability for growth characters in M_1 generation, following gamma irradiation in pea. Higher radiation doses resulted in increase in plant height, number of branches, biological yield and extended vegetative period as compared to control.

Kumar et al. (1993) reported reduction in plant height, number of branches, pods, seeds per pod and delay in maturity in fababean, following gamma irradiation in M_1 population. John (1995) studied the effect of gamma irradiation on grain yield in black gram. There was a highly significant negative relationship between seed yield and the gamma ray doses. All the genotypes responded similarly towards the radiosensitivity for grain yield.

Sarkar et al. (1996) reported the effects of gamma rays on yield characters in mungbean. They found that plant height showed a linear reduction with increasing gamma ray dosage. Pods per plant increased with increased dosage, while the seeds per pod showed a corresponding decrease. On the whole, yield of seed per plant was approximately equal to that of control except that at the 30 Kr treatment, where yield surpassed that of control. Plants subjected to this dosage exhibited more pods per plant but loss seeds per pod. Dosages above 40 Kr caused deleterious effects on these characters.

V: GENETIC VARIABILITY STUDIES

Crop improvement by conventional plant breeding depends to a large extent, on the amount and levels of genetic variability found in gene pool. Mutation induction is now a proven mean of creating or increasing genetic variability that differs from the kind obtainable through gene recombination, existing genes can be changed into different alleles, linkage groups can be separated, single gene or gene groups can change their position or can be eliminated. Different mutagens are used to understand the nature and magnitude of induced variation under different doses for various polygenic traits. Some of the studies on creation of genetic variability with mutagens in different crops is reviewed here:

Kasim et al. (1977) tested the effectiveness of gamma radiation for induction of quantitative variation in three varieties of broad bean. Analysis of M_2 generation revealed that there was no relationship between the magnitude of irradiation and the amount of induced genetic variance. The ranges of predicted heritability estimates for stem length and days to flowering also varied at different doses in the three varieties.

Shakoor et al. (1978 a) a studied the induced variation due to gamma irradiation in three cultivars of mung bean (*Vigna radiata* (L) Wilczek). They found that irradiation exposure generally had a depressing effect for plant height, number of pods per plant, number of grains per pod, pod length and yield per plant, number of grains per pod, pod length and

yield per plant. The magnitude of broadsense heritability estimates for plant height appeared to be related to the radiation exposure and was usually of a high order indicating the possibility of effective selection of M_2 generation.

Varietal differences regarding effect of irradiation in mungbean on plant height and pod number were studied in 1978 b by Shakoor et al. They also observed that the plant height, number of pods and grain yield per plant decreased in the M_2 population as compared to control. They found that there was a little relationship between the level of irradiation exposure and the magnitude of genetic variability in all the varieties.

Larik et al. (1980) critically examined the effects of various doses of gamma irradiation and fast neutrons in six cultivars of bread wheat for different quantitative characters i.e., spike length, spikelets per spike, grains per spike and grain yield. They found that genotypes varied significantly for all the characters. Irradiation treatments were instrumental in creating significant variability for all the characters, indicating that the varieties did not perform uniformly across different gamma rays. In M_2 generation there was a considerable increase in the variance for all the four metrical traits. Mutagen treatments shifted the mean values mostly towards the negative direction, but the shift was neither unidirectional nor equally effective for all the characters. They further observed that the estimates of genetic variability and heritability (b.s) increased with increasing doses of gamma rays. Genetic advance also exhibited a similar trend.

Majid et al. (1982) studied the genetic variability and correlation in 40 germ plasm of black gram and obtained highest correlation value between seed yield and number of pods per plant (0.827). Coefficient of genotypic variation ranged from 6.02% for days to maturity to 44.09% for number of pods per plant. Heritability was higher for number of pods per plant. Maximum genetic advance was obtained for number of pods per plant followed by 500-seed weight and seed yield per plant.

Khan et al. (1983) reported the data on 30 strains of gram (*Cicer arietinum* L) including black, brown and white type for estimation of heritability and correlations. They observed that number of seeds per pod had lowest heritability of 30.62%, while rest of the six characters including seed yield had moderate to high heritability values ranging from 44.49% for number of secondary branches to95.55% for number of pods per plant. Seed yield per

plant had significant positive correlation with plant height, number of primary branches and secondary branches number of pods per plant, 1000-seed weight and number of seeds per pod. The association between primary branches, secondary branches and number of pods per plant was also positive. The direct effect of secondary branches on yield was strong.

Khan (1984) studied induced variability in quantitative characters of mungbean after treatment with gamma rays in M_1, M_2 and M_3 generations. He reported that variability increased in almost all the characters in M_1 generation. The mean number of seeds per pod and 100-seed weight decreased while plant yield did not show any particular trend. There was an increase in mean value after gamma irradiation in M_2 generation and the maximum value obtained in M_3 generation. The estimates of heritability were higher for 100-seed weight followed by plant yield and seeds per pod in M_3 generation. Coefficient of genotypic variation and genetic advance were higher for plant yield in M_2 and M_3 generation. Generally, genotypic coefficient of variation, heritability and genetic advance observed more in M_3 as compared with M_2 indicating that significant gain could be possibly achieved through selection in M_3 generation.

Malik et al. (1987 b) studied the correlation and path coefficient analysis in 40 elite genotypes of *Vigna radiata*. Yield was significantly and positively correlated with plant height, primary branches per plant, pods per plant and biological yield. Path analysis revealed that plant height and biological yield had highest direct positive effect on seed yield per plant. The direct effect of days to flowering, days to maturity and seeds per pod on seed yield was high and negative.

Ramana and Singh (1987) studied the yield and yield related characters in 37 *Vigna radiata* varieties. Number of pods with relatively high values for heritability and genotypic coefficient of variation and significant positive correlation with yield are recommended as selection criterion for the improvement of yield.

Wadud and Yaqoob (1988) studied the phenotypic and genotypic correlation of some important characters in chickpea. The results revealed that the magnitude of GCV were higher than PCV for all the characters. The grain yield showed highly significant and positive association with number of branches and number of pods per plant. A negative and significant association was only observed between grain yield and days to maturity.

Rao et al. (1988) characterized the induced polygenic variability in pigeon pea with gamma radiation. Increase in means and estimates of genotypic coefficient of variation, heritability and genetic advance were observed for plant height, pods bearing branches, seed yield per plant and earliness in M_2 generation.

Vadher et al. (1988) reported earliness and reduction in plant height in M_2 generation of sorghum. Malik et al. (1988 b) irradiated the seeds of three local cultivars of mungbean. The results of M_4 generation revealed that seed yield was positively and significantly correlated with number of pods and branches in all the treatments.

Kumar and Sinha (1989) reported that gamma radiation induced genetic variability in M_2 population of *Cajanus cajan* and *Moghania* for yield characters. It was reported that these characters reach towards normal values at lower doses while at higher doses the effects were still present. Khan et al. (1989) studied the radiation induced variation of some genetic parameters in *Sorghum* cultivars in M_2 generation. They found highly significant difference in mean values due to cultivars for tillers per plant and days to 50% flowering except plant height for which the mean value were non-significant. The maximum increase was computed at the highest dose (45 Kr) for tillers per plant and plant height in both the cultivars. The delay in 50% flowering was observed at the highest dose.

Illhamuddin et al. (1989) studied 22 mungbean cultivars for 8 quantitative characters. They reported that the cultivars differed significantly for all the characters studied. Higher genoypic and phenotypic variance, heritability and genetic advance were recorded for plant height and 1000-seed weight, which indicated the additive gene effect on these characters.

Miah and Bhadra (1989) reported higher estimates of phenotypic coefficient of variability than the corresponding genotypic coefficient of variability for all the characters studied. High broadsense heritability with high genetic advance was observed for days to flowering, pods per plant, plant height and seeds per pod. Number of branches, seeds per pod and 1000-seed weight showed high heritability but low genetic advance suggested non-additive gene effect. They further reported that most of the characters were highly influenced by environment as the environmental variance was high than their corresponding genetic part.

Kalia and Gupta (1989) studied the induced polygenic variation in lentils. Sufficient genetic variability due to gamma radiation was induced in seed yield, biological yield, harvest

index, number of pods per plant, 100-seed weight, plant height, time to 50% flowering and days to maturity of polygenic traits.

Sinha and Bharati (1990) studied the effect of gamma radiation in urdbean with a view to enlarge the genetic base. In M_4 yield characters were studied and maximum mean value for pods per plant was observed. Coefficient of variability followed the same pattern as that of characters, for pods per plant and pod length. Heritability (b.s) was 79% for plant height and minimum 41% for seeds per pod.

Kamala (1990) studied the gamma ray effects on polygenically controlled characters in sesame. They noted highest levels of genotypic and phenotypic variance for seed yield followed by capsule per plant, branches per plant, plant height and seeds per pod. 1000-seed weight had low value. High levels of heritability and expected genetic advance were found for branches per plant. 1000-seed weight and seed yield in M_2 and M_3 generation.

Ghafoor et al. (1990) reported from correlation and path coefficient studies in 48 local genotypes of mash (*Vigna mungo* L) that days to maturity, plant height, branches per plant, pods per plant seeds per pod, 100-seed weight, biological yield and harvest index had positive correlation with grain yield. Path analysis revealed that branches per plant, harvest index and biological yield had positive effect on grain yield. Biological yield and harvest index may be exploited in selecting high yield cultivars in mash bean improvement.

Yaqoob et al. (1990) studied 12 genotypes of chickpea for estimation of genootypic and phenotypic correlation coefficients for some important traits. The results indicated that grain yield was positively correlated with number of branches, number of pods, plant height and 100 gain weight. While relation of grain yield with days to maturity were found negative. The genotypic coefficients of variation were higher in magnitude than their respective phenotypic coefficient for number of pod, plant height and 1000 grain weight.

Ramani and Jadon (1991) studied induced variability in groundnut in M_2 generation. They recorded that plant height was reduced while flowering was delayed in treated populations. Moderate to high levels of heritability accompanied by high levels of genetic advance was recorded for pod weight per plant, pods per plant and seeds per plant.

Alexieva and Nikolov (1991) studied the radiosensitivity and mutability due to gamma

irradiation in six cultivars of soybean. The cultivars tested manifested differential response to radiosensitivity. Various types of chlorophyll, morphological, physiological and sterile plants were observed.

Bhatnagar (1991) isolated a large number of macro and micromutations with gamma irradiation in M_2 population of chickpea. He observed a high genotypic and phenotypic coefficient of variability as well as heritability with moderate to low genetic advance in different metrical traits. The studies revealed the possibility of isolating desirable mutants for traits such as earliness, tallness, high branching and an increase number of pods per pod. Sarma et al. (1991) studied the effects of gamma radiation in green gram. They observed an enormous genetic variability in M_2 and M_3 generations for various yield characters.

Jayamanne and Jayasuriya (1991) obtained highly divergent mutants in green gram with various doses of gamma radiation. They found a highest genetic variation and genetic advance for 100 seeds weight and also a fairly high genetic variation and genetic advance for number of pods per plant and days to flowering.

Ignacimuthu and Babu (1992) evaluated the induced genetic variation by gamma radiation in yield traits of wild and cultivated urd and mungbean. They found that there was a broad spectrum of induced variability in most of the yield traits. Genetic variance, heritability and genetic advance were high in most yield traits. Their results demonstrated that induced mutations were random, polydirectional and quantitative in nature.

Aslam et al. (1992) estimated the phenotypic and genotypic correlation and path coefficient analysis in 10 genotypes of soybean. It was observed that grain yield had positive and significant genotypic correlation with plant height and 100-seed weight. It was generally observed that phenotypic coefficient of variation were larger than the genotypic coefficient of variation. The genotypic coefficient of variation ranged from 5.99 for days to maturity to 16.15 for pod length Number of pods per plant, 100-seed weight and plant height also indicated high degree of genetic variability with a coefficient of variation 13.73, 8.19 and 8.06% respectively. Heritability estimates varied from 26.20% for grain yield to 84.10% for days to maturity. Path coefficient indicated that plant height had a very high direct path followed by 100-seed weight.

Charumathi et al. (1992) obtained a high amount of variation in black gram, induced

by various doses of gamma radiation in M_2 and M_3 generations for number of branches, number of pods per plant, pod length, seeds per pod, seed yield per plant and 100-seed weight, plant height, days to 50% flowering and days to maturity.

Ignacimuthu and Babu (1993) studied some quantitative characters in M_2 generation of wild and cultivated urd and mungbean for the range of variability and genetic advance. Their results indicated that induced mutations are random, polydirectional and quantitative in nature. They also were about heritable changes in polygenic system. From the pattern of induced variability, it was clear that the threshold action of certain proportion of mutant loci was the basis for phenotypic modifications.

Baruah and Talukdar (1994) studied the variability and pattern of association for yield and other yield contributing physiological traits in micromutants of. green gram. They found high genetic variations for harvest index and biological yield per plant. Among these high heritability coupled with high genetic advance was observed for biological yield per plant which also exhibited a strong association with seed yield per plant. A strong negative association of seed yield per plant with harvest index coupled high genotypic coefficients of variation, heritability and low genetic advance was indicated.

Mehetre et al. (1994) studied the genetic variability, heritability and yield correlation on 7 yield related trails in M_2 and M_3 generation of four gamma irradiated soyabean varieties. The characters viz, plant height, 100-grain weight, pods per plant, branches per plant and grain yield per plant exerted higher variability indicating better scope in genetic improvement. High heritability associated with genetic gain and genotypic coefficient of variation was observed for 100-grain weight, plant height, branches per plant and pods per plant suggesting intensive selection for these characters to achieve increase in yield. On the basis of correlation and path analysis studies, it is revealed that the characters pods per plant, 100-grain weight and branches per plant are most important yield contributing characters and should be given due importance during process of selection.

John (1995) studied the effect of gamma rays on yield in black gram. He found that the grain yield was decreased in M_1 generation while there was a positive shift for the M_2 and M_3 generation. Seed yield increased at higher doses in the M_2 and at lower doses in the M_3 generation. Kumari (1995) studied the effect of gamma radiation in *Vicia sativa* and *Vicia*

hirsuta. The M_1 and M_3 generations were evaluated for 5 yield components. In *V. Sativa* the GKR resulted in an increase in all characters compared to control. While 12 Kr resulted in an increase in shoot length, number of flowers per plant and number of seeds per plant only. In *V. hirsuta*, the 6 and 12 Kr doses resulted in increase in the number of primary branches per plant, number of flowers per plant and number of pods per plant. Higher doses resulted in a decrease in all characters.

Gupta and Sharma (1996) reported the radiation induced variation for yield and its components in horsegram. Seeds of HPK2 and HPK4 horsegram irradiated with 10, 20 and 30 Kr gamma rays showed that change in mean was independent of variety, radiation dose and character in M_3 generation. Increased mean was observed for seed yield per plant, biological yield per plant and pods per plant for both the varieties under 10, 20 and 30 Kr doses. Sufficient genetic variability was observed in both the varieties under 10, 20 and 30 Kr doses for seed yield per plant, biological yield per plant, pods per plant, pod length, seeds per pod and 100- seed weight.

Kumari (1996) reported the induced variability, heritability and genetic advance due to gamma irradiation for yield and 8 yield related attributing characters of two cultivars of fababean in M_2 generation. The mean values shifted bidirectionally for most of the characters studied. Both phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were higher for seeds/plant, seed yield per plant, pods per plant and branches per plant. PCV was, in general, higher than GCV, but the gap between them was narrow. High hiritability coupled with high genetic advance were observed for 7 characters, including seed yield, indicating the presence of additive gene action for these traits.

Dev and Gupta (1997) studied the effects of gamma radiation on two varieties of Kidney bean in M_1, M_2 and M_3 generation. Phenotypic and genotypic coefficient of variability were generally over 20% after radiation treatment for seed yield/ plant, number of seeds / pod and harvest index. Estimates of heritability were high for most tracts in both varieties (24.74 to 97.44%)

Fahmy et al. (1997) studied the effect of gamma rays (7.5, 10 and 15 Kr) on three cultivars of soyabean. The studies were carried out upto M_3 generation. Increasing doses of gamma rays were negatively associated with plant height and days to maturity. Pods/plant

showed little variability among cultivars, generations and treatments except at 15 Kr in the M_3, making it suitable and predictable treatment for isolating mutants in later generations. Branches / plant, seeds / plant and seed weight/ plant varied with dose and generation.

VI: CHLOROPHYLL AND MORPHOLOGICAL MUTATION SPECTRUM IN M_2 GENERATION

Mutation breeding is rapidly becoming a significant method of modern plant breeding. Induced mutations have been recognized as an important tool for crop improvement and are believed to have sufficient scope in pulses. Several types of chlorophyll deficiencies and morphological mutants occur in mutagenically treated plant material. The frequency of occurrence of chlorophyll deficient seedlings and other morphological mutants in the treated population is generally considered an appropriate measure for determining the effectiveness of a mutagen. A review of chlorophyll deficiencies and other morphological mutations reported in different crops is given here:

Athwal et al. (1970) reported that *albina* constituted the largest single category of mutants observed in gamma rays treated M_2 generation of one desi and one Kabuli type of chickpea variety. Nerker and Mote (1978) studied in Bengal gram different type of chlorophyll mutations that were specific to genotypes and a relationship was noted between mutagen dose and the frequency of morphological mutations.

Kalia et al. (1981) treated five improved selections of chickpea with different doses of gamma rays and observed predominant occurrence of *xantha* mutants irrespective of the variety. On the overall basis of these genotypes the mutants can be arranged in the following order; xan*tha-chlorina-viridis-albina.*

Filippetti et al. (1982) with gamma radiation in M_2 generation of *Vicia faba* L. observed *xantha, chlorina* and *chlorotica* chlorophyll mutants. Other variations regarding morphological, short stature, leaf shape, seed coat colour, pod dimension, and semi sterility were noticed. One of the mutants showed greatly enlarged foliage, flowers, stem and pods. The leaves and stipules were two to three times larger than the normal type.

Dixit and Dubey (1983) recorded several plant height and chloromutations, leaf mutants, flower mutants in lentil with gamma irradiation. Kharkwal (1983) reported different types of morphological mutants with gamma irradiation on culinary and desi varieties of chickpea with good genetic make up and yield performance. Dixit and Dubey (1986) studied the chloromutations and morphological mutants in lentil induced by gamma rays. They reported various categories of chlorophyll mutations including *xantha, viridis, alboxantha, viridoxantha, striata* and *tigrina*. A number of seedling mutants were also found like curled apex, stunted, barren apex, and giant seedlings. The chlorophyll mutants produced were ranked according the frequency of occurrence; *xantha> tigrina>viridis>striata>alboxantha> viridoxantha.*

Yadav and Singh (1988) determined that chlorophyll mutation frequency with gamma rays in M_2 generation of mungbean increased in a linear fashion up to medium doses and was erratic at higher doses. The chlorophyll mutation spectrum included *albina, xantha, chlorina, virescence, viridis* and *maculata*.

Singh et al. (1989) reported effect of gamma radiation on type and frequency of chlorophyll mutations in lentil. The spectrum of chlorophyll mutations was quite narrow as only two kinds namely, *xantha* and *viridis* occurred in different treatments. *Xantha* were more frequent as to *viridis.*

Svetleva and Petkova (1991) reported the different chlorophyll mutants. *Viridissima, chloroviridis, flavovirids, viridocostata,* and *xanthomarginata* with gamma rays in *Phaseolus vulgaris*. Tyagi and Gupta (1991) reported induced macromutations in lentil with gamma rays. A number of mutations screened for a variety of morphological and agronomic characters were stunted dwarf, bushy dwarf, open flower, partial sterile, complete sterile and large leaved plants.

Tripathi and Dubey (1992 a) studied frequency and spectrum of mutation induced by gamma rays in two microsperma varieties of lentils. Mutants isolated in the M_2 generation were grouped into chlorophyll, vital mutations and sterile mutations. The isolated chloromutants included *xantha, viridis* and *viridoxantha* types. Vital mutations which produced viable seeds included the mutations for plant height, branching, leaf characteristics, plant colour and texture, maturity and floral arrangement.

Singh and Yadav (1993) studied chlorophyll mutations in mungbean with radiation and obtained *albina, xantha, chlorina* and *viridis* mutants in M_2 generation.

Haq et al. (1994) reported the frequency and spectrum of chlorophyll and other morphological mutations with gamma rays in three Kabuli Chickpea genotypes. An inverse relationship between radio sensitivity in M_1 and mutability in M_2 was observed. The mutagenic treatments induced mutations affecting plant height, growth habit, branching and stem structure, stem and foliage colour, leaf type, flowering and maturity, seed and pod type. There were differences in mutation spectrum between the genotypes. A wide spectrum of chlorophyll mutations was observed which included *xantha, viridis, chlorina.* and *albina.*

VII: GIBBERELLIC ACID (GA_3)

The gibberellins (GAs) are a large family of diterpene acids. They were originally isolated as metabolites of the fungus *Fusarium moniliforme*, the imperfact stage of *Gibberella fujikuroi*, and were shown to cause a wide range of often spectacular growth responses when applied to intact plants. The GAs are now known to be of wide spread and probably universal, occurrence in higher plants where they are generally accepted to function as hormones. More than 60 individual GAs are now known to exist having different structural types and the various functions in plant growth and development may be ascribed due to those structural types.

The effect of GA_3 on growth, development and metabolism is to involve the action of GA_3 at several biochemical levels. It has been established that GA_3 can promote growth of plants by affecting either cell expansion or cell division or both. Cell division can contribute to growth only by producing more cells, which can undergo expansion. GA_3 increases the size of the meristematic regions and also increases the proportion of cells undergoing division (Loy, 1977). These effects of GA_3 on cell division can be readily accounted for by an effect on cell cycle. GA_3 reduced the duration of cell cycle primarily by reducing the length of G1 and S phase of cell cycle (Liu and Loy, 1976).

It is well established that GA_3 have been involved in the synthesis of RNA and proteins in the treated plants (Bewley and Black, 1978; Jacobsen, 1977; Jacobsen et al., 1979). An increase of reaction rate for the formation of rapid starting complex shows that GA_3

affects the chromatin in a way which results in binding of enzyme to chromatin more easily for the enhancement of template activity of chromatin in pea (Tomi et al., 1983). They also found that the quantity of enzyme binding to chromatin was also increased 1-4 fold by GA_3 treatment. Martin et al. (1984) studied that GA_3 in castor beans stimulate nonspecifically the rate of transcription and in turn protein synthesis. Martin and Northcote (1983) determined the amount of protein synthesis in germinating castor bean seeds by qualitative and quantitative examination of polysomes from the seeds in the presence and absence of gibberellic acid. Ribonuclease activity and polysome formation in seeds extracts increased by about two fold. It also stimulated the amount of mRNA associated with polysomes by two fold during germination. Berry and Sachar (1981) reported the hormonal control of Poly(A) polymerase activity in wheat seeds by the exogenous application of GA_3. Martin and Northcote (1982) determined that GA_3 stimulates isocitrate lyase activity of the endosperm during germination of castor bean seeds. The stimulation of isocitrate lyase activity by exogenous GA_3 may be accounted for the action of the growth substance in advancing the over all production of rRNA and mRNA which accelerate the rate of total protein synthesis during germination.

Narsinghani and Kumar (1976), Uppal and Maherchandani (1988) and Khanna (1992) investigated the radioprotective effect of gibberellic acid in different crops. The results revealed that the frequency of chromosomal aberrations decreased with post mutagenic treatment of GA_3 and this reduction was more pronounced at higher radiation doses. Arora et al. (1989) suggested that GA_3 probably reduced the cytological damage by preventing the potential damage from becoming actual detectable cytological damage and promoting the repairing process. Post mutagenic application of GA_3 increased the peroxidase activity may be as a radio protective measure (Khanna, 1992).

Now it is well established that higher doses of gamma radiation resulted in the impaired cellular activity and consequently the retarded growth of the plant. Treatment with GA_3 again restored the growth of the plants by minimizing the potential toxic effects of gamma irradiation at different metabolic processes (Callebaut et al., 1980, Pak et al., 1982, Khanna, 1992).

Effect of growth regulators IAA and Kinetin on germination, seedling growth and some biochemical constituents in gamma irradiated seeds of chickpea was studied by Ali and

Ansari (1989). Seed treatment with both growth regulators reduced the inhibition of germination, shoot and root length caused by irradiation. Uppal and Maherchandani (1988) studied the radioprotective effect of GA_3 in wheat. Seedling height was increased by the GA_3 treatment but there was no relationship between the concentration of growth regulator and seedling height. Arora et al. (1989) studied the modulation of gamma radiation effects in wheat by growth regulators. Post mutagenic treatment with GA_3 treatment resulted in higher germination and significantly higher seedling height.

Modulation of radio sensitivity was also observed with other radio protective compounds. Protective effect of N.allyl and Phenyl-N,2-pyridyl thiourea in *Pisum sativum* and *Glycine max* against gamma radiation were studied by Vasilev and Mekhandzhiev (1991). On the basis of germination percentage they determined that the application of chemicals increased the resistance against radiation. It was thought that treatment with them in the course of mutation breeding would increase the frequency of induced mutant survival.

Babaev et al. (1989) studied the protective effect of hydroquinone following gamma irradiation of wheat seeds. They observed that hydroquinone treatment increased seedling survival and plant height, normalized yield component values and significantly reduced the frequency of dominant mutations in M_1 generation.

Narsinghani and Kumar (1978) reported that EMS and MMS induced various types of chlorophyll mutations in M_2 and M_3 generation of pea. Post mutagenic treatment with GA_3 reduced the frequency and modified the mutational spectrum in both generations.

MATERIALS AND METHODS

MATERIALS AND METHODS

This project was initiated to evaluate the effect of gamma irradiation and consequently the modulation of radiosensitivity with gibberellic acid at cytogenetical, biochemical and genetic spectrum in chickpea. In this experiment three chickpea varieties with different seed coat colors namely Noor 91 (white), Punjab 91 (brown) and C141 (white) were used. Seed samples of the genotypes were collected from the genuine seed stock maintained at Pulses Programme, National Agriculture Research Centre (NARC). The details of the various experiments is given here:

I: SEED TREATMENT

Moisture content in seed at the time of mutagenic treatment was brought to 11% by keeping them in a dessicater containing calcium chloride. Seeds were exposed to gamma irradiation at doses of 10, 20, 30, 40, 50, 60, 70, 90 and 110 Kr to 1000 seeds for each treatment in three genotypes. Gamma irradiation was carried out at Nuclear Institute for food and Agriculture (NIFA), Peshawar at room temperature (22-25 ^{0}C) in Coboll^{60} gamma cell delivering 198.926 Kr hr^{-1} at the time of irradiation.

For different studies prior to gibberellic acid (GA_3) treatment, irradiated seeds were pre-soaked in distilled water. After one hour of soaking under continuous aeration excess water was drained and the swollen irradiated seeds were subjected to 0.5 mM aqueous solution of gibberellic acid for 16 hours with constant shaking. The volume of GA_3 used was three times more than that of seed volume. Non irradiated seeds soaked in water were kept as control in the case. After treatment seeds were washed in running tap water and then were dried on blotting paper. All these operations were carried out at room temperature (22-25^{0}C).

II: LABORATORY EXPERIMENTS

To appraise the modulation of radiosensitivity with gibberellic acid for cytogenetical, biochemical, seedling physiology and also to determine the appropriate dose for inducing

genetic variability different laboratory experiments were conducted. Methodology of these experiments is presented below;

i: CYTOGENETICAL STUDIES

i: MITOSIS IN ROOT TIP CELLS

Twenty seeds of irradiated and post mutagenically treated with gibberellic acid (GA_3) along with control (soaked and dry) were grown in the laboratory, Quaid-i-Azam University, Islamabad in petri plates having moistened whatmann filter paper. The petri plates were placed at room temperature (22-25^{0}C). Root tips 10-15 mm length were excised and fixed in Carnoy's solution (ethanol 3:1 glacial acetic acid) for 12 hours, washed with distilled water and stored in 70% ethanol. Root tips were hydrolyzed with 1.0 N HCl at 60^{0}C for 8 minutes, washed with distilled water thoroughly and left in aceto orcein stain for about an hour.

Stained root tips were squashed on a glass slide in 45 percent acetic acid. A cover glass was placed gently over tissues and was tapped to get a monolayer of cells. The slides were then scrutinized and data of all normal and abnormal stages were recorded. For each slide five fields at random were studied and the whole process was repeated for ten samples of each treatment. In all the experiments, the parameters used for the identification of potentially cytotoxic/genotoxic treatment were:

Mitotic index = Calculated as percentage of mitotic stages to the total number of cells observed.

Mitotic anomalies encountered were expressed as percentage to the total number of dividing cells. The data were subjected to the analysis of variance.

ii: LIGHT AND TRANSMISSION ELECTRON MICROSCOPY OF NUCLEOLUS

About 0.5 mm long root tips of 10, 60 and 110 Kr and post mutagenically treated with gibberellic acid along with control (untreated) seedlings were excised and fixed immediately in freshly prepared 2.5 % phosphate buffered glutaraldehyde (pH = 7.2) for 4-5

hours. Post fixation was carried out in 1% cold aqueous osmium tetraoxide (OsO4) for 2 hours at room temperature. The tissues were dehydrated in ascending series of acetone, cleared in propylene oxide and embedded in Spurr resin (Sigma Chemical Co., St. Louis, MO, USA). For polymerization the moulds were placed in an incubator at 60^0C for 48 hours.

Semithin and ultrathin sections were made using glass knives on a LKB 2088 ultramicrotome V. The semithin sections were stained with 1% toluidine blue and were studied under a Nikon Optiphot research microscope (Type-104). Two diameter (D and d) of each nucleolus were measured at right angle with the help of micrometer. The volume of nucleoli was calculated according to Martini and Flavell (1985).

$V = (4\ \wedge/3)\ (D + d)/64$

For electron microscopy ultra thin sections of silver to pale gold color collected on copper grids and then contrasted with Reynold's (1963) lead citrate and uranyl acetate. The observations were carried out under 100 SX Joel Transmission Electron Microscope.

ii: BIOCHEMICAL STUDIES

Forty seeds of treated and untreated were grown in laboratory at Quaid-i-Azam University, Islamabad at room temperature (22-25 ^{0}C) in petri plates having sand with four replications. Following studies were carried out from 3 to 8 days after sowing.

i: FRESH WEIGHT

Whole shoots were excised at the point of emergence from the seed from 3, 4, 5, 6, 7, and 8^{th} days after sowing and weighed. It was expressed as mg per shoot.

SAMPLE PREPARATION – ENZYME ASSAY

The excised shoots were thoroughly homogenized with a pestle in an ice cold motor containing cold 5.0 ml of cold 0.1 M phosphate buffer (pH 7.5) and acid washed sand. Phase microscopy of the homogenate showed complete disruption of cells and organelles. The slurry so obtained was filtered through eight layers of muslin and the filterate centrifuged at 4000 xg for 20 minutes at 5 ^{0}C. The supernatant solution was decanted and immediately used for the estimation of the enzyme activities and soluble protein.

ii: DETERMINATION OF CATALASE ACTIVITY (1.11.1.6)

The catalase activity was determined by the method described by Cohen et al. (1970). First order velocity constants were determined for each sample at 2-3 time intervals upto 60 seconds. Crude enzyme extract 0.5 ml was mixed with 2.0 ml of 0.2 M phosphate buffer (pH 7.0) and 1.0 ml of 30 mM / litre of hydrogen peroxide in 1.0 cm wide silica cell, the latter solution being absent from the reference cell. The experiment was conducted at room temperature 20-22 ^{0}C. The catalase activity was measured at 240 nm and expressed as milli mole (mM) mg^{-1} protein $minute^{-1}$.

iii: DETERMINATION OF PEROXIDASE ACTIVITY (1.11.1.7)

Peroxidase activity was estimated quantitatively by the method of David and Murray (1965). Crude enzyme extract 0.2 ml was mixed with 2.5 ml of 0.1 M phosphate buffer (pH 7.0) and 0.2 ml of 1.0% (W/V) guaiacol aqueous solution in a 1 cm wide silica cell, the latter solution being absent from the reference cell. The cells were left for several minutes to equilibrate the temperature of 30^{0}C and then 0.1 ml of 0.3 % H_2O_2 solution was added and stirred quickly. Absorption was noted at 470 nm. The specific activity of the enzymes was measured as the increase in optical density (O.D) at 470 nm per mg protein per 30 seconds.

iv: DETERMINATION OF INDOLE-3-ACETIC ACID (IAA) OXIDASE ACTIVITY

IAA oxidase activity was determined by the method of Shinshi and Noguchi (1975) by using salkowski's reagent (Gordon and Weber, 1951; 0.5M $FeCl_3$ and 35% perchloric acid in the ratio of 1:50). The reaction mixture was prepared by adding; 1.0 ml IAA solution (10 mg IAA in 100 ml containing 0.5 mM $MnCl_2$), 0.25ml of 0.1 mM 2,4 dichlorophenol, 3.5 ml of 0.05M phosphate buffer pH 6.5 and 1.0 ml of plant tissue extract. It was incubated at 30^{0} C for one hour in the dark. Then 2 ml of this reaction mixture was taken in a test tube having 2 ml of Salkowski's regent. After 30 minutes of incubation absorbance was recorded at 530 nm. For controls, samples were drawn at zero time while, adding Salkowski's reagent. Absorbance values obtained in the reaction tubes subtracted from that of control tubes. This

gave the measure of indole-3-acetic acid oxidized by the enzyme action and the activity was recorded in terms of μg IAA oxidized per mg protein per hour.

v: DETERMINATION OF SOLUBLE PROTEINS

Soluble protein contents were estimated quantitatively by the method of Lowry et al. (1955). 0.5 ml of the tissue extract was taken in a test tube containing 1.0 ml of 10% cold trichloroacetic acid. Centrifuged at 5000 xg for 10 minutes and decant the supernatant. Precipitate was dissolved in 5.0 ml of 0.1 N NaOH solution and 0.1 ml was used for protein estimation. 0.1 ml of protein suspension was taken in a test tube having 5.0 ml of freshly prepared reagent (c) by mixing reagents (a) and (b) (50:1) shortly before use and incubated for 10 minutes (Reagent a; 2.0% $Na_2 CO_3$ in 0.2% NaOH, and reagent b; 0.3% $CuSO_4$ in 1.0% sodium potassium tartarate). Finally, 1:1 diluted 0.1 ml Folin phenol reagent was added. Mixed and incubated for 30 minutes again. The absorbance of each tube was read at 650 nm using the water blank to zero the spectrophotometer. Standard curve was prepared by using 0.05 ml to 0.5 ml solution of Bovine Serum Albumin (BSA) in a test tube (BSA was prepared at 0.2 mg/ml in 0.1 N NaOH). Absorbance was plotted against protein content for the standard BSA protein. And from the final calibrated BSA curve, soluble protein contents were determined and expressed as mg per shoot.

NUCLEIC ACID CONTENTS

Preparation of samples

10.0 ml of boiled ethanol was poured on the tissue placed in a centrifuge tube. The tissue was crushed with the help of a glass rod and the contents of the centrifuge tube allowed to boil for 5 minutes with occasional shaking. The tubes were cooled, centrifuged and the residue was washed 3-4 times with cold ethanol and dried over $CaCl_2$ under vacuum. The dried tissue was treated with 1.0 ml of 10 % perchloric acid at 4^0C overnight. The cold extract was used for the estimation of RNA.

To each centrifuge tube was added 5.0 ml of 10 % perchloric acid and the suspension was heated at 60^0C for 25 minutes. The hot extract was used for the estimation of DNA

vi: ESTIMATION OF RIBONUCLEIC ACID (RNA) CONTENTS

0.1 ml of perchloric acid (PCA) extract was suitably diluted with distilled water and its optical density was determined at 260 nm. The amount of RNA was calculated from the relationship.

$E^{1\%}$ 1cm (260 nm) = 0.03 (Ogur et al. 1952) and was expressed as µg per shoot.

vii: ESTIMATION OF DEOXYRIBONUCLEIC ACID (DNA) CONTENTS

Estimation of DNA was made by an improved diphenylamine method of Giles and Myers (1965). To 2 ml PCA hot extract DNA + 2.0 ml of 4% diphenylamine solution in glacial acetic acid and then was added 0.1 ml of acetaldehyde (1.6 mg /ml water), The reaction mixture was shaken and incubated at 37^0C for 18 hours. The optical density 595 nm was determined and the amount of DNA was calculated by comparing optical density to those obtained from standard solution prepared from calf thymus DNA.

iii: SEEDLING STUDIES

The treated and control seeds were grown at Quaid-i-Azam University, Islamabad in petri plates having sand in four replications each comprising of ten seeds. The petri plates were placed at room temperature 22-25 ^{0}C. The following parameters were studied.

i: GERMINATION PERCENTAGE

Data on germination percentage was recorded on ten day after sowing by counting total number of plants germinated in each treatment. The emergence of radical was taken as the criteria for germination.

ii: SHOOT LENGTH

Shoot length of seedling was recorded in centimeters on 5 randomly selected plants from each treatment on 10^{th} day after sowing.

iii: ROOT LENGTH

Data on primary root length was recorded in centimeters on 5 randomly selected plants from each treatment on 10^{th} day after sowing.

iv: NUMBER OF SECONDARY ROOTS

Data on number of secondary roots was recorded on 5 randomly selected plants from each treatment on 10^{th} day after sowing.

III: FIELD EXPERIMENTS

Seedling physiology studies provided that dose range of 40 to 60 Kr was the best for inducing genetic variability in the three genotypes of chickpea. This criterion was based on the relative decrease in lengths of shoots and roots with gamma irradiation as compared to control.

I: M_1 GENERATION

i: SOWING AND HANDLING OF TRIAL

450 seeds exposed at 40, 50 and 60 Kr and with post mutagenic treatment of gibberellic acid along with controls were sown at Barani Agriculture Research Institute (BARI), Chakwal on 26-10-1995. The trial was laid out according to split plot arrangement in a complete block design replicated three times of 150 seeds with a plot size of 5.0 x 1.0 meter. Row to row distance of 33 cm was kept. Varieties were assigned to main plots, while radiation doses including check were kept in sub plots. For obtaining unbiased data, the crop was grown under normal field conditions. Data on plant height, number of primary and secondary branches and number of pods were recorded in the field of 20 consecutive plants in the middle row of each treatment. However, other characters like number of seeds per pod, 100-seed weight, biological yield, grain yield, harvest index, flowering and maturity days were recorded by different methods depending upon the requirement of the study. After recording the field data plant samples were harvested, packed in plastic bags and brought to laboratory for measuring yield characters.

ii: CHARACTERS STUDIED

The important yield and agronomic characters were recorded for each genotype over all treatment. All the characters were observed at appropriate growth and developmental stages of plants. The characters included in the study were; plant height, number of primary

and secondary branches, number of pods per plant, seeds per pod, 100-seed weight, biological yield, grain yield, harvest index, flowering and maturity days.

i: PLANT HEIGHT

Height of tagged plants was measured in centimeters from ground level to top of the plant in the field at physiological maturity.

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

Number of primary branches were counted and expressed as number of primary branches per plant.

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

Data on number of secondary branches was recorded and expressed as number of secondary branches per plant.

iv: NUMBER OF PODS PER PLANT

Number of pods were counted on each plant and expressed as number of pods per plant.

v: SEEDS PER POD

Number of seeds on each plant was counted and divided by number of pods to get the number of seeds per pod.

vi: 100-SEED WEIGHT

100-seeds from each plant were taken and weighed. In case of seeds obtained less than 100, the weight was converted to 100-seed. It was expressed as 100-seed weight (g) per plant.

vii: BIOLOGICAL YIELD PER PLANT

Whole plant along with grain was weighed of each plant for recording biological yield. It was expressed as biological yield (g) per plant.

viii: GRAIN YIELD PER PLANT

Grain yield per plant (g) was calculated by threshing 20 consecutive plants from each plot manually and grains were weighed on electric balance for each plant.

ix: HARVEST INDEX PERCENTAGE

The harvest index is the ratio of grain yield and biological yield and is calculated by the following formula:

Harvest Index percentage = Grain yield x 100 / Biological yield

x: FLOWERING DAYS

Data was recorded on plot basis in each treatment in number of days starting from planting date to when 50 percent plants had at least one fully opened flower.

xi: MATURITY DAYS

The maturity days were counted from the sowing date to the physiological maturity of the plants. The physiological maturity was considered when 90 percent plants become straw coloured.

II: M_2 GENERATION

i: SOWING AND HANDLING OF TRIAL

Seeds of 250 plants from treated populations were harvested separately, while from control population the seeds were bulked. The trial was conducted in a split plot design with three replications having 15 seeds in plot size of 1.5 x 0.33 meter at BARI, Chakwal in last week of September 1996. Data on various characters were recorded on 40 randomly selected plants.

ii: MUTATION SPECTRUM

Chlorophyll mutations were recorded in the 10-20 days old seedlings. All the viable morphological mutants were scored throughout growth period of plants. Variations in branching and stem structure, leaf type, flower type, flowering and maturity, pod and seed size were recorded. The mutation frequency was computed on M_2 family basis (% of mutated

progenies) and M_2 plant basis (% of mutants).

III: M_3 GERERATION

i: SOWING AND HANDLING OF TRIAL

The seeds obtained from 40 randomly selected plants of M_2 generation were bulked to produce M_3 population. The trial was laid out in a randomized complete block design with a split plot arrangement in last week of September 1997. Data on 20 consecutive plants were recorded for each treatment as detailed in M_1 experiment.

IV: STATISTICAL ANALYSIS

The data collected on the above mentioned characters were statistically analyzed using analysis of variance and co-variance, phenotypic and genotypic coefficient of variation, heritability and genetic advance, correlation coefficient and path coefficient for all the characters.

i: ANALYSIS OF VARIANCE

The variance components of genotypes, treatments and their interaction were determined by the analysis of variance using the methodology given by Steel and Torrie (1960). The significance of variance components was determined by an F-test. Individual comparison of varietal and treatment means were accomplished by placing them in descending order and applying Duncan's Multiple Range Test (DMRT).

ii: PHENOTYPIC AND GENOTYPIC CORRELATION COEFFICIENT

Phenotypic and genotypic correlation coefficient were calculated using the procedure described by Kwon and Torrie (1964) as under:

$$rp = Mij / (Mii)\ (Mjj)$$

Where:

rp = The estimate of phenotypic correlation coefficient.

Mij = The mean product of varieties for ith and jth traits.

Mii & Mjj = Variety mean squares for ith and jth traits, respectively.

$$rg = cov.\ gij / (var.\ gi)\ (var.\ gj)$$

Where:

rg = The estimate of genotypic correlation coefficient.

cov. gij; var. gi and var. gj are the estimate of lines are varieties components of covariance for ijth traits, variance of ith trait and jth trait, respectively.

iii: PATH COEFFICIENT ANALYSIS

Path coefficient was studied according to the method prescribed by Dewey and Lu (1959), by solving simultaneous equations, using genotypic correlations where seed yield per plant was kept as a resultant variable and other contributing characters as casual variables.

1. ray = Pay + rab Pby + rac Pcy + rad Pdy + rae Pey + raf Pfy + Pgy + rah Phy + rai Piy + raj Pjy

2. rby = Pby + rab Pay + rbc Pcy + rbd Pdy + rbe Pey + rbf Pfy + rbg Pgy +rbh Phy + rbi Piy + rbi Pjy

3. rcy = Pcy + rac Pay + rbc Pby + rcd Pdy + rce Pey + rcf Pfy + rcg Pgy + rch Phy + rci Piy + rej Pjy.

4. rdy = Pdy + rad Pay + rbd Pby + rcd Pcy + rde Pey + rdf Pfy + rdg Pgy + rdh Phy + rdi Piy + rdj Pjy

5. rey = Pey + rae Pay + rbe Pby + rce Pcy + rde Pdy + ref Pfy + reg Pgy + reh Phy + rei Piy + rej Pjy.

6. rfy = Pfy + raf Pay + rbf Pby + rcf Pcy + rdf Pdy + ref Pey + rfg Pgy

+ rfh Phy + rfi Piy +rfj Pjy

7. rgy = Pgy + rag Pay + rbg Pby + rcg Pcy + rdg Pdy + reg Pey + rfg Pfy + rgh Phy + rgi Piy + rgj Pjy

8. rhy = Phy + rah Pay + rbh Pby + rch Pcy + rdh Pdy + reh Pey + rfh Pfy + rgh Pgy + rhi Piy + rhj Pjy.

9. riy = Piy + rai Pay + rbi Pby + rci Pcy + rdi Pdy + rei Pey + rfi Pfy + rgi Pgy + rhi Phy + rij Pjy

10. rj = Pjy + raj Pay + rbj Pby + rcj Pcy + rdj Pdy + rej Pey + rfj Pfy + rgi Pgy + rhj Phy + rij Piy

Where: r is the genetic correlation coefficient and Pay, Pby Pcy, Pdy, Pey, Pfy, Pgy, Phy, Piy and Pjy are standard regression coefficients.

iv: ESTIMATION OF VARIABILITY

Genetic variability estimates of different characters were computed on the data recorded earlier for each treatment. The genetic parameters determined were:

PHENOTYPIC VARIANCE ($\sigma^2 p$)

Total phenotypic variance was calculated for each treated population by analysis of variance.

Phenotypic variance = $\Sigma(X_i - \bar{x})/n$

GENOTYPIC VARIANCE ($\sigma^2 g$)

It was the induced genetic variance in radiated population for different treatment and was calculated by the formula:

Genotypic variance = Phenotypic variance – Environmental variance

COEFFICIENT OF VARIABILITY

Coefficient of variability was determined by computing the standard deviation and

mean of the respective treatments. It was calculated by the following formula:

Phenotypic coefficient of variability (C.V% p)= $\sqrt{(\sigma^2 p/Mean)}$ 100

Genotypic coefficient of variability (C.V% g) = $\sqrt{(\sigma^2 g/Mean)}$ 100

HERITABILITY (BROADSENSE)

The broadsense heritability (h^2) of a character was estimated according to the method of Burton (1952)

i.e. % $h^2 = \sigma^2 g/\sigma^2 p$x 100.

Where h^2 is broadsense heritability, and $\sigma^2 p$ and $\sigma^2 g$ are phenotypic and genetypic variances repectively.

GENETIC ADVANCE

The statistical approach proposed by Singh and Chaudhry (1985) was used to estimate genetic advance (G.A.) at 5% selection intensity and computed by the following formula:

G.A. = (k) (σp) (H)

where σp = phenotypic standard deviation of the mean performance of radiated population H= heritability coefficient and K= 2.06 constant for selection differential. The G.A. was expressed as percentage of the mean for the purpose of comparison.

v: CORRELATION ANALYSIS

Simple correlation of chlorophyll mutations with morphological mutations was determined by calculating correlation co-efficient (r) by using the standard formula,

$r = \Sigma xy / \sqrt{\Sigma x^2 . \Sigma y^2}$ as mentioned by Gomez and Gomez (1976).

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The present studies were carried out on three commercially grown genotypes having different seed coat colors. Gamma irradiation separately and in combination with gibberellic acid was applied to estimate the modulation of radiosensitivity for cytogenetical, biochemical and genetic spectrum induced in chickpea and also to evaluate the appropriate dose for inducing mutations. Field trials were conducted for three successive years to determine the variability, establish the correlation of grain yield with various yield contributing characters and to find out direct and indirect effects of yield characters on yield itself through path coefficient analysis in three chickpea genotypes.

I: CYTOGENETICAL STUDIES

Growth of a plant or any organ depends upon the cell division and increase in volume of cells. It is a very complex phenomena; require efficient metabolic process and duplication of the chromosomes in a very precise manner. Any deformity in the biological steps, structure or in the replication of chromosomes leads to decrease in mitotic activity and consequently reduces the growth. Gamma irradiation is known to interfere with a number of biochemical processes and also directly disintegrates the structures involved in cell division and biosynthesis of various metabolites. Gibberellic acid on the other hand regulates the growth of plants by acting directly at various biochemical and cellular levels. In this study seeds of three chickpea genotypes were treated with gamma irradiation and post mutagenically with GA_3 to determine their effects on mitotic activity, chromosomal abnormalities and nucleolus size and structure. The results obtained were presented here.

i: MITOTIC INDEX

Table 1 presents analysis of variance for mitotic index. The results show significant differences ($p<0.01$) among treatments and genotypes. The treatment-genotype interaction was also highly significant. The results (Table 2) revealed that gamma irradiation decreased the mitotic index in root tip cells as compared to control in Noor 91 and C 141 at all the treatments, while an increase in mitotic index was observed in Punjab 91 at 20, 30 and 40 Kr doses. This decrease in mitotic index was dose dependent. Higher doses of irradiation 70, 90 and 110 Kr was intensively mitodepressive. Noor 91 exhibited a maximum decrease of 19.82 % in mitotic index followed by 17.76 and 16.67 % in C 141 and Punjab 91 at 110 Kr of gamma irradiation as compared to their respective controls.

Sensitivity of mitotic index in all the genotypes changed and an increase was recorded at all the treatments with the post mutagenic application of gibberellic acid. The results also revealed that mitotic index increased over their respective controls in all the genotypes up to 40 Kr of gamma irradiation except at 30 Kr in C 141 genotype.

From table 2, it is clear that gamma irradiation altered the movement of cells through the nuclear cycle. In all the three genotypes at lower doses up to 50 Kr prophase frequency was higher as compared to metaphase and anaphase frequencies, while at higher intensities, reverse was the case. Treatment with GA_3 reduced this difference by modulating the effects of gamma irradiation.

Table 1: Analysis of variance for mitotic index in root tip cells of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F. value
Varieties (V)	2	190.33	95.16	66.54**
Treatments (D)	18	18725.44	1040.30	727.48**
(VxD) interaction	36	414.82	11.52	8.05**
Error	513	737.70	1.43	
Total	569	20068.29		

** Indicates significance at 1% level of probability

S.E.for variety means=0.0867

S.E. for treatment means=0.2183

S.E. (VxD) interaction means=0.3781

Table 2: Effect of gamma radiation on mitotic index in root tip cells of three chickpea genotypes.

Noor 91																				
Treatment	Total mitotic index				% of prophases				% of metaphases				% of anaphases				% of telophases			
Control	26.55	±	0.30		8.68	±	0.28		6.47	±	0.31		5.70	±	0.22		5.66	+	0.34	
10 Kr	25.96	±	0.38		7.53	±	0.27		6.60	±	0.21		5.91	±	0.24		6.02	±	0.34	
20 Kr	26.00	±	0.36		7.45	±	0.24		6.30	±	0.27		6.24	±	0.21		6.00	±	0.27	
30 Kr	25.50	±	0.36		7.00	±	0.30		6.45	±	0.19		5.98	±	0.23		6.03	±	0.27	
40 Kr	25.81	±	0.35		7.34	±	0.28		6.69	±	0.24		6.41	±	0.24		5.34	±	0.31	
50 Kr	23.14	±	0.40		6.95	±	0.23		5.46	±	0.26		5.82	±	0.23		4.88	±	0.32	
60 Kr	21.23	±	0.34		4.64	±	0.23		5.85	±	0.22		5.80	±	0.24		4.91	±	0.26	
70 Kr	16.27	±	0.34		3.09	±	0.21		4.89	±	0.23		5.22	±	020		3.40	±	0.22	
90 Kr	10.60	±	0.37		2.40	±	0.20		3.14	±	0.22		3.20	±	0.23		1.83	±	0.21	
110 Kr	6.73	±	0.33		1.17	±	0.14		2.09	±	0.21		2.12	±	0.18		1.31	±	0.19	
10 Kr+GA_3	27.25	±	0.33		8.09	±	0.25		6.77	±	0.26		7.10	±	0.26		5.26	±	0.38	
20 Kr+GA_3	27.07	±	0.33		7.63	±	0.27		7.06	±	0.28		7.15	±	0.19		5.21	±	0.32	
30 Kr+GA_3	26.90	±	0.31		7.95	±	0.27		6.54	±	0.26		7.14	±	0.25		5.25	±	0.32	
40 Kr+GA_3	27.40	±	0.34		8.00	±	0.34		7.47	±	0.32		7.00	±	0.28		4.83	±	0.27	
50 Kr+GA_3	25.10	±	0.28		6.39	±	0.25		7.67	±	0.33		6.85	±	0.28		4.16	±	0.34	
60 Kr+GA_3	23.24	±	0.33		5.98	±	0.23		6.92	±	0.22		6.63	±	0.20		4.26	±	0.30	
70 Kr+GA_3	20.31	±	0.34		4.78	±	0.23		5.96	±	0.23		5.78	±	0.22		4.14	±	0.40	
90 Kr+GA_3	15.48	±	0.37		3.58	±	0.24		4.24	±	0.22		4.31	±	0.18		3.32	±	0.27	
110Kr+GA_3	9.33	±	0.31		2.23	±	0.17		2.71	±	0.18		2.80	±	0.16		1.56	±	0.18	

± S.E.

Punjab 91

Treatment	Total mitotic index	% of prophases	% of metaphases	% of anaphases	% of telophases
Control	26.55 ± 0.27	8.08 ± 0.30	7.46 ± 0.28	6.79 ± 0.25	4.19 ± 0.24
10 Kr	26.33 ± 0.29	7.62 ± 0.32	6.97 ± 0.29	6.99 ± 0.27	4.72 ± 0.26
20 Kr	26.63 ± 0.33	7.88 ± 0.16	6.59 ± 0.20	7.05 ± 0.28	5.09 ± 0.25
30 Kr	26.56 ± 0.29	7.89 ± 0.25	6.82 ± 0.25	7.10 ± 0.21	4.72 ± 0.28
40 Kr	26.79 ± 0.41	7.65 ± 0.31	6.98 ± 0.26	7.18 ± 0.24	4.95 ± 0.34
50 Kr	24.14 ± 0.35	6.21 ± 0.25	6.37 ± 0.19	6.61 ± 0.26	4.92 ± 0.23
60 Kr	21.34 ± 0.40	5.03 ± 0.26	5.77 ± 0.19	6.05 ± 0.30	4.46 ± 0.28
70 Kr	17.58 ± 0.34	6.02 ± 0.18	4.72 ± 0.20	5.24 ± 0.21	3.97 ± 0.28
90 Kr	13.61 ± 0.35	2.86 ± 0.17	3.75 ± 0.17	3.87 ± 0.23	3.09 ± 0.22
110 Kr	9.88 ± 0.34	2.21 ± 0.14	2.64 ± 0.13	2.90 ± 0.15	2.19 ± 0.24
10 Kr+GA_3	26.90 ± 0.37	8.04 ± 0.18	7.04 ± 0.21	7.17 ± 0.24	4.62 ± 0.28
20 Kr+GA_3	26.74 ± 0.35	7.64 ± 0.25	7.13 ± 0.23	7.37 ± 0.25	4.57 ± 0.30
30 Kr+GA_3	27.19 ± 0.35	7.92 ± 0.26	7.28 ± 0.24	7.37 ± 0.23	4.67 ± 0.28
40 Kr+GA_3	27.51 ± 0.38	7.62 ± 0.25	7.40 ± 0.26	7.76 ± 0.25	4.70 ± 0.28
50 Kr+GA_3	25.30 ± 0.41	6.03 ± 0.31	6.85 ± 0.19	7.24 ± 0.28	5.46 ± 0.36
60 Kr+GA_3	23.54 ± 0.37	5.25 ± 0.21	5.90 ± 0.17	6.47 ± 0.21	5.77 ± 0.29
70 Kr+GA_3	20.84 ± 0.38	4.65 ± 0.23	5.29 ± 0.24	6.12 ± 0.27	4.73 ± 0.30
90 Kr+GA_3	16.44 ± 0.40	3.90 ± 0.26	3.97 ± 0.25	4.36 ± 0.22	3.96 ± 0.31
110Kr+GA_3	13.21 ± 0.36	3.09 ± 0.19	3.40 ± 0.22	3.75 ± 0.23	2.93 ± 0.23

± S.E.

C141

Treatment	Total mitotic index	% of prophases	% of metaphases	% of anaphases	% of telophases
Control	26.88 ± 0.29	7.69 ± 0.27	7.03 ± 0.22	7.24 ± 0.23	4.89 ± 0.24
10 Kr	26.50 ± 0.35	7.40 ± 0.30	6.87 ± 0.21	7.42 ± 0.19	4.77 ± 0.27
20 Kr	26.82 ± 0.34	7.98 ± 0.25	7.23 ± 0.21	7.53 ± 0.21	4.06 ± 0.25
30 Kr	25.06 ± 0.37	6.98 ± 0.27	6.72 ± 0.18	7.23 ± 0.21	5.12 ± 0.28
40 Kr	25.53 ± 0.39	6.88 ± 0.31	6.75 ± 0.23	7.12 ± 0.18	4.89 ± 0.26
50 Kr	23.07 ± 0.32	5.74 ± 0.20	6.22 ± 0.20	6.43 ± 0.22	4.39 ± 0.24
60 Kr	21.32 ± 0.41	5.23 ± 0.19	6.10 ± 0.24	6.17 ± 0.17	3.79 ± 0.20
70 Kr	19.43 ± 0.33	4.55 ± 0.21	5.12 ± 0.17	5.38 ± 0.20	4.34 ± 0.23
90 Kr	12.03 ± 0.37	2.82 ± 0.18	3.34 ± 0.21	3.41 ± 0.22	2.61 ± 0.30
110 Kr	9.12 ± 0.39	2.24 ± 0.21	2.47 ± 0.23	2.56 ± 0.20	1.82 ± 0.22
10 Kr+GA_3	27.09 ± 0.38	7.39 ± 0.29	6.73 ± 0.24	6.85 ± 0.26	6.35 ± 0.28
20 Kr+GA_3	26.91 ± 0.37	7.76 ± 0.32	6.72 ± 0.24	6.94 ± 0.23	5.46 ± 0.32
30 Kr+GA_3	26.54 ± 0.27	7.85 ± 0.29	6.79 ± 0.18	6.70 ± 0.22	5.84 ± 0.32
40 Kr+GA_3	27.49 ± 0.42	7.27 ± 0.25	7.68 ± 0.20	7.43 ± 0.22	5.09 ± 0.31
50 Kr+GA_3	25.79 ± 0.40	6.79 ± 0.29	7.00 ± 0.24	7.05 ± 0.28	4.90 ± 0.30
60 Kr+GA_3	26.11 ± 0.31	6.52 ± 0.21	6.89 ± 0.17	7.04 ± 0.23	5.62 ± 0.27
70 Kr+GA_3	24.02 ± 0.41	6.10 ± 0.22	5.44 ± 0.20	6.56 ± 0.18	4.97 ± 0.32
90 Kr+GA_3	20.79 ± 0.50	5.00 ± 0.30	5.37 ± 0.26	5.59 ± 0.28	4.64 ± 0.34
110Kr+GA_3	14.76 ± 0.43	3.45 ± 0.20	3.83 ± 0.22	3.91 ± 0.28	3.53 ± 0.21

± S.E.

ii: CHROMOSOMAL ABERRATIONS

Analysis of variance for the percentage of chromosomal aberrations induced by gamma irradiation is presented in table 3. The results show highly significant ($p<0.01$) within treatments and genotypes. The interaction between genotype-treatment interaction was also highly significant. This indicates that the genotypes differed in their response to various treatments. The chromosomal aberrations were not recorded in controls and up to 30 Kr of gamma irradiation. Above this intensity frequency of chromosomal aberrations increased with an increase of gamma irradiation (Table 4). A drastic increase in the frequency of chromosomal aberrations was observed at 90 and 110 Kr of gamma irradiation in all the genotypes. Noor 91 showed the maximum abnormal frequency of 39.14 at 110 Kr followed by 16.75 and 13.11 in C 141 and Punjab 91, respectively.

GA_3 treatment of seeds decreased the frequency of chromosomal aberrations in the three genotypes by minimizing the potential damaging effects of gamma irradiation. The protective effects were more pronounced at higher doses of 90 and 110 Kr as compared to lower doses of gamma irradiation (Table 4).

Various abnormalities in root tip cells of the three genotypes encountered were fragments, bridges, and laggards at anaphase stage of the mitotic cycle (Fig 1 and 2). Chromosomal aberrations were not observed at other stages of the mitotic cycle. Bridge formation was the most frequent abnormality followed by lagging and fragmentation of the chromosomes. These abnormalities increased with an increase in gamma irradiation.

Seed treatment with GA_3 reduced the occurrence of various abnormalities in the root tip cells in the three genotypes. Fragments and laggards were not recorded at 40 Kr in Punjab 91 and C 141 with GA_3.

Table 3: **Analysis of variance for percentage of chromosomal aberrations in root tip cells of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F.	Sum of squares	Mean of squares	F. value
Varieties (V)	2	1938.76	969.38	262.72**
Treatments (D)	12	14307.13	1192.26	323.10**
(VxD) interaction	24	3970.95	165.45	44.83**
Error	351	1297.58	3.70	-
Total	389	21514.42		

** Indicates significance at 1% level of probability

S.E. for variety means=0.1687

S.E.for treatment means=0.3511

S.E. for (VxD) interaction means=0.6082

Table 4: **Chromosomal aberrations induced by different gamma radiation doses in root tip cells of three chickpea genotypes.**

Treatment	No. of dividing cells	Fragmentation %	Bridges %	Laggards %	Total chromosomal aberrations
NOOR 91					
Control	194.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
40 Kr	189.0	0.10 ± 0.07	2.11 ± 0.20	0.10 ± 0.06	2.31
50 Kr	168.0	0.36 ± 0.13	2.44 ± 0.22	0.23 ± 0.09	3.03
60 Kr	155.0	0.57 ± 0.14	3.66 ± 0.17	0.52 ± 0.16	4.75
70 Kr	118.0	0.84 ± 0.22	5.18 ± 0.23	1.12 ± 0.27	7.14
90 Kr	77.0	2.02 ± 0.38	14.16 ± 0.90	4.01 ± 0.32	20.19
110 Kr	49.0	4.26 ± 0.81	25.23 ± 1.28	9.65 ± 0.61	39.14
40 Kr+GA_3	200.0	0.05 ± 0.05	1.09 ± 0.13	0.05 ± 0.05	1.19
50 Kr+GA_3	183.0	0.15 ± 0.08	1.58 ± 0.17	0.11 ± 0.07	1.84
60 Kr+GA_3	171.0	0.28 ± 0.12	2.63 ± 0.10	0.29 ± 0.13	3.20
70 Kr+GA_3	148.0	0.40 ± 0.14	2.28 ± 0.27	0.45 ± 0.16	3.13
90 Kr+GA_3	113.0	0.68 ± 0.17	5.01 ± 0.37	0.91 ± 0.19	6.60
110 Kr+GA_3	68.0	1.50 ± 0.39	9.77 ± 0.95	2.80 ± 0.47	14.07

± S.E.

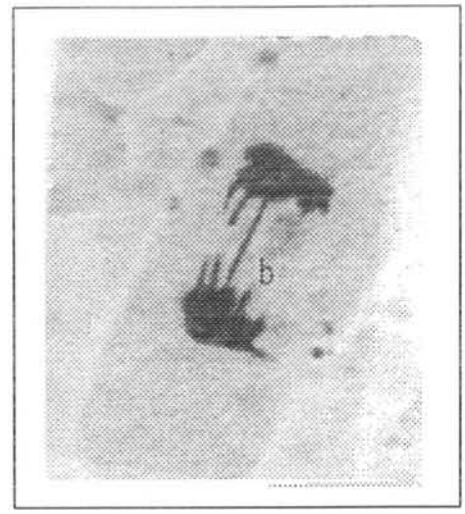
PUNJAB 91

Control	193.0	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00
40 Kr	195.0	0.05	± 0.04	0.91	± 0.18	0.05	± 0.05	0.09
50 Kr	176.0	0.17	± 0.08	1.20	± 0.19	0.17	± 0.08	1.54
60 Kr	155.0	0.31	± 0.10	1.66	± 0.25	0.39	± 0.13	2.36
70 Kr	129.0	0.54	± 0.12	2.68	± 0.31	0.69	± 0.13	3.91
90 Kr	99.0	0.78	± 0.23	3.99	± 0.37	1.11	± 0.18	5.88
110 Kr	73.0	2.33	± 0.35	8.59	± 0.56	2.19	± 0.48	13.11
40 Kr+GA_3	201.0	0.00	± 0.00	0.50	± 0.18	0.00	± 0.00	0.50
50 Kr+GA_3	185.0	0.05	± 0.05	0.81	± 0.16	0.05	± 0.05	0.91
60 Kr+GA_3	172.0	0.11	± 0.07	1.15	± 0.18	0.11	± 0.07	1.37
70 Kr+GA_3	152.0	0.32	± 0.10	1.69	± 0.18	0.32	± 0.10	2.33
90 Kr+GA_3	119.0	0.33	± 0.16	2.51	± 0.41	0.58	± 0.17	3.42
110 Kr+GA_3	96.0	1.07	± 0.34	3.98	± 0.32	1.05	± 0.28	6.10

C 141

Control	186.0	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00
40 Kr	186.0	0.10	± 0.06	1.11	± 0.15	0.00	± 0.00	1.21
50 Kr	169.0	0.18	± 0.12	1.41	± 0.19	0.18	± 0.13	1.77
60 Kr	156.0	0.44	± 0.13	2.12	± 0.23	0.39	± 0.14	2.95
70 Kr	142.0	0.70	± 0.14	3.52	± 0.28	0.48	± 0.14	4.70
90 Kr	88.0	1.37	± 0.22	6.60	± 0.48	1.19	± 0.28	9.16
110 Kr	67.0	3.30	± 0.40	10.54	± 0.72	2.91	± 0.39	16.75
40 Kr+GA_3	201.0	0.00	± 0.00	0.84	± 0.11	0.00	± 0.00	0.84
50 Kr+GA_3	190.0	0.00	± 0.00	1.05	± 0.13	0.05	± 0.05	1.10
60 Kr+GA_3	190.0	0.11	± 0.07	0.95	± 0.13	0.10	± 0.07	1.16
70 Kr+GA_3	176.0	0.28	± 0.09	1.11	± 0.17	0.29	± 0.13	1.68
90 Kr+GA_3	152.0	0.51	± 0.13	1.65	± 0.26	0.34	± 0.15	2.50
110 Kr+GA_3	108.0	0.76	± 0.24	2.53	± 0.40	0.65	± 0.24	3.94

± S.E.



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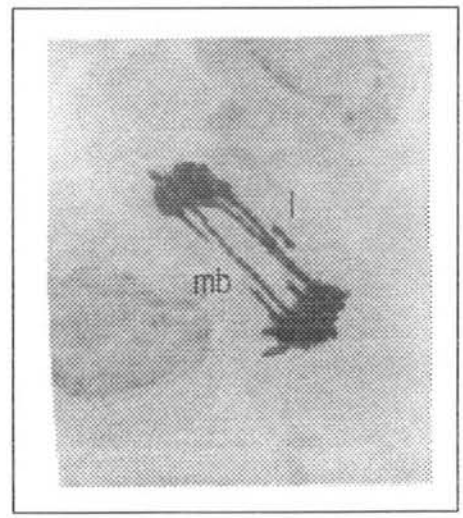
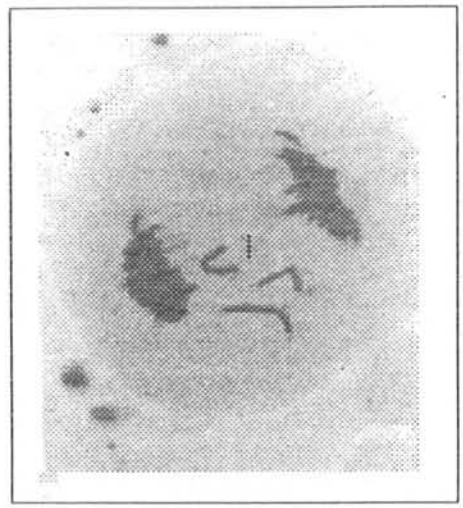


B

Fig. 1: Root tip cell showing A) single bridge (b) and B) multiple bridges (mb) with a fragment (f) at anaphase in Noor 91 (X 2400)



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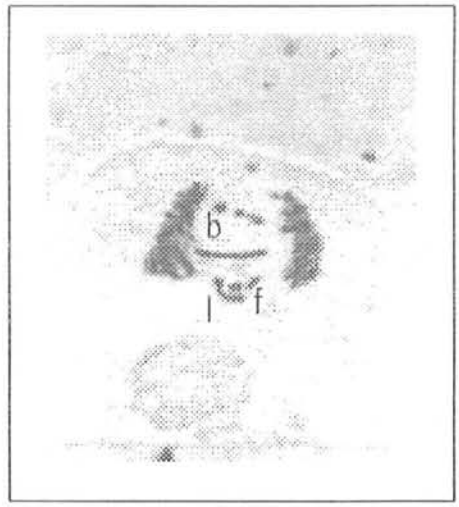


B

Fig. 2 Root tip cell showing A) laggards (l) at anaphase in Punjab 91 and B) laggard (l) fragment (f) with broken bridge (b) at anaphase in C141 (X 2400)

iii: CYTOLOGY AND NUCLEOLUS VOLUME

The root tips of untreated along with irradiated samples of Noor 91, Punjab 91 and C141 after being fixed in gluteraldehyde and osmic acid were cut in semi thin sections. The cells depicted in Fig. 3 show an organized nucleus, nucleolus and cytoplasm. Treatment with gamma irradiation at 10 Kr separately and with GA_3 increased the size and vacuolation of cells (Fig. 4). However, at 60 Kr of gamma irradiation had not an appreciable affect on cell wall, while the cells were seen with considerable vacuolation. Application of GA_3 increased the elasticity of cell wall along with the increase in vesiculation (Fig. 5). At highest dose of 110 Kr these effects became more pronounced (Fig. 6-7). Cell size increased and large vacuoles were formed on collapsing the smaller ones with gamma irradiation. GA_3 application increased the cell size and vacuolation appreciably by diminishing the damaging effects of gamma irradiation.

The results of frequency distribution of nucleolus volume in Noor 91, Punjab 91 and C 141 following seed treatment with various doses of gamma radiation and with post mutagenic treatment of GA_3 are summarized in table 5-7.

In Noor 91 minimum and maximum nucleolus volumes were 4-19 μm^3 and 33-52 μm^3, respectively. Gamma irradiation increased the frequency of cells having greater nucleolar volume, while the application of GA_3 increased the frequency of cells having smaller nucleolar volume and these effects were more pronounced at higher doses (Table 5).

Minimum and maximum nucleolar volumes in Punjab 91 were 4-19 and 41-64 μm^3, respectively. In control the nucleolus volume was up to 33-52 μm^3, while the radiation treatment increased the volume up to 41-64 μm^3. With an increase of irradiation the frequency of cells having greater nucleolar volume increased, however, the application of GA_3 altered this situation and the cells with smaller nucleolar volume became more frequent (Table 6).

In C 141 minimum and maximum nucleolar volumes were 4-19 and 38-80 $\mu m,^3$ respectively. Various gamma irradiation doses increased the frequency of cells having greater nucleolar volume, while GA_3 decreased this frequency (Table 7).

Table 5: Frequency distribution of nucleolus volume in Noor 91 treated with gamma radiation and post mutagenic treatment with GA_3.

Nucleolus volume	Control	10 Kr	10 Kr+GA_3	60 Kr	60 Kr+GA_3	110 Kr	110 Kr+GA_3
4.19	26	0	24	0	6	6	0
4.85	16	10	0	0	24	4	4
5.20	0	0	0	0	0	4	0
5.57	16	0	4	18	14	8	12
5.96	0	0	0	0	0	10	0
6.37	16	4	10	4	4	8	0
7.24	0	6	8	6	16	0	26
8.18	0	0	0	0	18	12	10
9.20	0	6	4	10	0	0	12
10.30	0	0	6	6	14	0	6
10.89	0	0	0	0	0	6	0
11.49	8	20	8	14	0	4	0
12.77	12	28	16	20	0	4	18
33.52	6	26	20	22	4	34	12

Table 6: Frequency distribution of nucleolus volume in Punjab 91 treated with gamma radiation and post mutagenic treatment with GA_3.

Nucleolus volume	Control	10 Kr	10 Kr+GA_3	60 Kr	60 Kr+GA_3	110 Kr	110 Kr+GA_3
4.19	8	0	0	0	0	0	0
4.85	0	0	0	0	0	4	0
5.57	6	0	0	0	4	0	4
6.37	8	0	0	0	8	6	0
7.24	12	0	10	0	0	0	16
8.18	16	8	26	8	16	14	14
9.20	20	0	12	0	14	0	0
10.30	14	16	8	16	0	12	12
11.49	8	22	0	12	18	0	0
12.77	4	0	0	8	0	10	14
33.52	4	14	8	14	12	0	0
36.09	0	8	10	0	8	16	12
38.80	0	10	12	16	4	18	12
41.64	0	20	14	26	16	20	16

Table 7: **Frequency distribution of nucleolus volume in C 141 treated with gamma radiation and post mutagenic treatment with GA_3.**

Nucleolus volume	Control	10 Kr	10 Kr+GA_3	60 Kr	60 Kr+GA_3	110 Kr	110 Kr+GA_3
4.19	18	0	0	0	0	6	0
4.85	12	0	16	4	4	0	14
5.57	6	0	20	0	0	8	0
6.37	0	18	0	14	10	0	14
7.24	14	0	14	0	12	10	0
8.18	0	14	0	8	12	8	12
9.20	20	0	0	10	0	0	14
10.30	0	10	10	0	12	4	0
11.49	18	0	8	4	0	12	4
12.77	0	0	0	0	8	16	10
35.52	12	16	8	18	14	6	8
36.09	0	24	14	20	12	10	8
38.80	0	18	10	22	16	20	16

iv: NUCLEOLUS STRUCTURE

At ultrastructural level, the nucleolus of untreated sample of Noor 91, was seen as a compact structure of three components (i) pale fibrillar (ii) dark fibrillar and (iii) granular (Fig.7). The rest of nucleus is filled with patches of chromatin. At 10 Kr of gamma irradiation separately and with GA_3 the nucleolus structure had the similar organization as that of control samples except that granular region was increased (Fig. 8-9). Nucleolus was faintly stained. In samples treated at 60 Kr separately and with GA_3 nucleolar associated bodies were seen on one side of nucleolus (Fig. 10-11). The dark clumps in the form of big and small patches are distributed along the periphery of the nucleolus. However, with GA_3 dark clumps are scattered throughout the nucleolus (Fig. 11). The highest dose of 110 Kr had deformed the nucleolus and clumps of dark patches were seen evenly distributed in the nucleolus. However, with the application of GA_3 dark clumps were located towards the central side. Nucleolar associated bodies were seen with both types of treatments (Fig. 12-13).

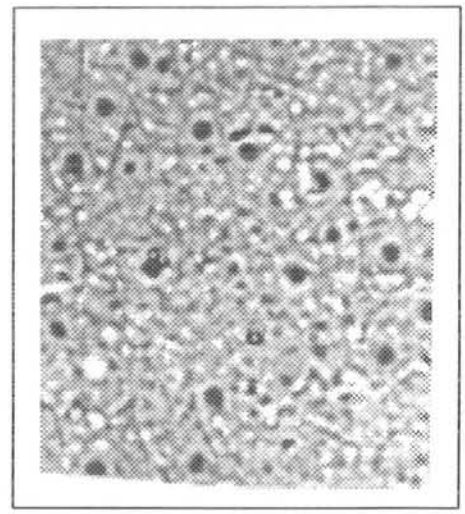
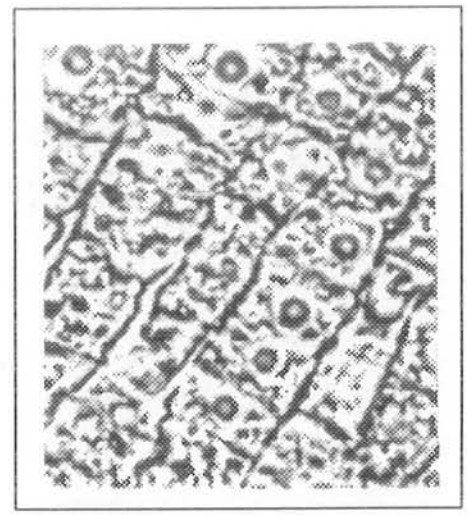


Fig. 3: Photograph of non-irradiated (Control) root tip cells of Noor 91 showing nucleoli (X 960)



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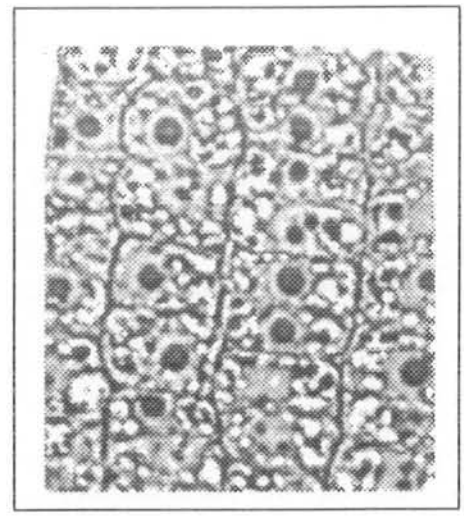
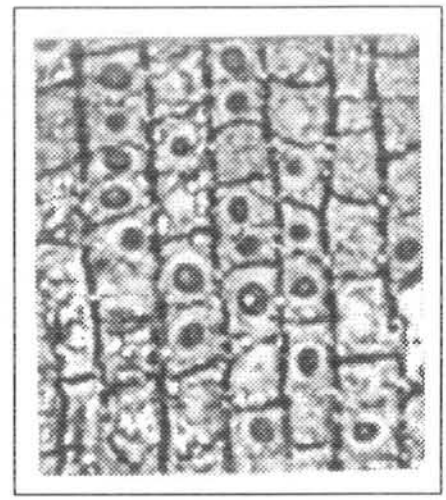


B

Fig. 4: Photograph of irradiated A) 10 Kr and B) 10 Kr + GA_3 in root tip cells of Punjab 91 showing nucleoli (X 960)



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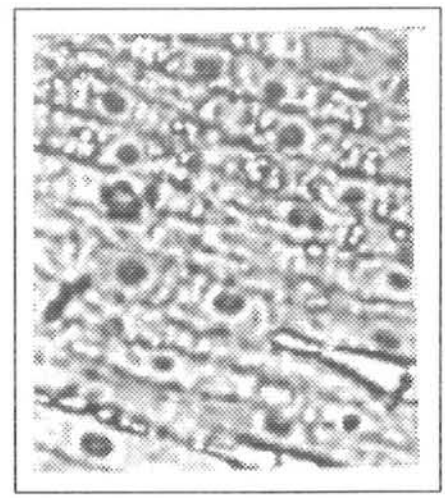
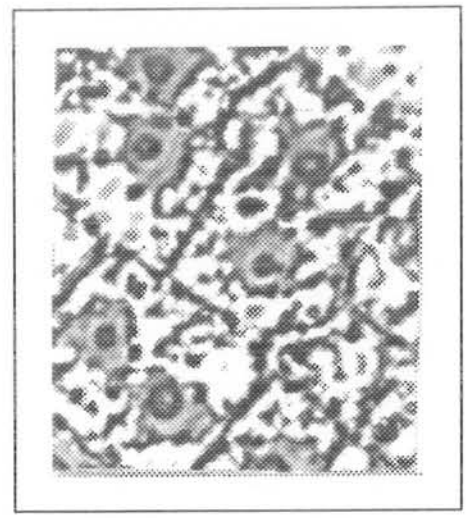
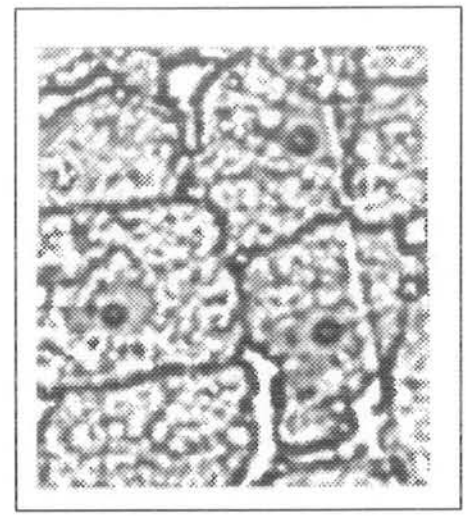


B

Fig. 5: Photograph of irradiated A) 60 Kr and B) 60 Kr + GA_3 in root tip cells of C141 showing nucleoli (X 960)



A



B

Fig. 6: Photograph of irradiated A) 110 Kr and B) B) 110 Kr + GA_3 in root tip cells of Noor 91 showing nucleoli (X 960)

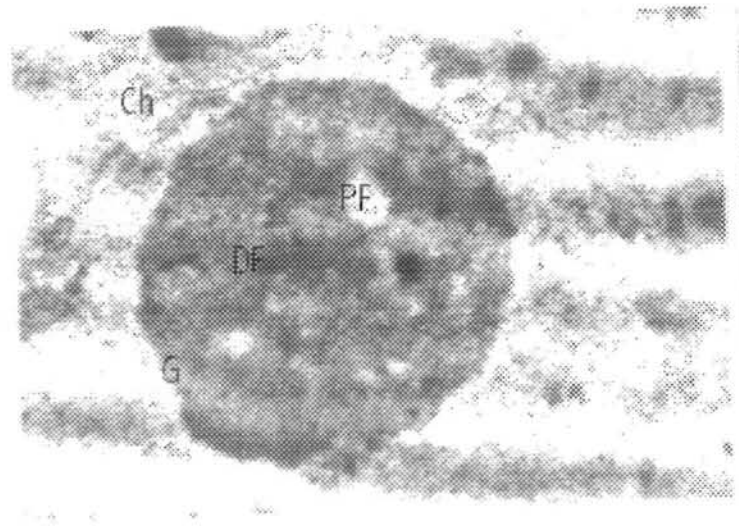


Fig. 7: Electron micrograph of an interphase cell of Noor 91 untreated material after classical staining with uranyl acetate and lead citrate. Nucleolus present in the centre of the nucleus showing Pale fibrillar (PF) regions irregularly scattered, encircled by Dark fibrillar (DF) regions, while Granular (G) region is at the periphery of the nucleolus. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.10,000)

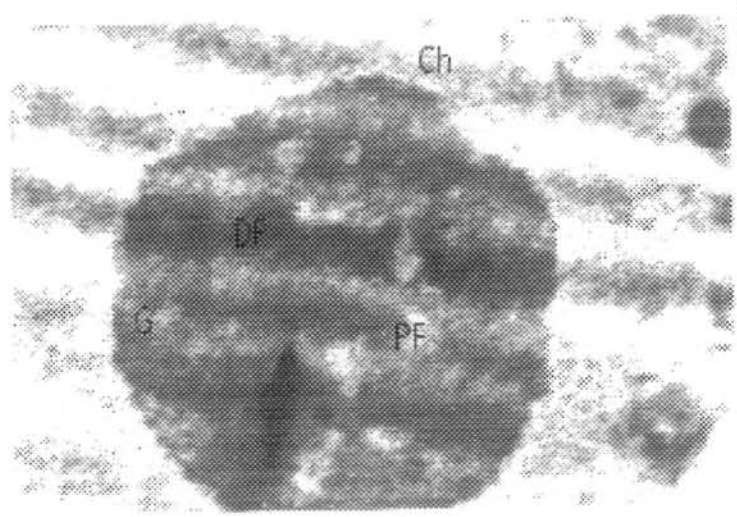


Fig. 8: Electron micrograph of an interphase cell of Punjab 91 following seed treatment with 10 Kr of gamma irradiation after classical staining with uranyl acetate and lead citrate. Nucleolus present in the centre of the nucleus showing Pale fibrillar (PF) regions irregularly scattered, encircled by Dark fibrillar (DF) regions, while Granular (G) region is at the periphery of the nucleolus. Nucleolus is faintly stained. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.10,000)

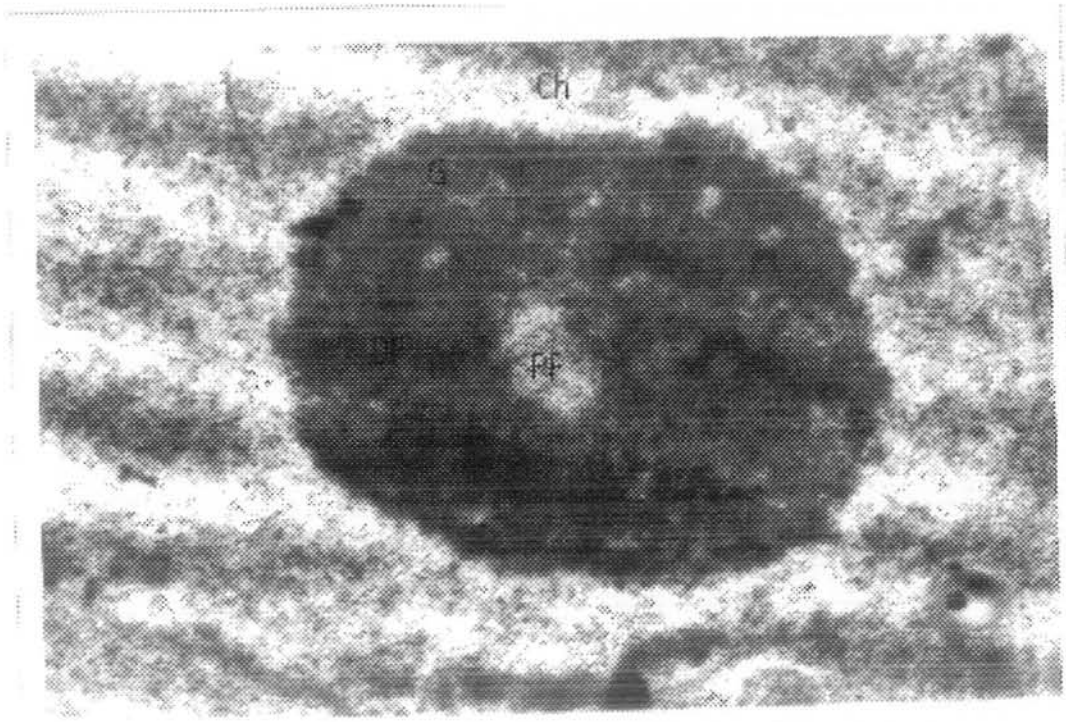


Fig. 9: Electron micrograph of an interphase cell of Punjab 91 following seed treatment with 10 Kr + GA_3 of gamma irradiation after classical staining with uranyl acetate and lead citrate. Nucleolus present in the centre of the nucleus showing Pale fibrillar (PF) regions irregularly scattered, encircled by Dark fibrillar (DF) regions, while Granular (G) region is at the periphery of the nucleolus. Nucleolus is faintly stained. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.10,000)

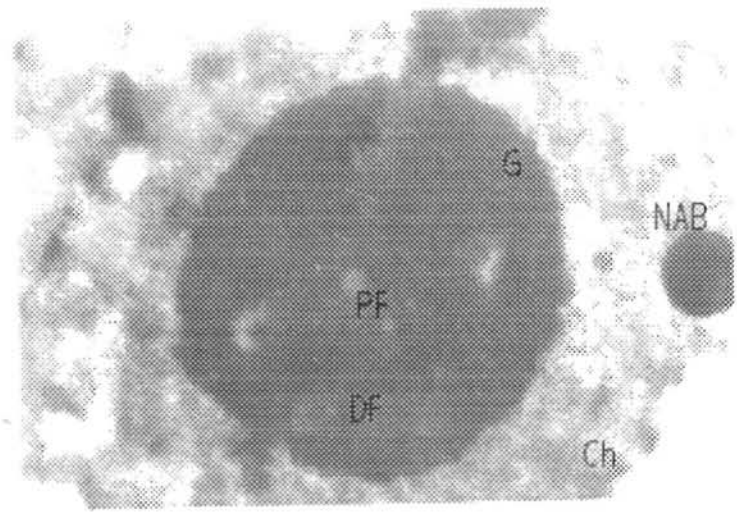


Fig.10: Electron micrograph of an interphase cell of C141 following seed treatment with 60 Kr of gamma irradiation after classical staining with uranyl acetate and lead citrate. Nucleolus present in the centre of the nucleus showing Pale fibrillar (PF) regions irregularly scattered, encircled by Dark fibrillar (DF) regions, while Granular (G) region is at the periphery of the nucleolus. A nucleolus associated body (NAB) is also seen at one side of the nucleolus. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.10,000)

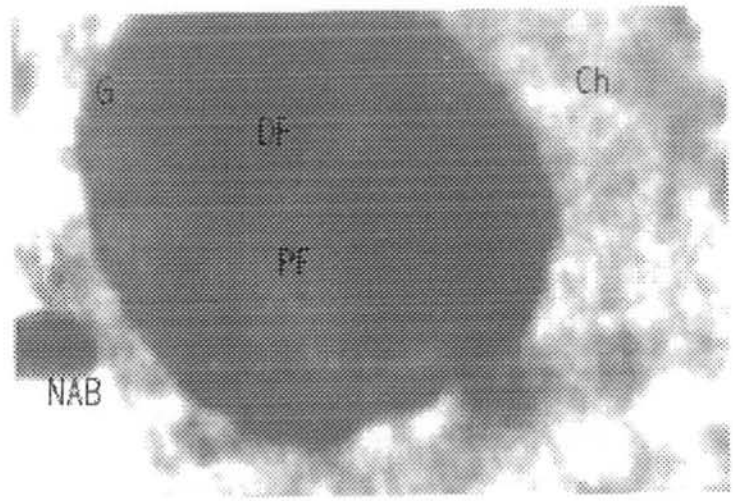


Fig.11: Electron micrograph of an interphase cell of C141 following seed treatment with 60 Kr + GA_3 of gamma irradiation after classical staining with uranyl acetate and lead citrate. Nucleolus present in the centre of the nucleus showing Pale fibrillar (PF) regions irregularly scattered, encircled by Dark fibrillar (DF) regions, while Granular (G) region is at the periphery of the nucleolus. A nucleolus associated body (NAB) is also seen at one side of the nucleolus. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.13,000)

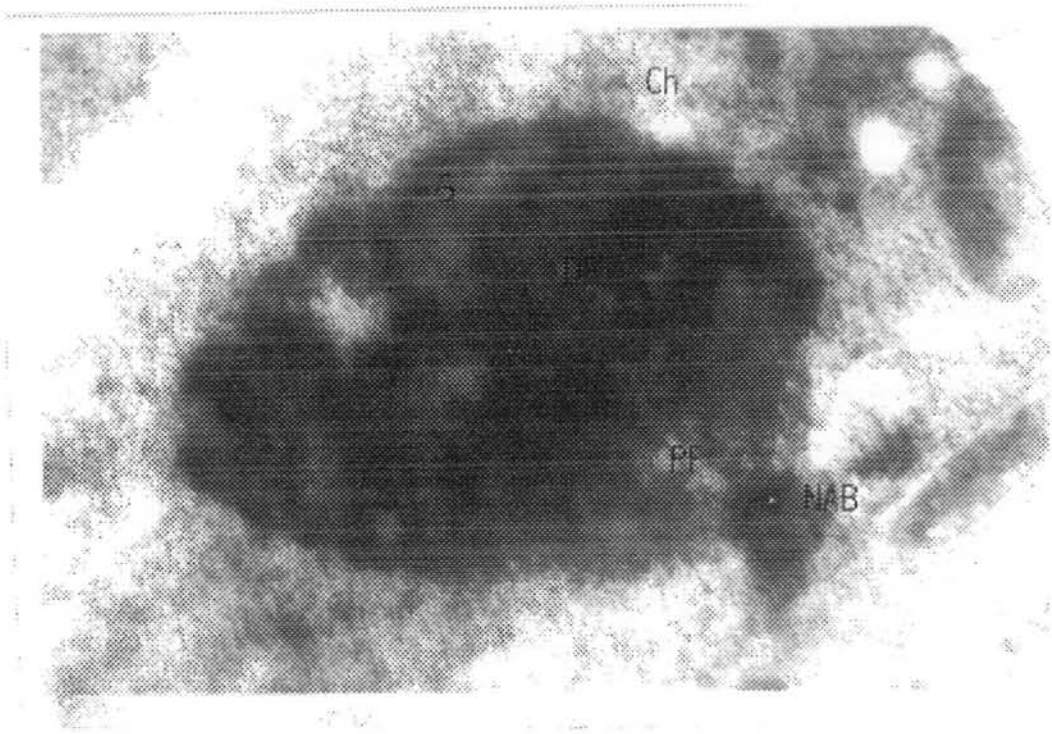


Fig.12: Electron micrograph of an interphase cell of Noor 91 following seed treatment with 60 Kr of gamma irradiation after classical staining with uranyl acetate and lead citrate. Nucleolus is present in the centre of the nucleus and is greatly deformed showing mixture of Pale fibrillar (PF) Dark fibrillar (DF) and Granular (G) regions. A nucleolus associated body (NAB) is also seen at one side of the nucleolus. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.10,000)

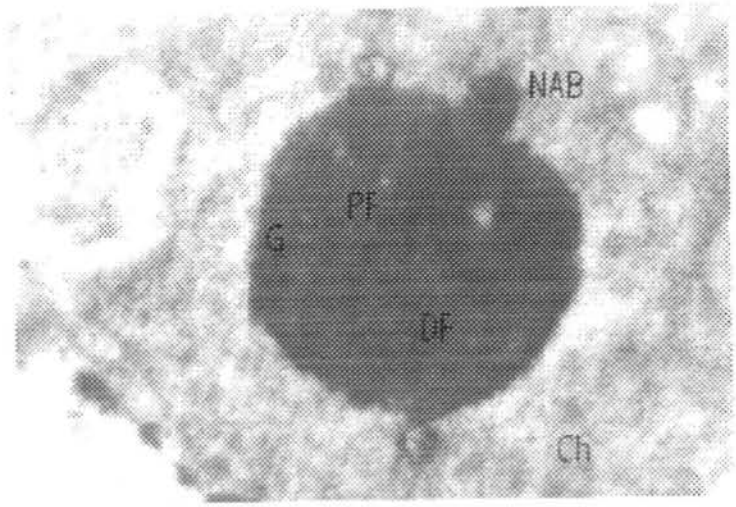


Fig.13: Electron micrograph of an interphase cell of Noor 91 following seed treatment with 110 Kr + GA_3 of gamma irradiation after classical staining with uranyl acetate and lead citrate. Nucleolus present in the centre of the nucleus showing Pale fibrillar (PF) regions irregularly scattered, encircled by Dark fibrillar (DF) regions, while Granular (G) region is at the periphery of the nucleolus. A nucleolus associated body (NAB) is also seen at one side of the nucleolus. Chromatin material (Ch) is also spread evenly throughout the nucleus (X.13,000)

II: BIOCHEMICAL STUDIES

Growth is a very intricate phenomenon controlled at several biochemical levels. Gamma irradiation plays a stress on metabolic activities, resulting in a differential response at various doses. To determine the physiological activities of the enzymes and chemicals, which are directly related to growth, are of utmost important. Growth regulators like GA_3 express their physiological role at different levels of cellular activities. It is very essential to determine the modulatory effects of GA_3 created through the use of gamma irradiation. The results obtained in this context are described below:

i: FRESH WEIGHT PER SHOOT (mg)

Effects of gamma irradiation and the modulation of radio sensitivity with GA_3 on fresh weight are shown in fig 14-16. It is apparent from the results that fresh weight reduced with an increase in gamma irradiation in all the three chickpea genotypes. Maximum fresh weight per shoot was observed at 10 Kr with GA_3, while minimum fresh weight was recorded at the highest dose 110 Kr of gamma irradiation on various days in the three chickpea genotypes. Stimulation in fresh weight was observed at 10 Kr of gamma irradiation in all the genotypes. At higher doses of gamma irradiation Noor 91 appeared to be more radio sensitive, while Punjab 91 more radio resistant as the increase in fresh weight at 90 and 110 Kr at different days was more pronounced (Fig. 14-15).

Post mutagenic treatment with GA_3 increased the fresh weight per shoot at all the irradiation doses in the three genotypes. However, more pronounced increase in fresh weight per shoot with GA_3 was observed after six and seven days in the three genotypes at 50, 60, and 70 Kr of gamma irradiation doses.

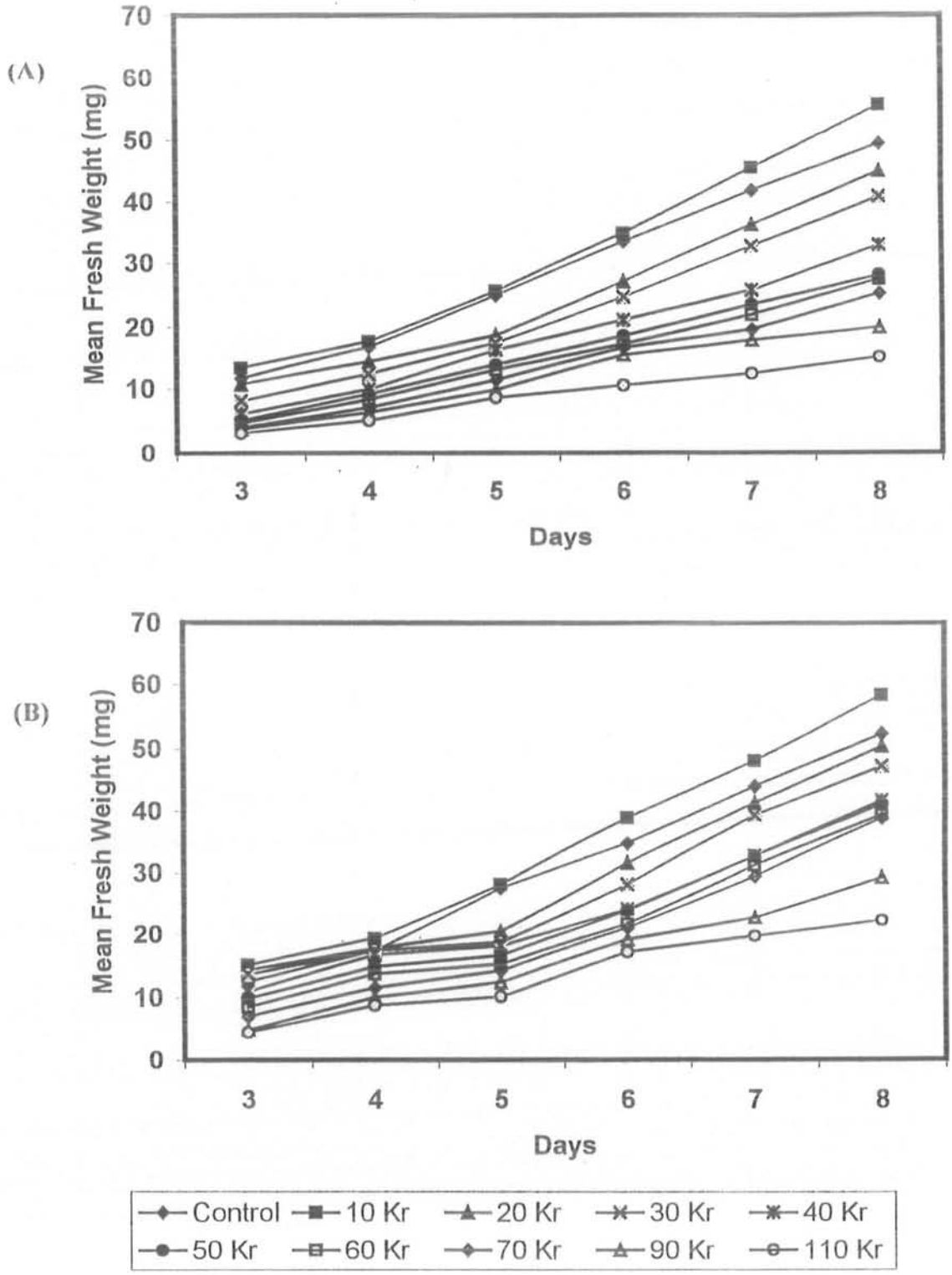


Fig. 14: The response of genotype Noor 91 for fresh weight to (A) gamma irradiation separately and (B) with GA_3.

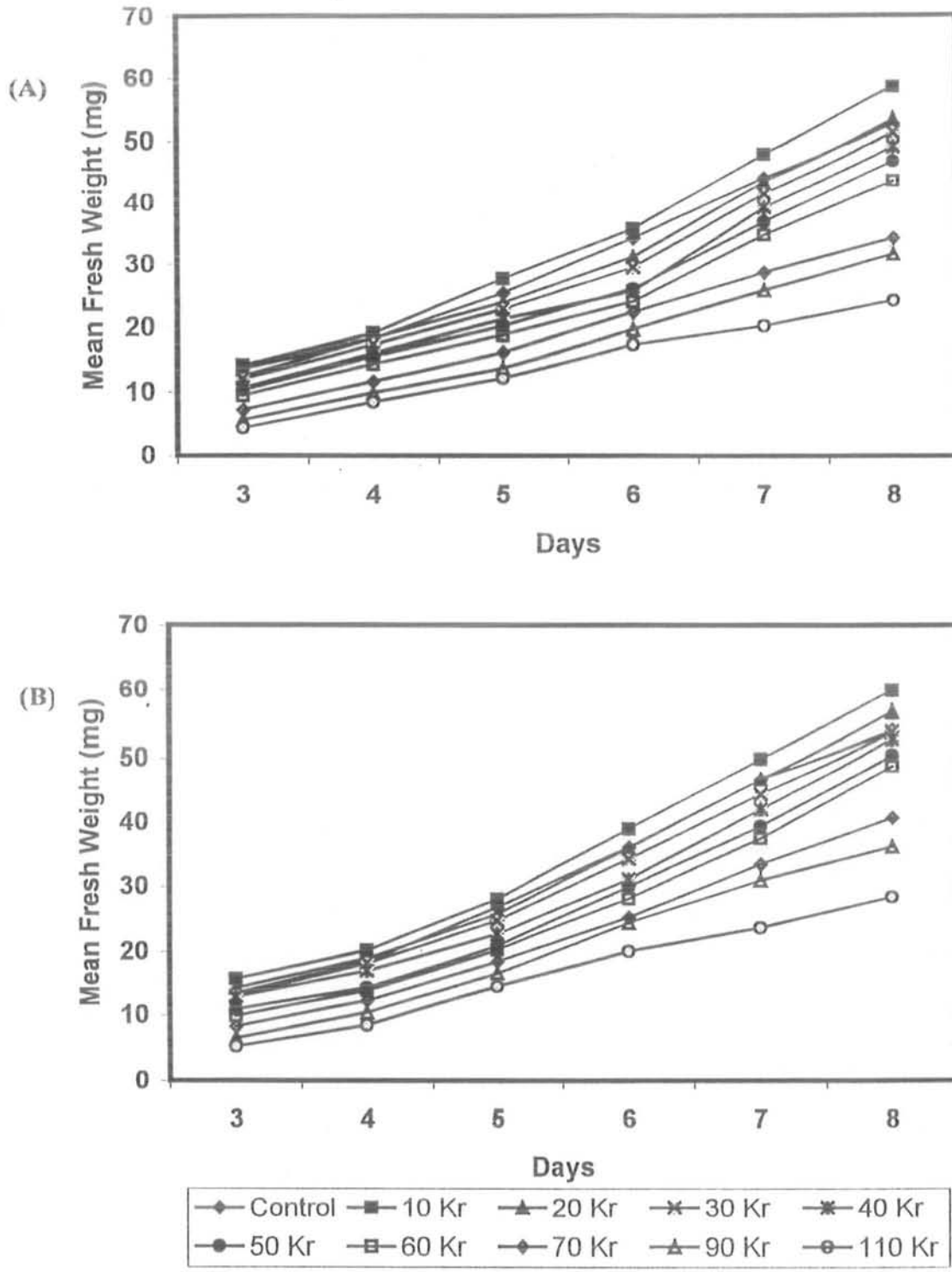
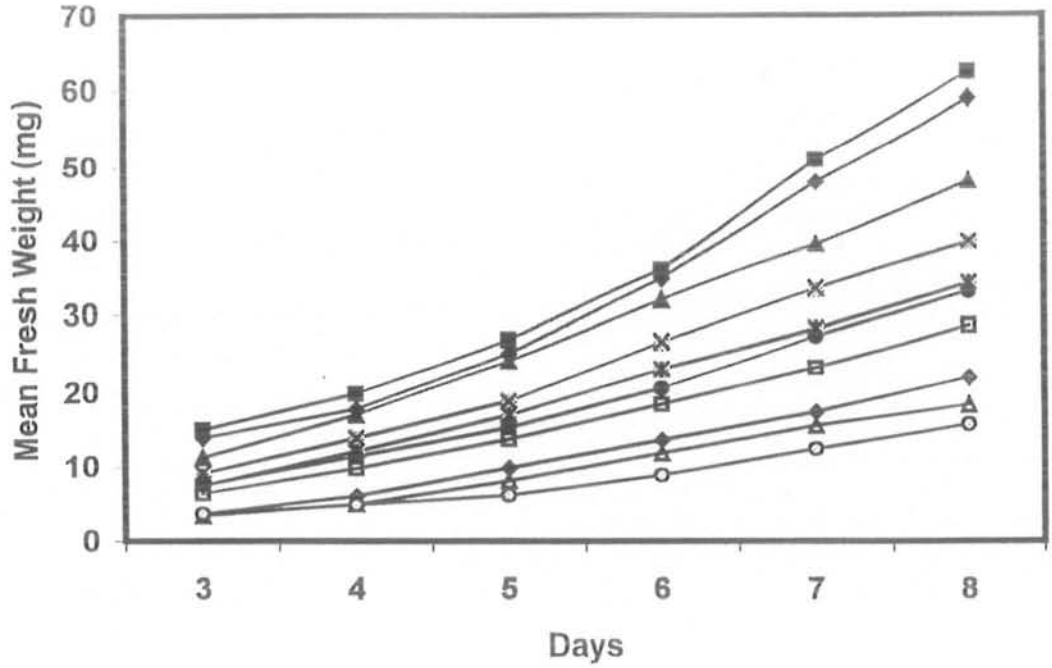


Fig. 15: The response of genotype Punjab 91 for fresh weight to (A) gamma irradiation separately and (B) with GA_3.

(A)



(B)

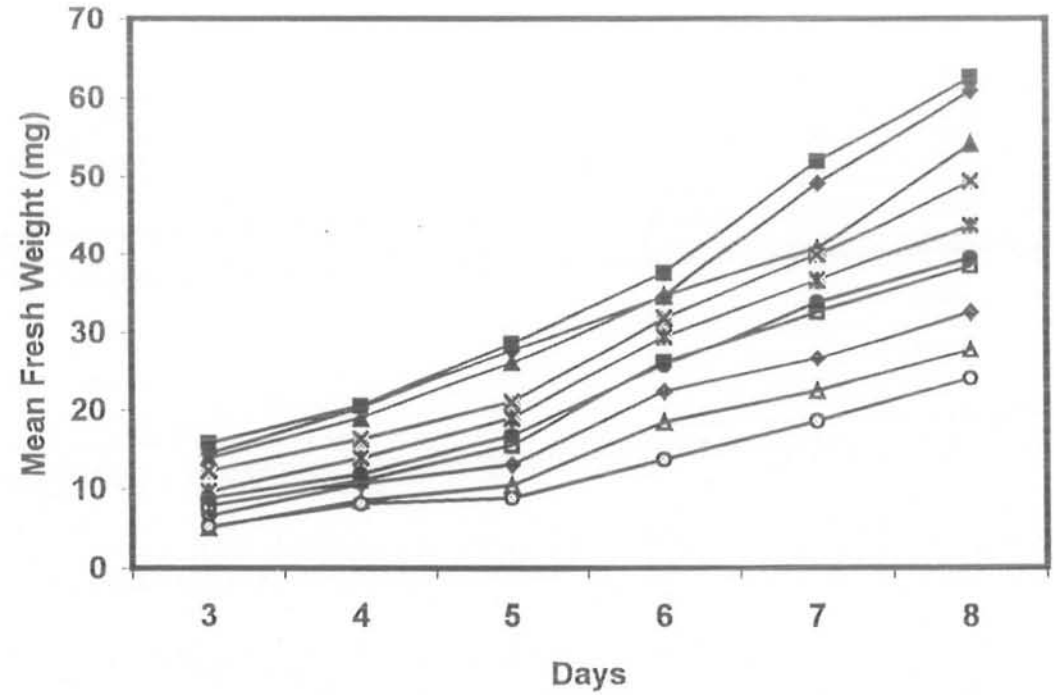


Fig. 16: The response of genotype C141 for fresh weight to (a) gamma irradiation separately and (b) with GA_3.

ii: CATALASE ACTIVITY (mM)

Effects of gamma irradiation separately and with the post mutagenic treatment of GA_3 on catalase activity was depicted in Fig 17-19. It is seen from the results that the three chickpea genotypes responded similarly to both mutagenic treatments. Catalase activity, a microbody marker, at 10 and 20 Kr of gamma irradiation decreased except on 5^{th} growth day where, an increase in activity was observed. A gradual decrease in catalase activity with an increase in gamma irradiation higher than 20 Kr was observed on third developmental day as compared to control. However, an increase in catalase activity was found at doses of 30 and 40 Kr on 4^{th} and 5^{th} day of seedling growth, while a gradual decrease in catalase activity was observed following the 5^{th} day.

Post mutagenic treatment of GA_3 increased the catalase activity with an increase of irradiation dosages except in Punjab 91 at 10 and 20 Kr doses as compared to the respective control.

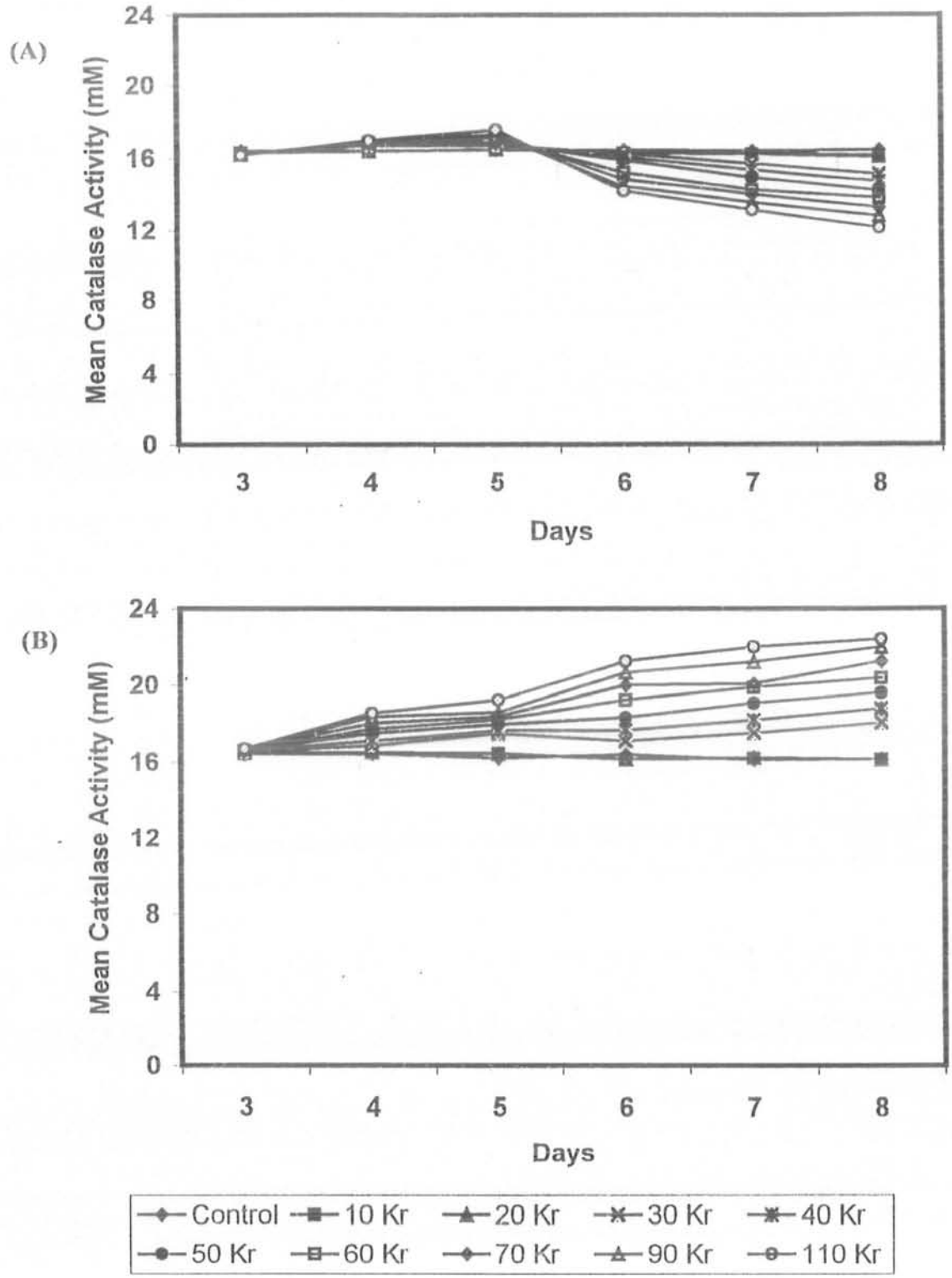


Fig. 17: The response of genotype Noor 91 for Catalase activity to (A) gamma irradiation separately and (B) with GA_3.

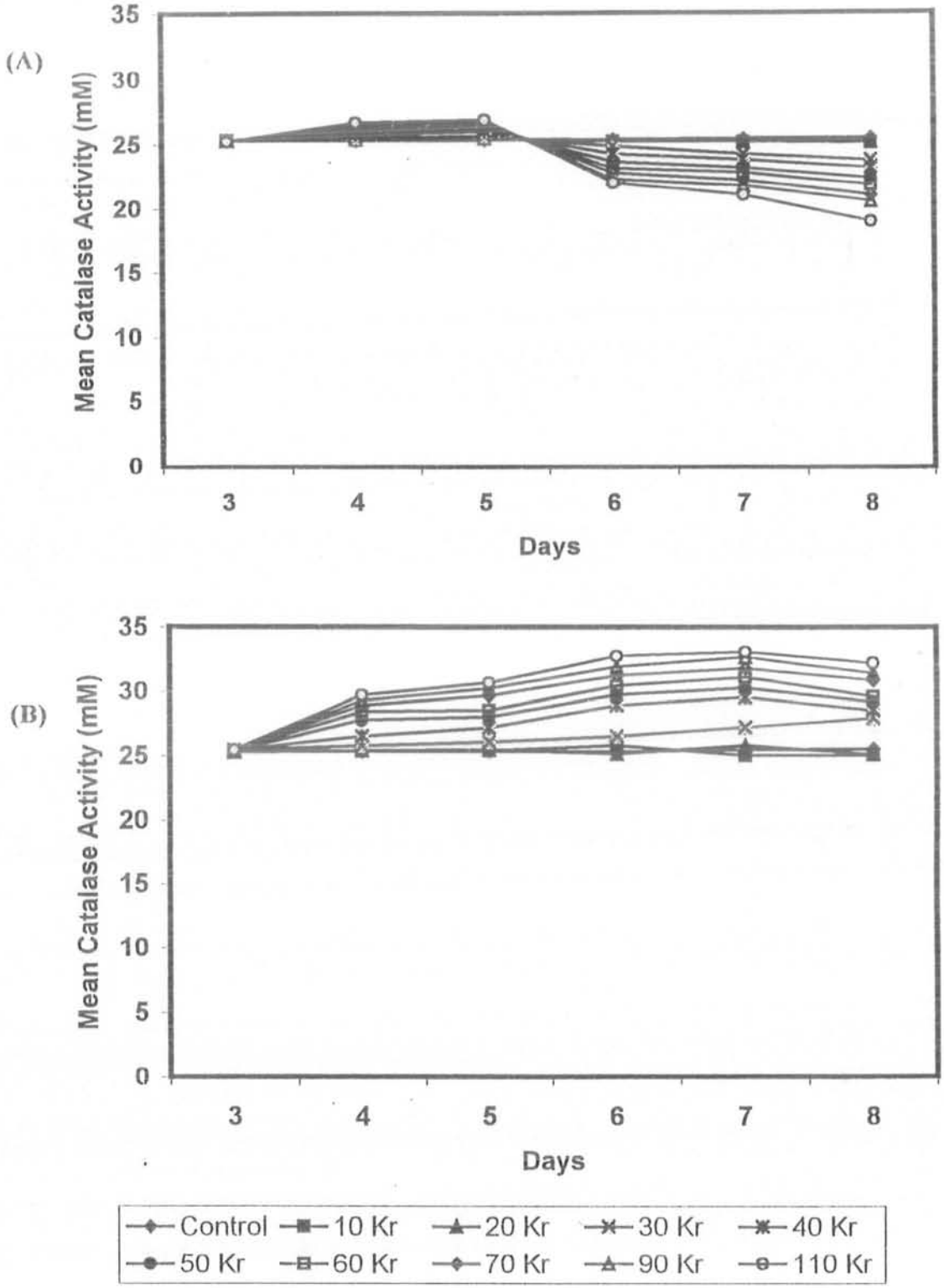


Fig. 18: The response of genotype Punjab 91 for Catalase activity to (A) gamma irradiation separately and (B) with GA_3.

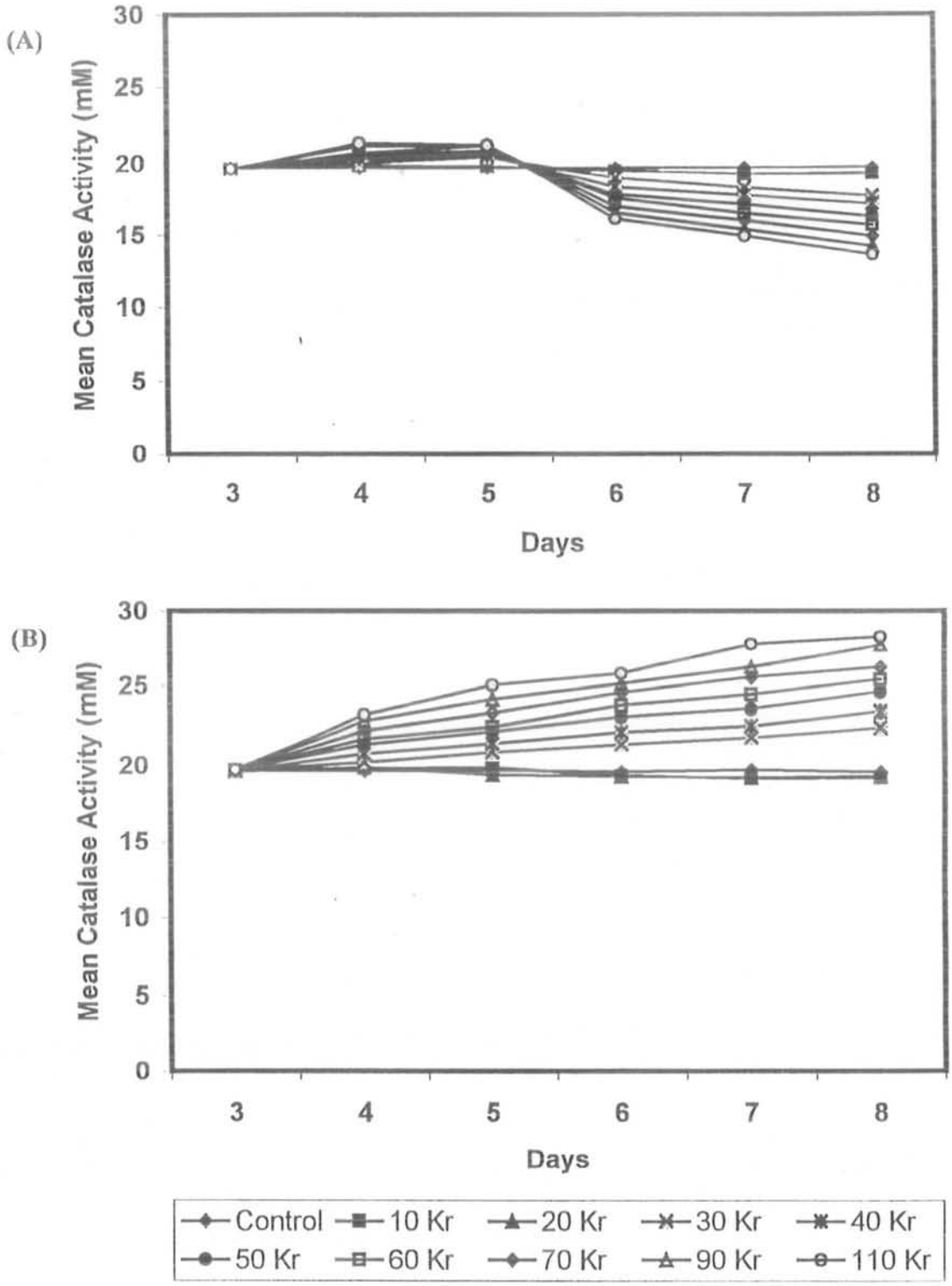


Fig. 19: The response of genotype C141 for Catalase activity to (A) gamma irradiation separately and (B) with GA_3.

iii: PEROXIDASE ACTIVITY (O.D.)

Effects of gamma irradiation separately and with post mutagenic treatment of GA_3 on peroxidase activity at different days of seedling developmental is shown in Fig 20-22. Peroxidase activity in Noor 91 increased regularly with gamma irradiation from 10 to 60 Kr until 5th day, while, with 70 to 110 Kr until 6th seedling developmental day, and following this a decrease in peroxidase activity was observed (Fig 20). Application of GA_3 increased the peroxidase activity at various doses by modulating the effects of gamma irradiation.

In Punjab 91 10 and 20 Kr of gamma irradiation did not had much effect on peroxidas activity. However, at higher doses peroxidase activity increased until 5th and 6th day after sowing and then decrease gradually for the following days. At 50 and 60 Kr of gamma irradiation peroxidase activity increased throughout the developmental period. Application of GA_3 increased the peroxidase activity throughout the developmental study at 70 Kr, while at 90 and 110 Kr this increase was until 6th and 5th day, respectively (Fig 21).

C 141 exhibited an erratic behaviour at 10, 20 and 30 Kr with both mutagenic treatments. Peroxidase activity increased throughout the developmental period from 40 to 70 Kr doses with both mutagenic treatments. However, this increase in peroxidase activity at 90 and 110 Kr with both mutagenic treatments was until 6th developmental day (Fig 22).

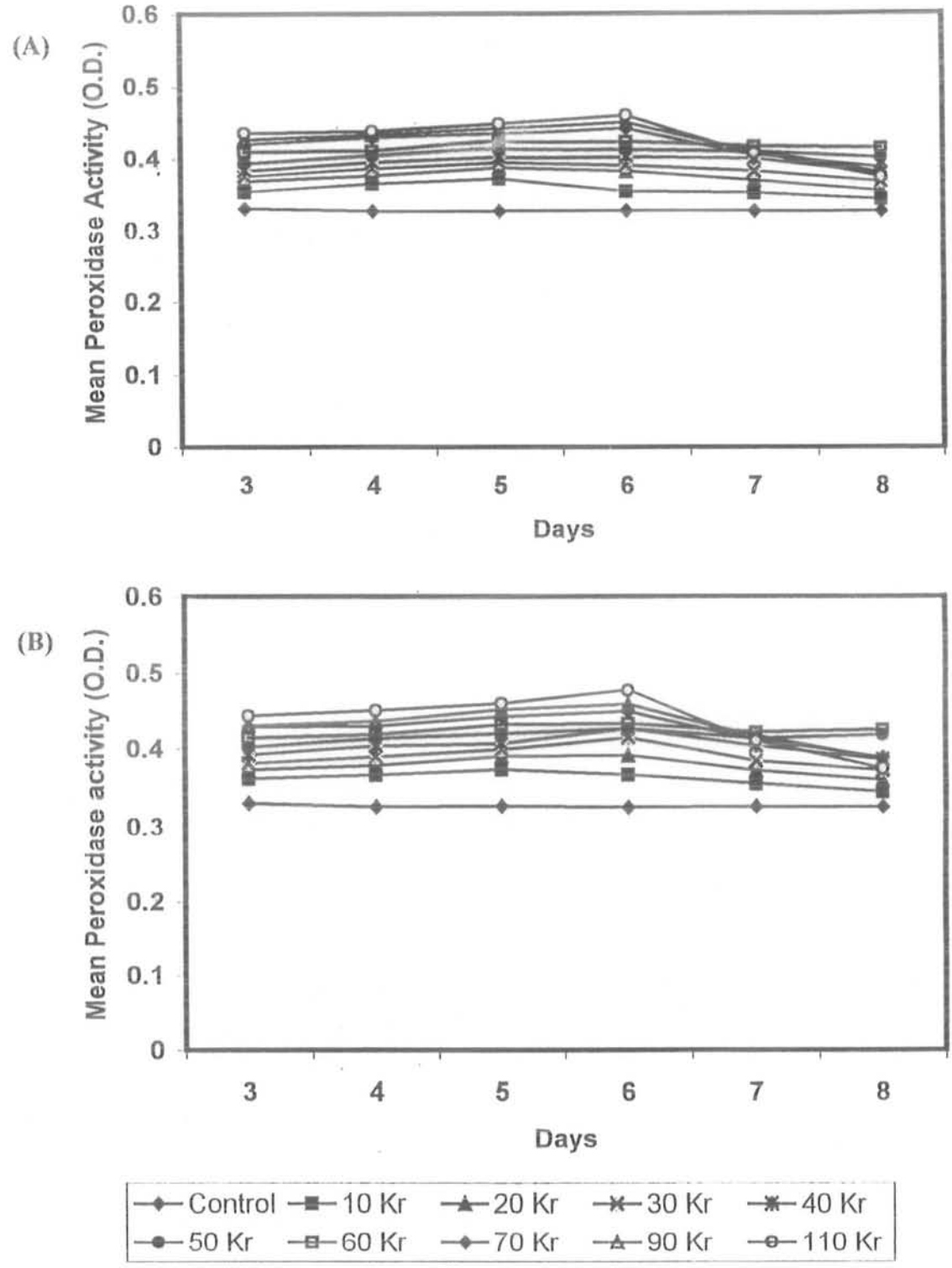


Fig. 20: The response of genotype Noor 91 for peroxidase activity to (A) gamma irradiation separately and (B) with GA_3.

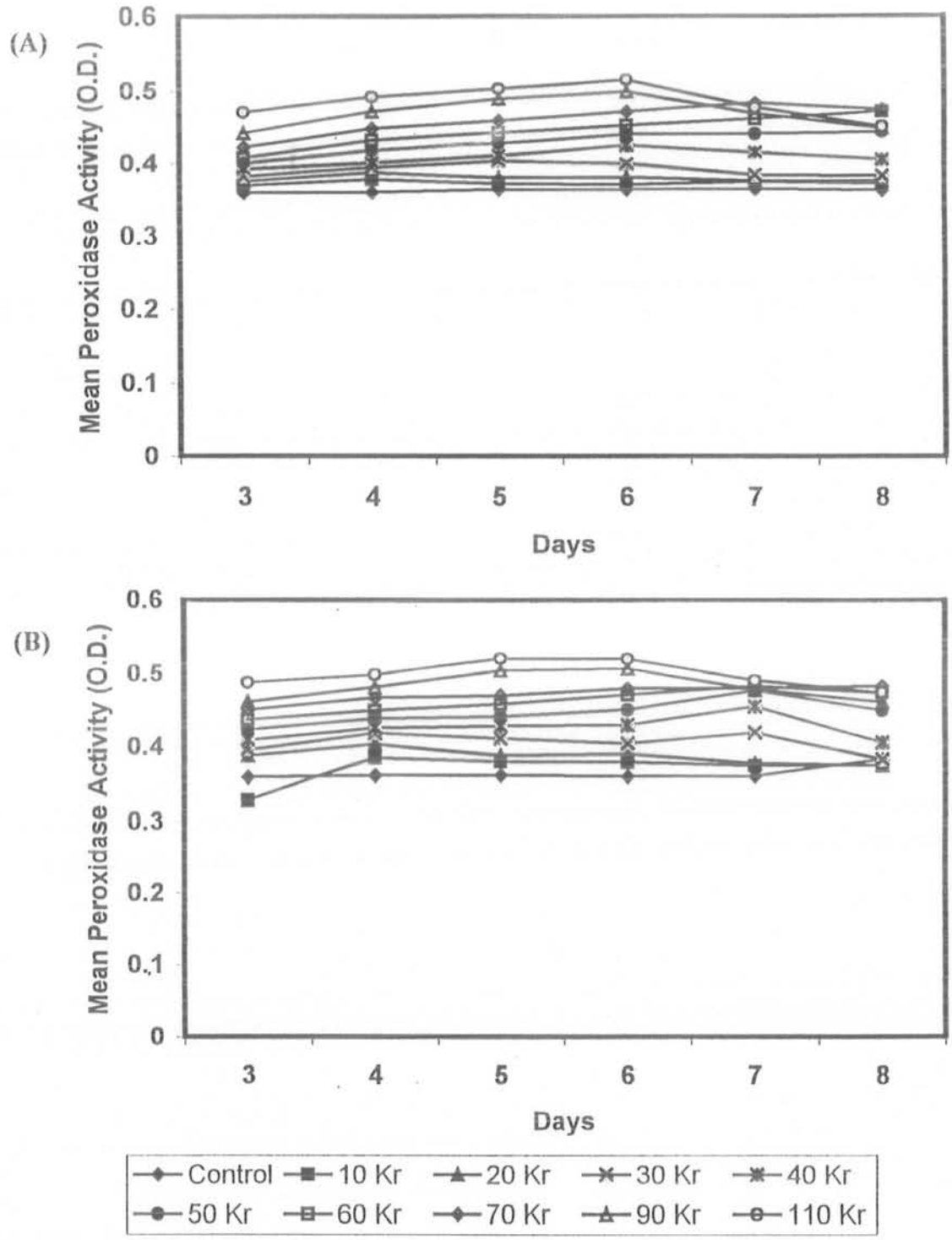


Fig. 21: The response of genotype Punjab 91 for peroxidase activity to (A) gamma irradiation separately and (B) with GA_3.

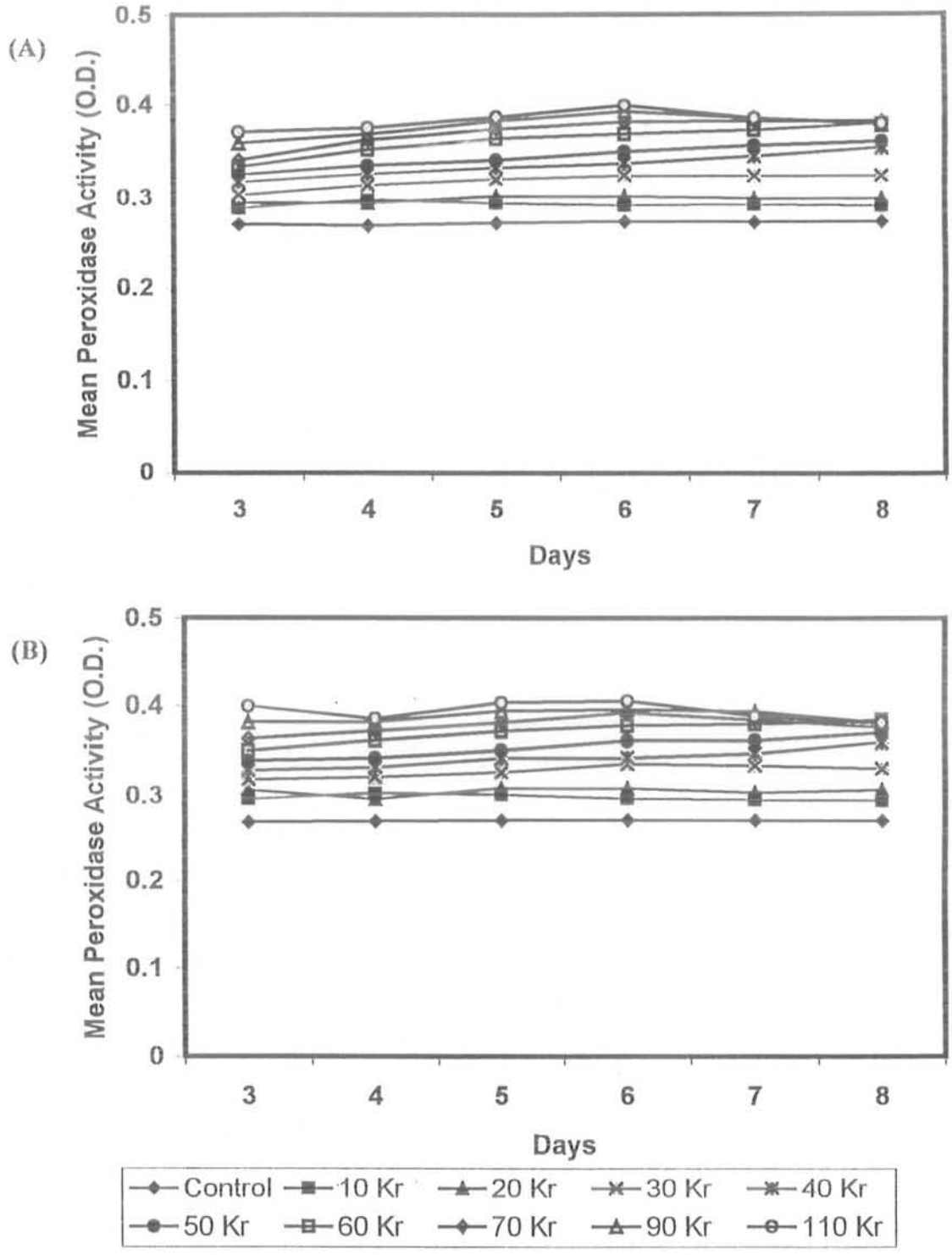


Fig. 22: The response of genotype C141 for peroxidase activity to (A) gamma irradiation separately and (B) with GA_3.

iv: INDOLE-3-ACETIC ACID (IAA) OXIDASE ACTIVITY (μg)

Effect of gamma irradiation separately and with postmutagenetic treatment of gibberellic acid on Indole Acetic Acid (IAA) oxidase activity are shown in Fig 23-25. It is evident from the results that the three genotypes responded similarly for IAA oxidase activity in shoot towards the both mutagenic treatments. IAA oxidase activity stimulated at 10 and 20 Kr doses of gamma irradiation and it was further increased with the treatment of GA_3 in all the three genotypes. From 30 Kr dose of gamma irradiation IAA oxidase activity decreased gradually with an increase in irradiation dosages and minimum activity was recorded at 110 Kr. However, application of GA_3 increased the IAA oxidase activity at different doses. In all the genotypes IAA oxidase activity increased throughout the developmental period at 10 and 20 Kr of gamma irradiation. However, from 30-110 Kr with both mutagenic treatments IAA oxidase activity decreased throughout the seedling growth.

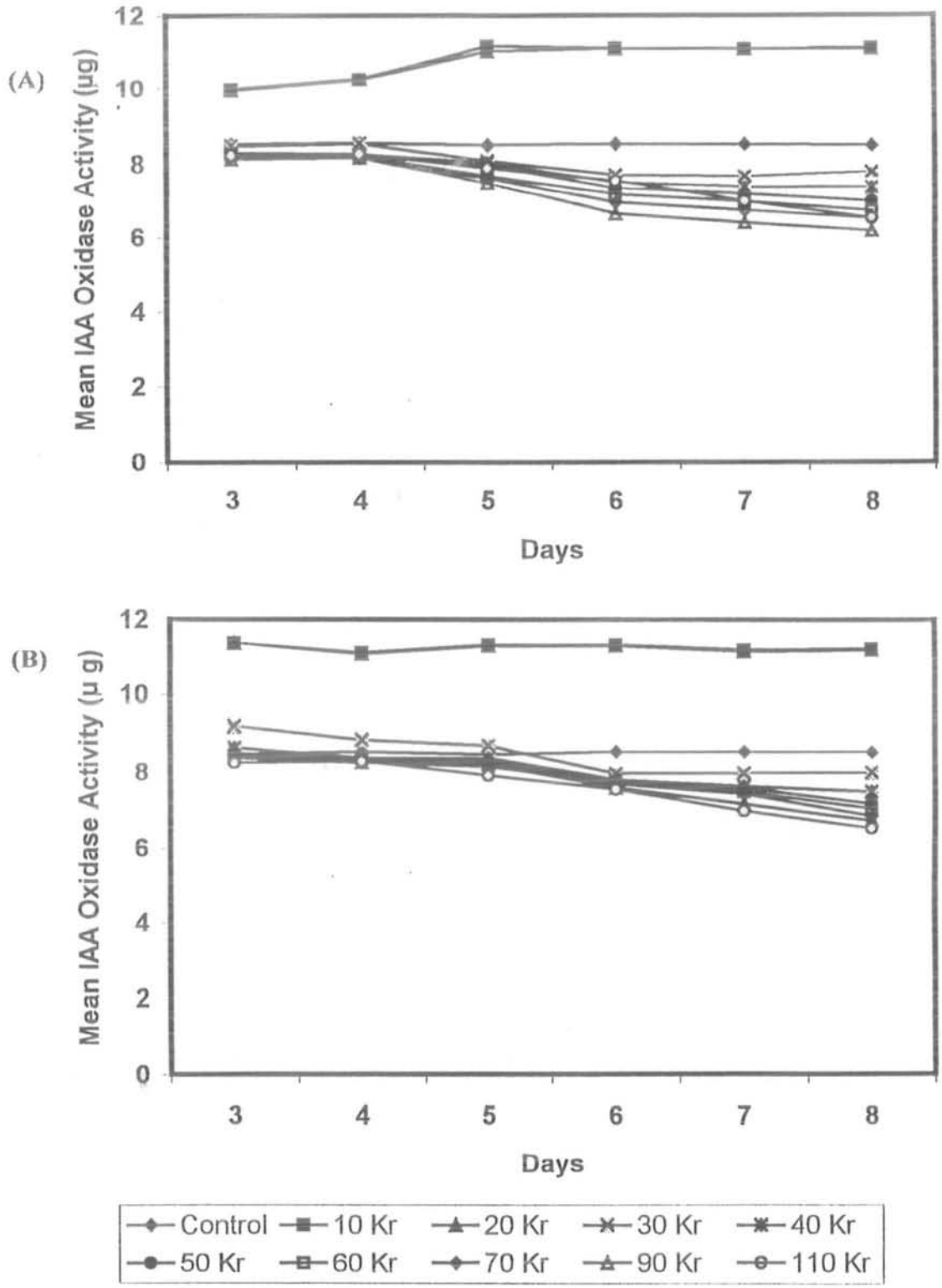


Fig. 23: The response of genotype Noor 91 for IAA oxidase activity to (A) gamma irradiation separately and (B) with GA_3.

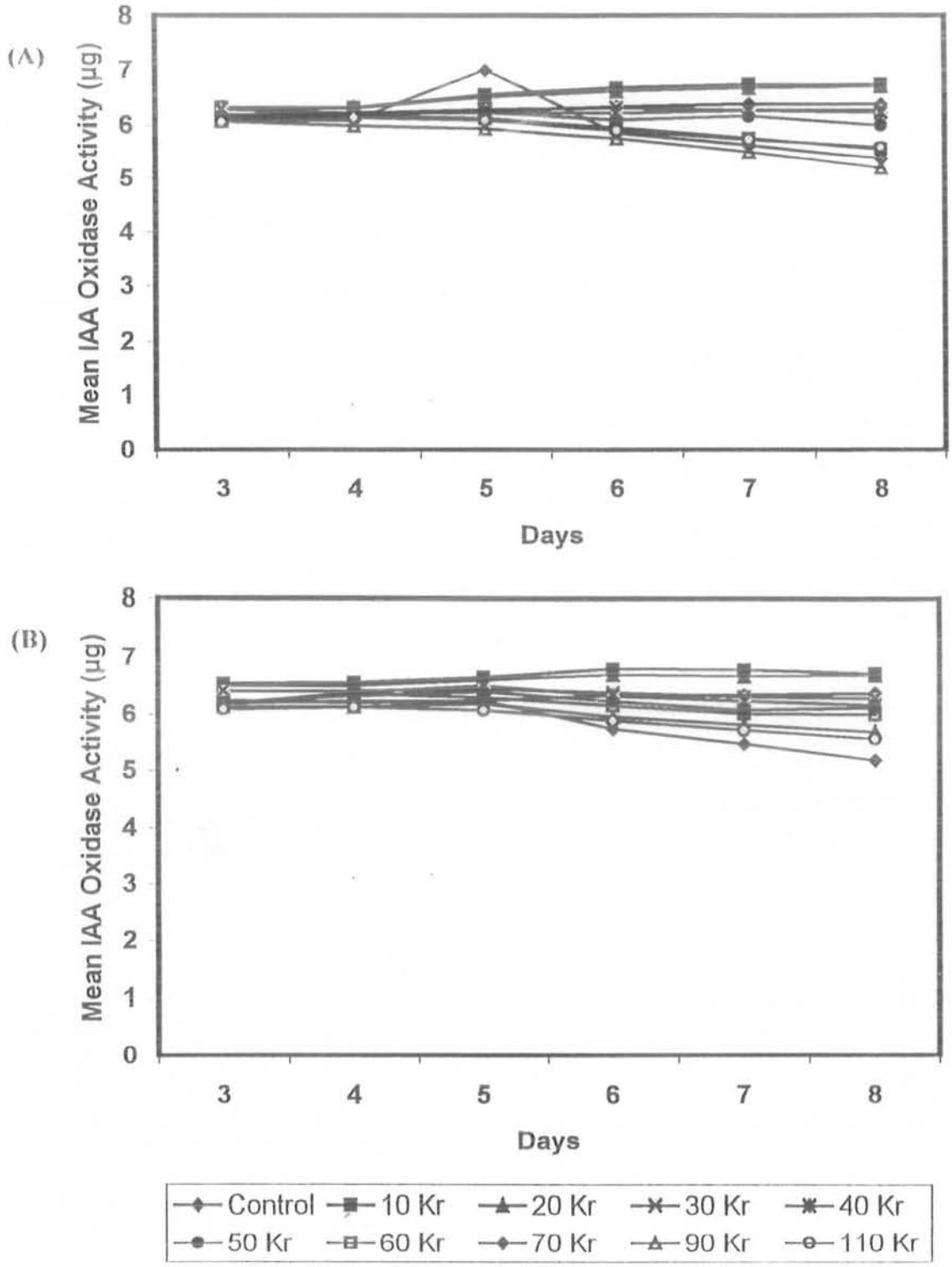


Fig. 24: The response of genotype Punjab 91 for IAA oxidase activity to (A) gamma irradiation separately and (B) with GA_3.

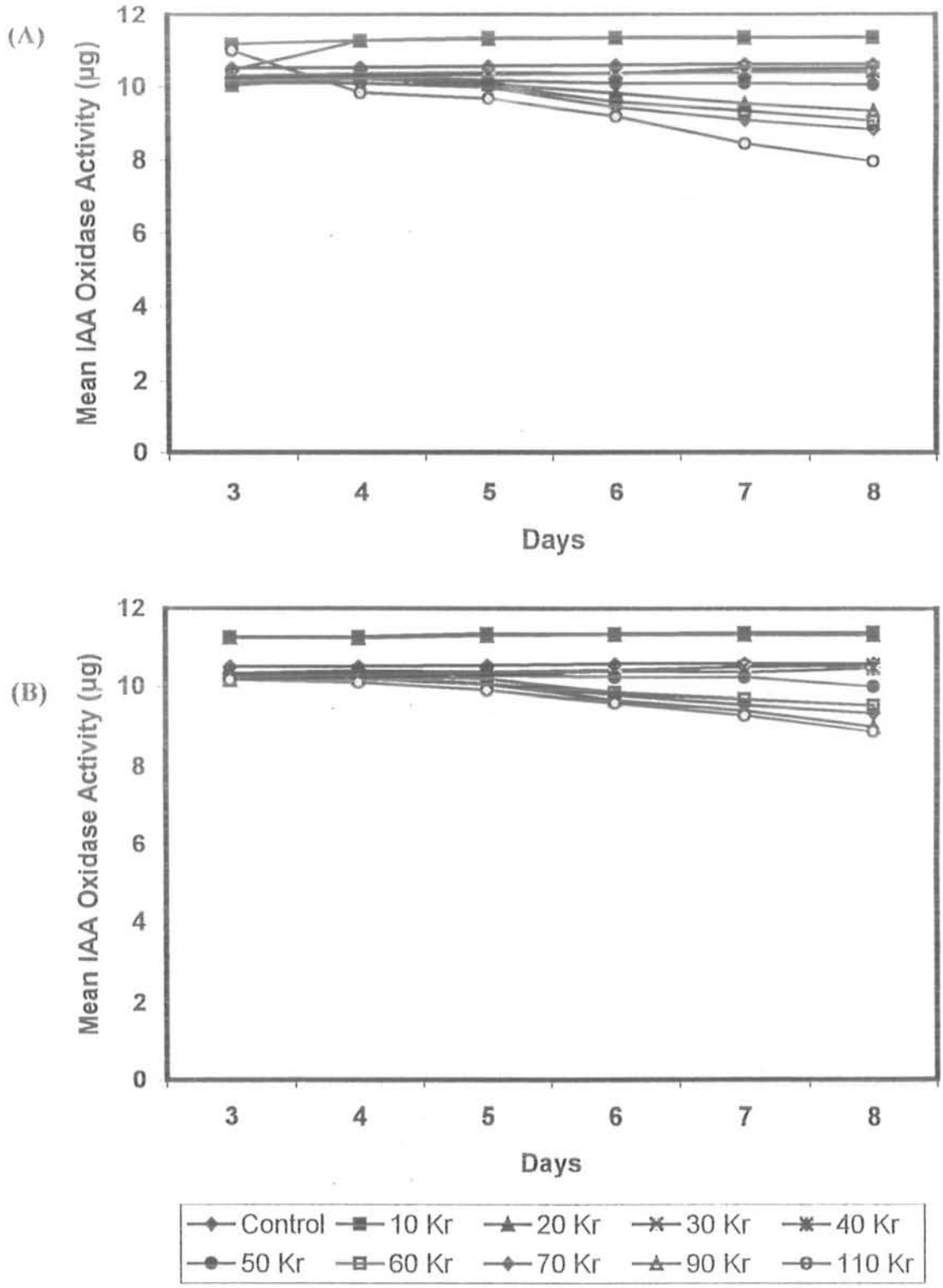


Fig. 25: The response of genotype C141 for IAA oxidase activity to (A) gamma irradiation separately and (B) with GA_3.

v: PROTEIN CONTENTS (mg)

Effect of gamma irradiation with and without the post mutagenic treatment of gibberellic acid on protein contents in shoot are shown in Fig 26-28. It is seen from the results that the three genotypes responded similarly to both mutagenic treatments. Protein contents decreased gradually with an increase in gamma irradiation except at 10 Kr treatment where stimulation as compared to control was noticed. Application of GA_3 increased the protein contents at different irradiation dosages.

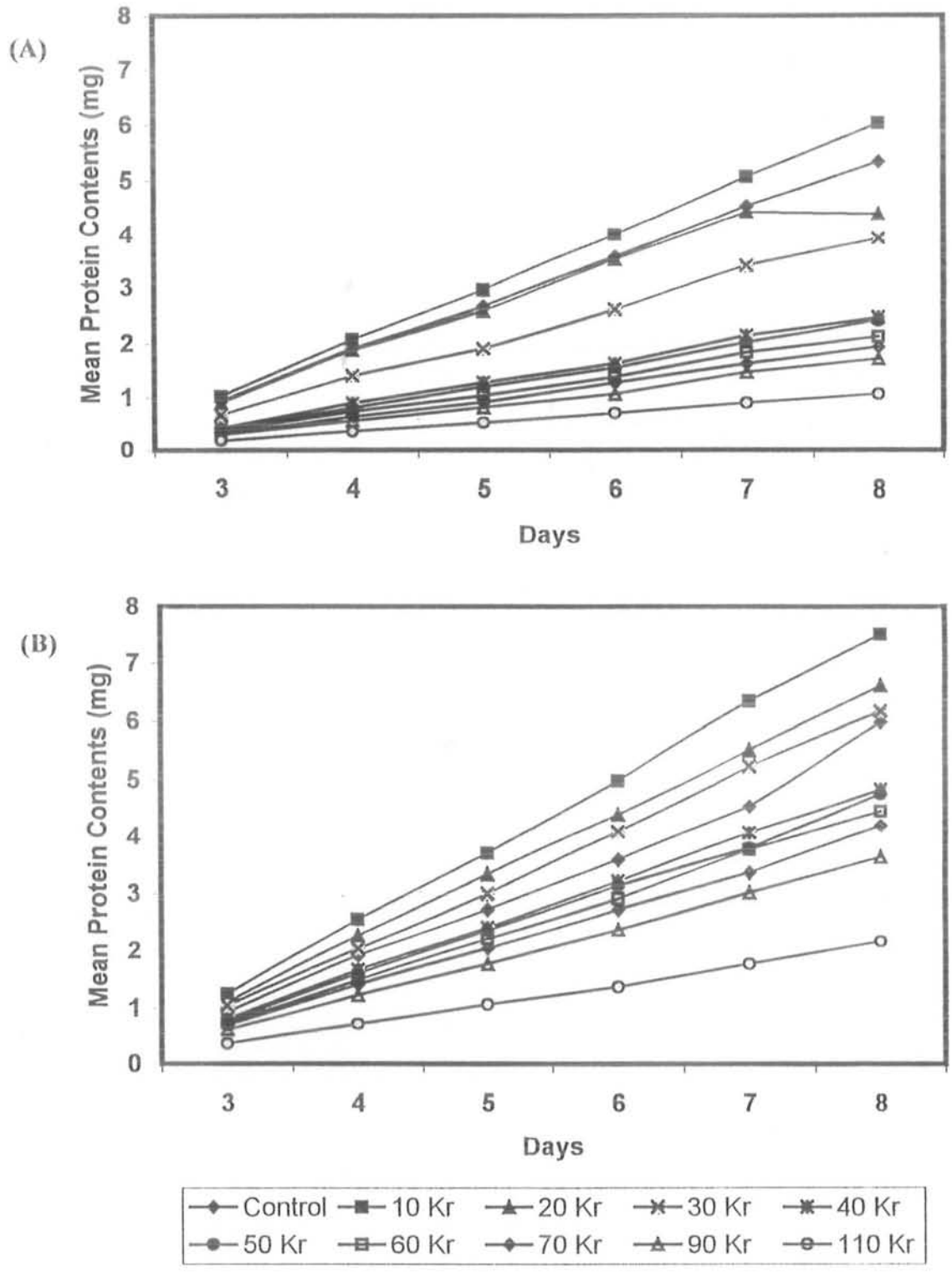


Fig. 26: The response of genotype Noor 91 for protein contents to (A) gamma irradiation separately and (B) with GA_3.

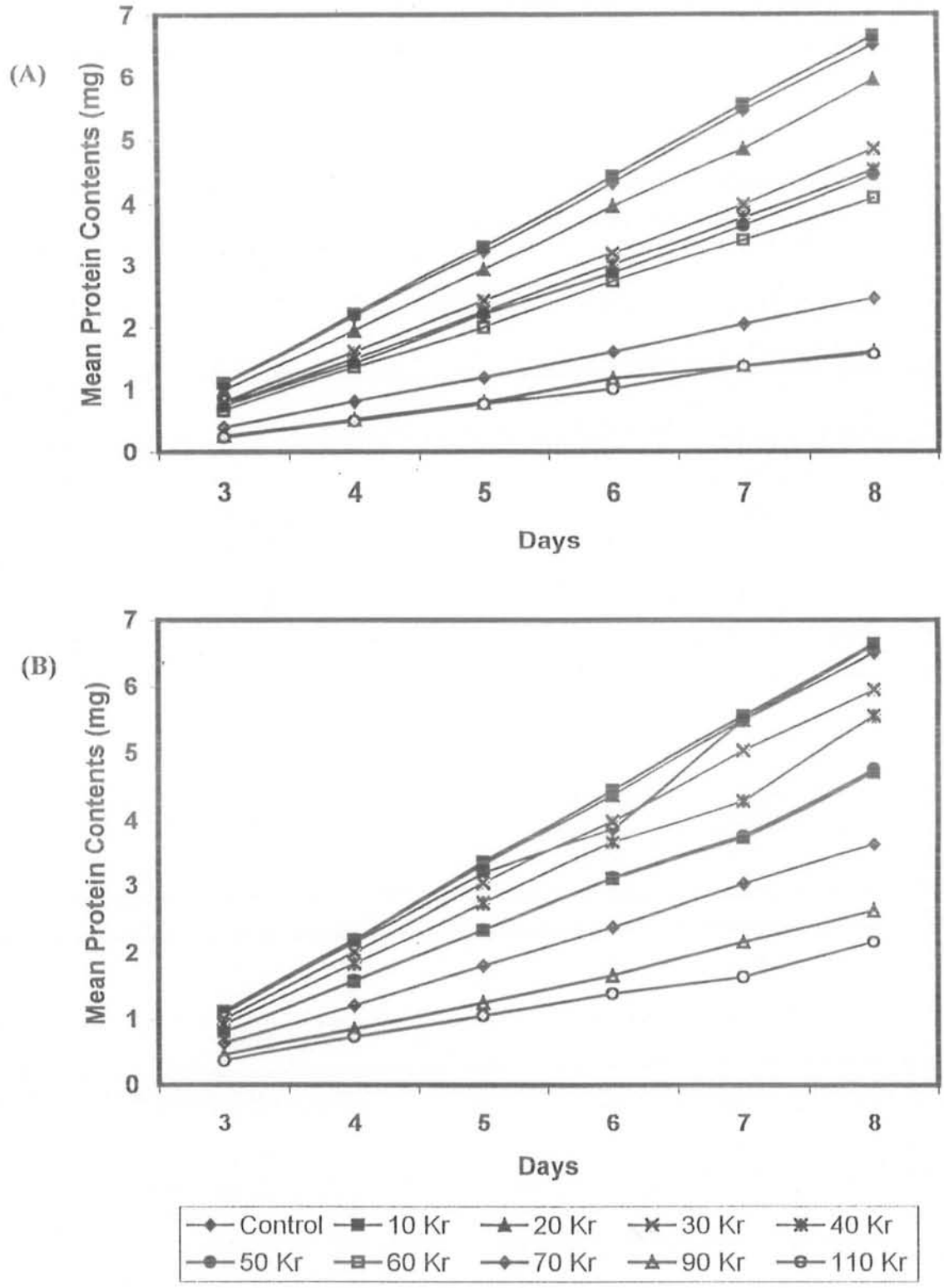


Fig. 27: The response of genotype Punjab 91 for protein contents to (A) gamma irradiation separately and (B) with GA_3.

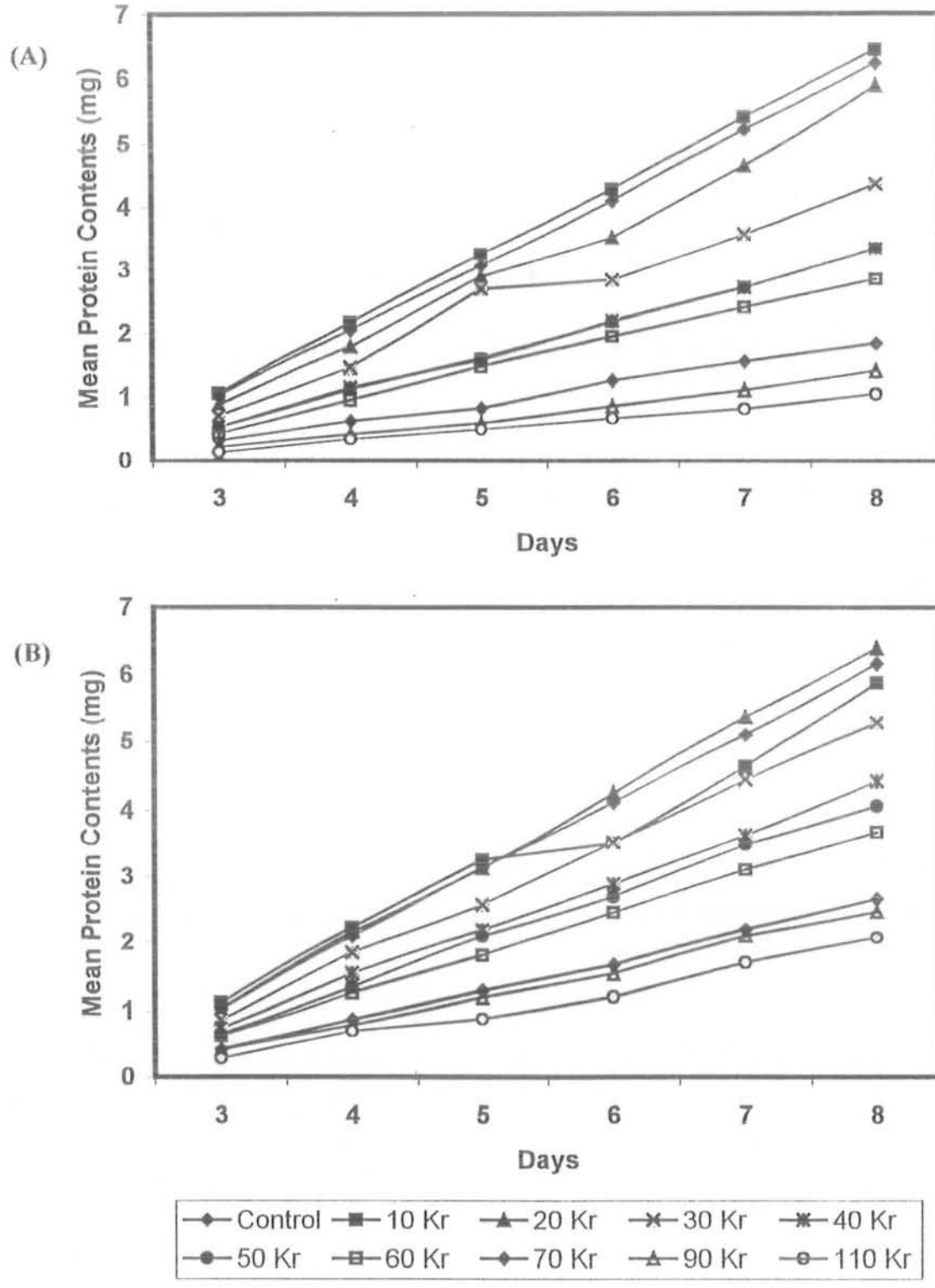


Fig. 28: The response of genotype C141 for protein contents to (A) gamma irradiation separately and (B) with GA_3.

vi: RIBONUCLEIC ACID (RNA) CONTENTS (µg)

Effect of gamma irradiation separately and with the post mutagenic treatment of gibberellic acid on RNA contents in shoot is shown in Fig 29-31. It is evident from the results that RNA contents in three genotypes responded similarly towards the both mutagenic treatments. RNA contents decreased regularly with an increase in gamma irradiation except at 10 Kr in Noor 91 and C 141, while at 10 and 20 Kr in Punjab 91. Treatment with GA_3 increased the RNA contents as compared to control at 10, 20 and 30 Kr in Noor 91, at 10 and 20 Kr in Punjab 91 and at 10 Kr in C 141. More RNA contents were observed with GA_3 at various irradiation treatments.

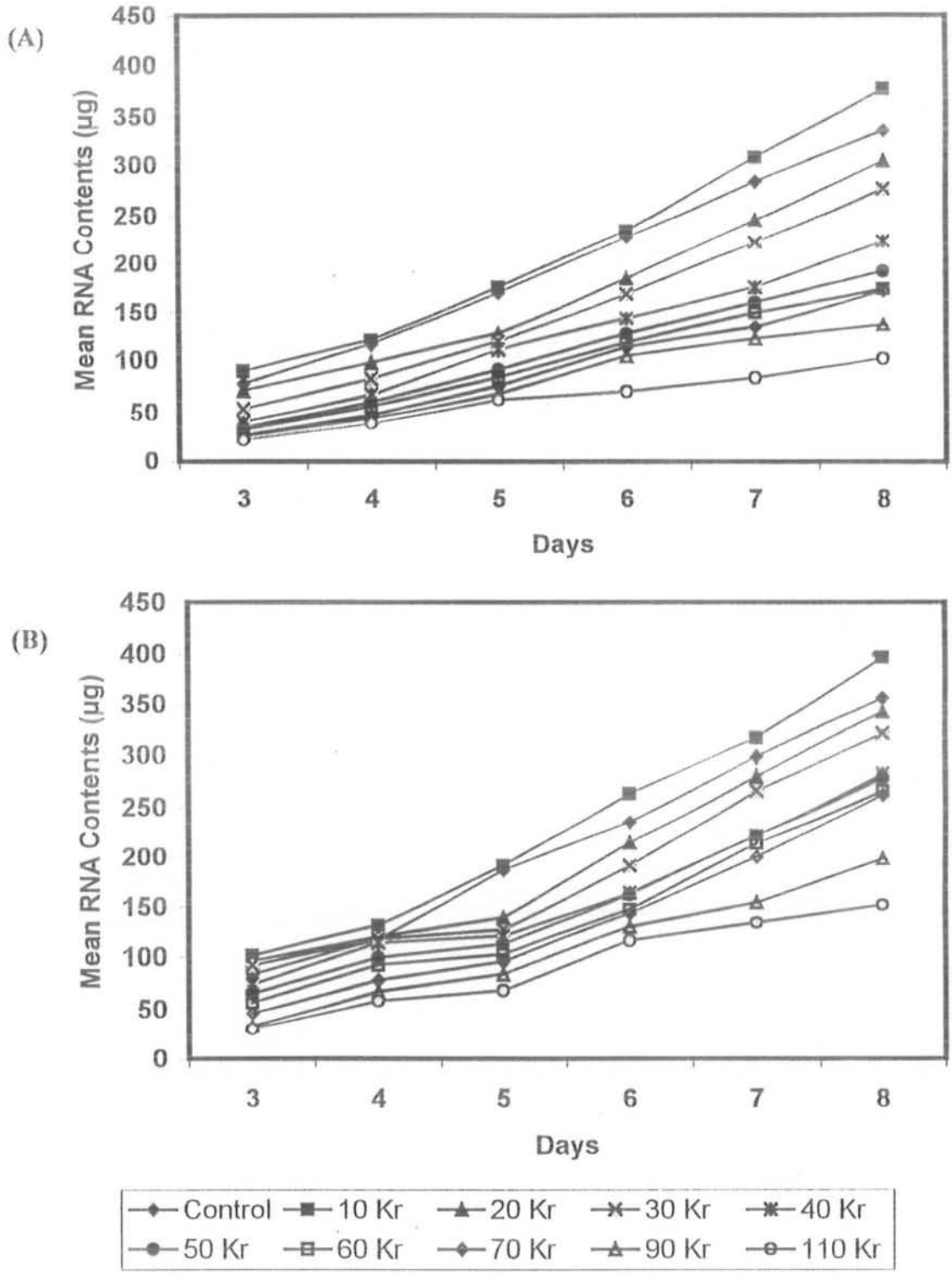


Fig. 29: The response of genotype Noor 91 for RNA contents to (A) gamma irradiation separately and (B) with GA_3.

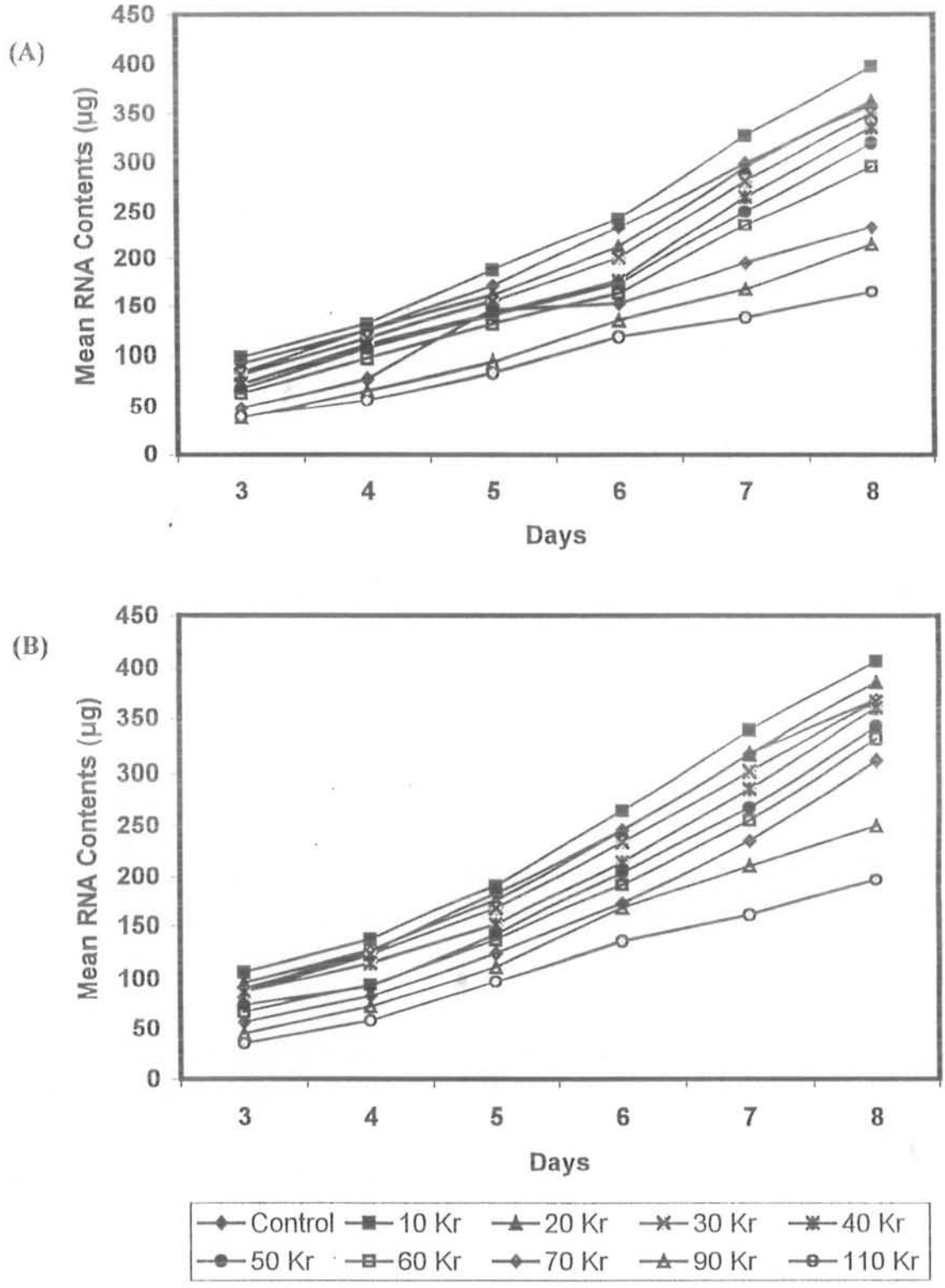


Fig. 30: The response of genotype Punjab 91 for RNA contents to (A) gamma irradiation separately and (B) with GA_3.

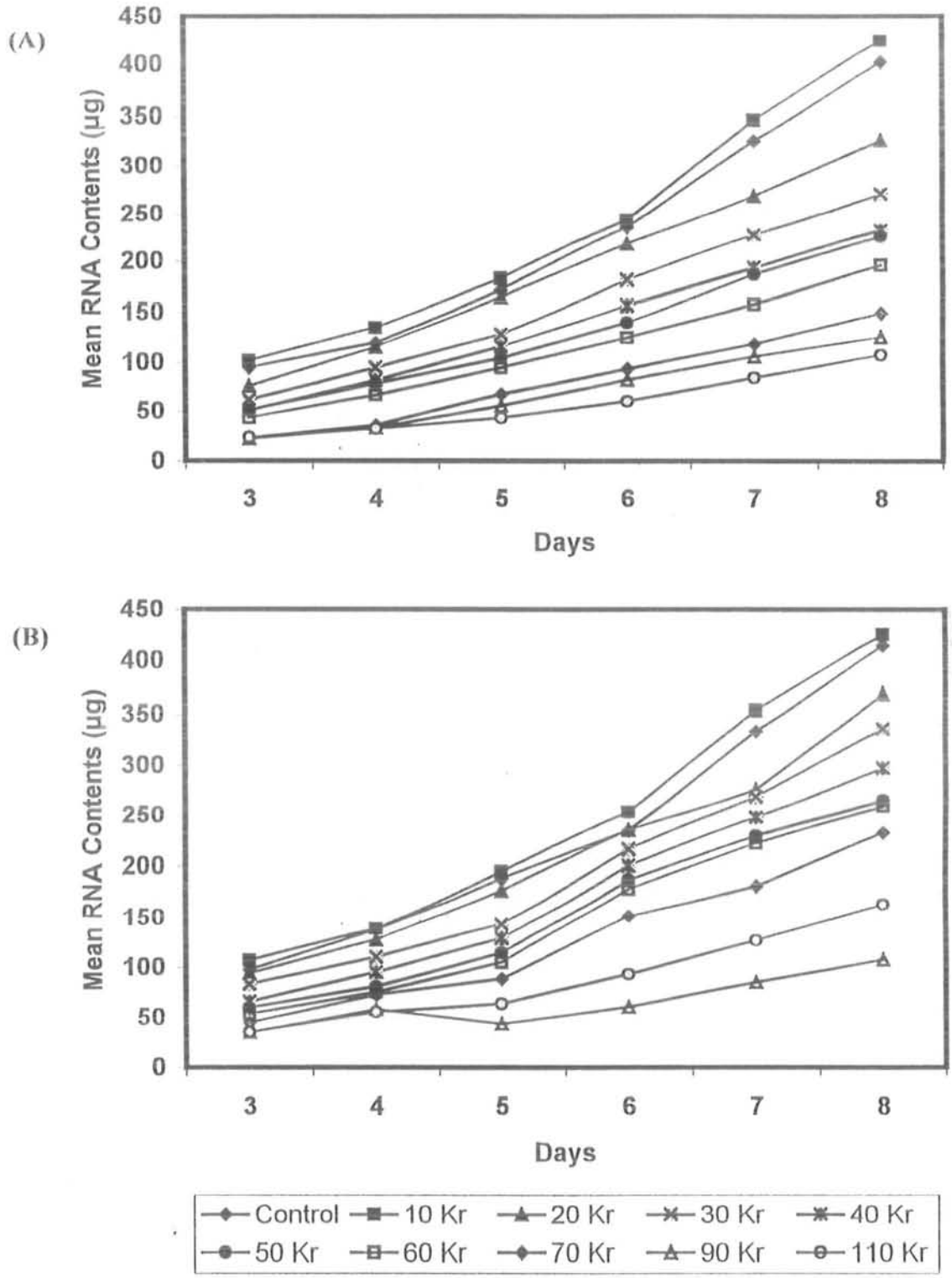


Fig. 31: The response of genotype C141 for RNA contents to (A) gamma irradiation separately and (B) with GA_3.

vii: DEOXYRIBONUCLEIC ACID (DNA) CONTENTS (µg)

Effect of gamma irradiation alone and with the post mutagenic treatment of gibberellic acid on DNA contents (µg shoot^{-1}) is shown in Fig 32-34. It is evident from the results that all the genotypes responded similarly for DNA contents towards the both mutagenic treatments. Treatment of gamma irradiation decreased the DNA contents except at 10 Kr dose, gradually with an increase in gamma irradiation dosage. On the other hand application of GA_3 at 10 and 20 Kr doses increased the DNA contents as compared to control. However, at higher doses more DNA contents were observed with GA_3 treatment.

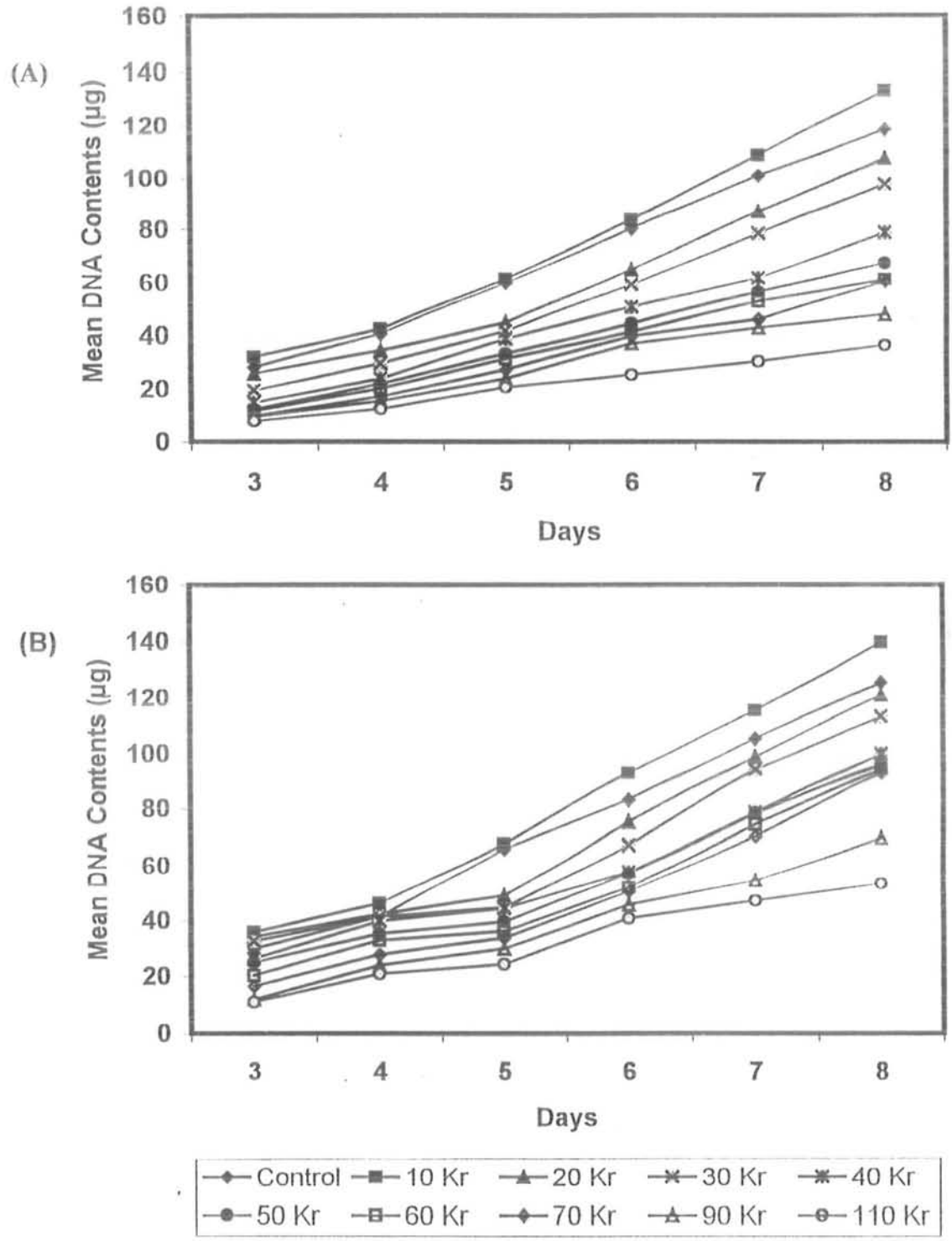


Fig. 32: The response of genotype Noor 91 for DNA contents to (A) gamma irradiation separately and (B) with GA_3.

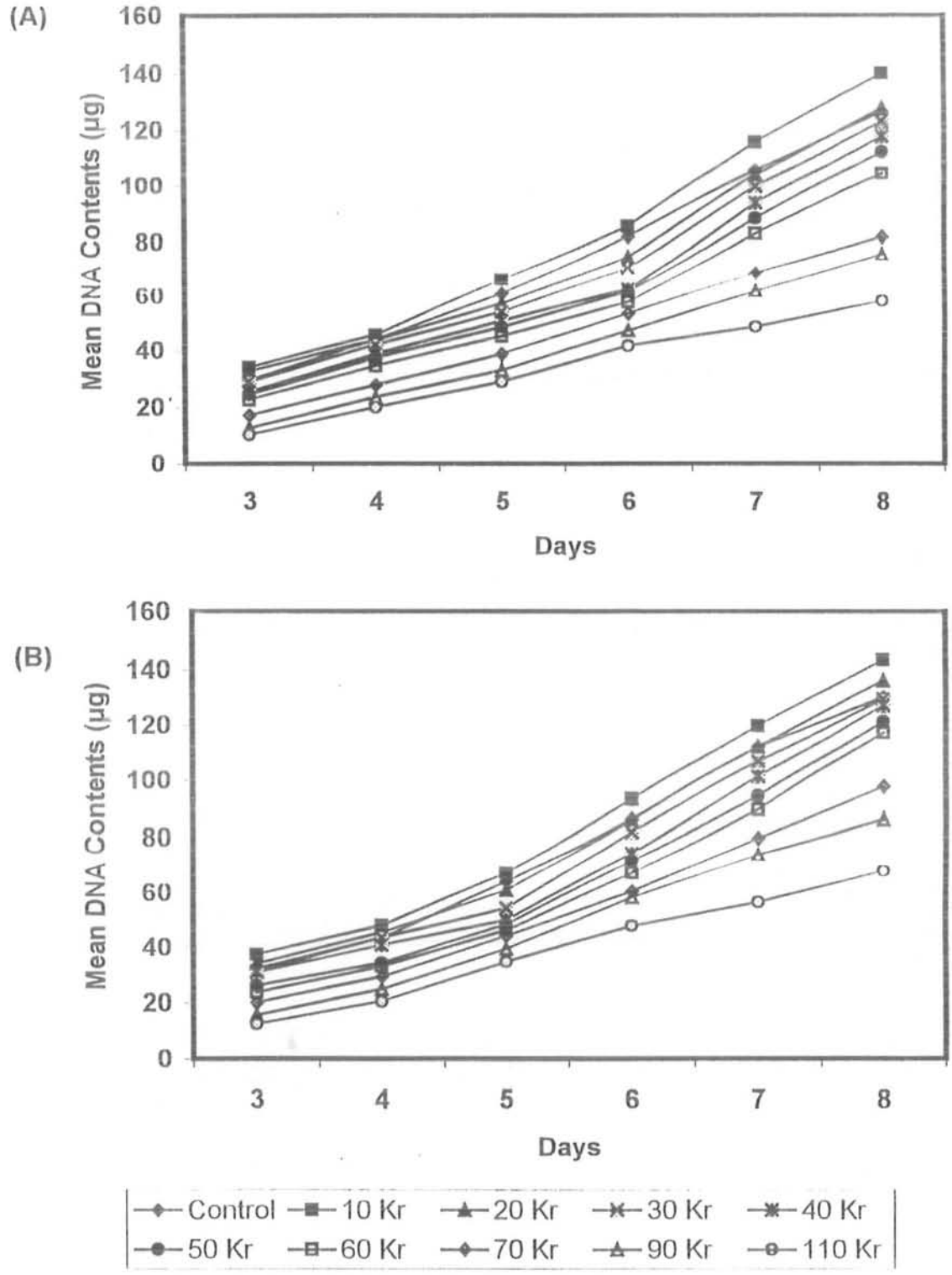


Fig. 33: The response of genotype Punjab 91 for DNA contents to (A) gamma irradiation separately and (B) with GA_3.

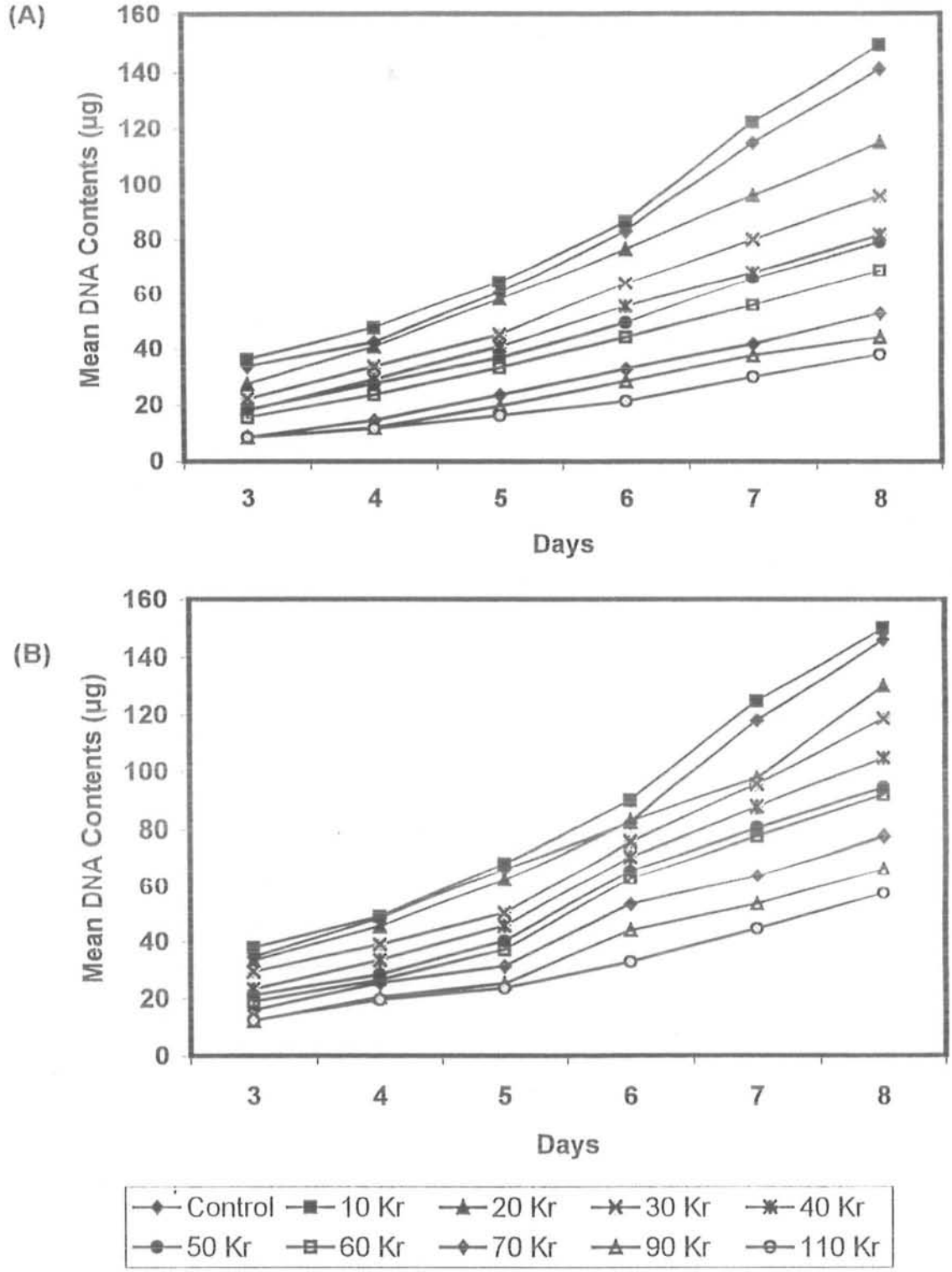


Fig. 34: The response of genotype C141 for DNA contents to (A) gamma irradiation separately and (B) with GA_3.

III: SEEDLING STUDIES

Seedling studies are considered as early manifestation of various mutagenic treatments. Gamma radiation are acknowledged to interfere with the biological system of an organism. A particular plant may die, or show altered pattern of growth in response to a particular treatment. These early studies are used to manipulate the particular dose of gamma radiation used to enhance the breeding programme by creating extra genetic variability. In mutation breeding, doses with low physiological effects and higher genetic effects are desirable. Gibberellic acid modulates the radio sensitivity by acting on various biochemical processes. So in this seedling study seeds of three chickpea genotypes were subjected to gamma irradiation to manipulate the proper dose.

i: GERMINATION PERCENTAGE

Analysis of variance for germination percentage is presented in table 8. The results show significant ($p<0.05$) and highly significant ($p<0.01$) differences among the genotypes and treatments, respectively. The interaction between genotype-treatment was non-significant ($p>0.05$). This reflects that the genotypes responded consistently across the various doses for this character.

Punjab 91 exhibited the highest germination percentage of 98.25% as compared to 97.62 and 95.25% of C 141 and Noor 91, respectively (Table 9). Germination percentage was not affected in Punjab 91 upto 30 Kr. The effect of radiation was gradual and with an increase in irradiation doses, germination percentage decreased with minimum at 110 Kr, 82.50, 90.00 and 90.00% in Noor 91, Punjab 91 and C141, respectively.

Germination percentage increased in irradiated seeds with gibberellic acid at various levels of irradiation. Germination was not affected upto 50, 60 and 70 Kr in Noor 91, C 141 and Punjab 91, respectively. Maximum recovery of 12.5% was observed in Noor 91 at 110 Kr treatment.

Table: 8 Analysis of variance for germination percentage under laboratory conditions in three chickpea genotypes with different doses of gamma radiation separately and with GA_3 (1995-96).

Source of variation	D. F.	Sum of squares	Mean of squares	F-value
Replicates	3	12.80	4.26	2.04
Varieties (V)	2	40.08	20.04	9.63*
Error (a)	6	3.58	0.59	-
Treatments (D)	19	314.12	16.53	7.94**
(VxD) interaction	38	53.24	1.40	0.67
Error (b)	171	356.18	2.08	-
Total	**239**	**780.00**		

*,** indicate significance at 5% and 1% level of probability, respectively.

Table: 9 **Effect of different doses of gamma radiation separately and with GA_3 on germination percentage in three genotypes of chickpea**

Treatment	Noor 91	Punjab 91	C 141	Treatment mean
Control				
Unsoaked	100.00 A	100.00 A	100.00 A	100.00 A
Soaked	100.00 A	100.00 A	100.00 A	100.00 A
Gamma radiation				
10 Kr	100.00 A	100.00 A	100.00 A	100.00 A
20 Kr	97.50 AB	100.00 A	100.00 A	99.16 AB
30 Kr	97.50 AB	100.00 A	97.50 AB	98.33 B
40 Kr	87.50 EF	97.50 AB	97.50 AB	94.16 E
50 Kr	92.50 CD	97.50 AB	97.50 AB	95.83 D
60 Kr	90.00 DE	97.50 AB	97.50 AB	94.16 E
70 Kr	90.00 DE	95.00 BC	92.50 CD	92.50 F
90 Kr	85.00 FG	92.50 CD	92.50 CD	90.00 G
110 Kr	82.50 G	90.00 D	90.00 D	87.50 H
Gamma radiation+GA_3(0.5 mM)				
10 Kr + GA_3	100.00 A	100.00 A	100.00 A	100.00 A
20 Kr + GA_3	100.00 A	100.00 A	100.00 A	100.00 A
30 Kr + GA_3	100.00 A	100.00 A	100.00 A	100.00 A
40 Kr + GA_3	100.00 A	100.00 A	100.00 A	100.00 A
50 Kr + GA_3	100.00 A	100.00 A	100.00 A	100.00 A
60 Kr + GA_3	97.50 AB	100.00 A	100.00 A	99.16A B
70 Kr + GA_3	95.00 BC	100.00 A	97.50 AB	97.50 C
90 Kr + GA_3	95.00 BC	97.50 AB	97.50 AB	96.66 CD
110 Kr + GA_3	95.00 BC	97.50 AB	95.00 BC	95.83 D
Means	**95.25 C**	**98.25 A**	**97.62 B**	

S.E. for variety mean =0.0858
S.E. for treatment mean=0.4163
S.E. for (VxD) interaction mean=0.8326
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

ii) SHOOT LENGTH

Analysis of variance for the effect of different doses of gamma irradiation on shoot length (table 10) revealed highly significant ($p<0.01$) differences among genotypes and treatments. Varieties x treatment interaction was also highly significant ($p<0.01$). It indicates highly inconsistent performance of genotypes for this character across different doses.

Punjab 91 exhibited the highest overall shoot length of 13.88 cm as compared to 10.30 and 9.73 cm of Noor 91 and C141, respectively (Table11). At lower doses of gamma irradiation stimulation in shoot growth was observed in all the genotypes. In Punjab 91, stimulating effect was observed at 10, 20, 30 Kr, while in Noor 91 and C 141 at 10 Kr dose. The shoot length decreased at higher doses with an increasing dose of gamma irradiation and maximum decrease in shoot length was observed at 110 Kr dose.

In case of post mutagenic treatment of gibberellic acid stimulation in shoot growth was recorded at all treatments in the three varieties. Maximum stimulating effect with an increase of 5.65 cm was observed in Noor 91 at 20 Kr treatment.

Table 10: Analysis of variance for shoot length in ten days old seedlings under laboratory conditions in three chickpea genotypes with different doses of gamma radiation separately and with GA_3 (1995-96).

Source of variation	D. F.	Sum of squares	Mean of squares	F-value
Replicates	3	3.71	1.23	2.51
Varieties (V)	2	813.17	406.58	829.75**
Error (a)	6	0.83	0.13	
Treatments (D)	19	3556.08	187.16	381.95**
(VxD) interaction	38	278.75	7.33	14.95**
Error (b)	171	85.21	0.49	
Total	**239**	**4737.75**		

** Indicate significance at 1% level of probability.

Table 11: Effect of different doses of gamma radiation separately and with GA_3 on shoot length of ten days old seedlings in three genotypes of chickpea.

Treatment	Noor 91	Punjab 91	C 141	Treatment means
Control				
Unsoaked	12.92 C	15.35 CDE	14.95 B	14.40 DE
Soaked	13.02 CD	15.60 CD	15.55 B	14.72 D
Gamma radiation				
10 Kr	14.25 BC	17.92 B	15.87 B	16.00 C
20 Kr	11.62 DE	17.70 B	11.70 C	13.67 E
30 Kr	10.50 EF	15.45 CDE	9.62 DE	11.85 G
40 Kr	6.92 G	15.00 CDEF	8.20 F	10.00 H
50 Kr	6.67 G	13.67 FG	8.32 EF	9.55 H
60 Kr	6.55 G	12.65 GH	6.40 H	8.531
70 Kr	5.02 HI	9.75 JK	3.821	6.20 KL
90 Kr	4.57 IJ	9.15 K	3.521	5.75 L
110 Kr	3.52 J	6.42 L	2.951	4.30 M
Gamma radiation + GA_3 (0.5 mM)				
10 Kr + GA_3	18.15 A	19.52 A	16.15 A	17.94 A
20 Kr + GA_3	17.27 A	18.22 B	15.37 B	16.96 B
30 Kr + GA_3	14.57 B	16.17 C	12.27 C	14.34 DE
40 Kr + GA_3	13.97 BC	16.10 C	10.37 D	13.48 EF
50 Kr + GA_3	12.47 D	14.55 DEF	9.60 DE	12.20 G
60 Kr + GA_3	11.82 DE	14.37 EF	9.52 DE	11.90 G
70 Kr + GA_3	10.22 F	11.45 HI	7.82 FG	9.83 H
90 Kr + GA_3	6.27 GH	10.65 IJ	6.62 GH	7.85 J
110 Kr + GA_3	5.72 GHI	8.00 K	5.82 H	6.50 KL
Means	**10.30 B**	**13.88 A**	**9.73 C**	

S.E. for variety mean =0.0403
S.E. for treatment mean=0.2020
S.E. for (VxD) interaction means=0.3500
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

iii: ROOT LENGTH

Table 12 shows the analysis of variance for the effect of mutagenic treatments on root length. The results revealed highly significant differences ($p<0.01$) among the genotypes and treatments. The interaction between genotype-treatment was also highly significant ($p<0.01$). This indicates high variation in their performance across the various treatments for this character.

On the average, maximum root length of 11.72 cm was observed in Punjab 91 as compared to 10.82 and 9.20 cm of C141 and Noor 91, respectively (Table 13). The effect of gamma irradiation was gradual and with an increase in irradiation dose, the root length decreased with minimum at 110 Kr, 4.92, 5.05 and 3.27 cm in Noor 91, Punjab 91 and C 141, respectively. In Punjab 91 a buffering action for root length was noticed at 20, 30, 50, 60 and 70 Kr and then a very sharp decrease of 4.65 cm was observed from 70 Kr to 90 Kr.

Gibberellic acid treatment stimulated the root growth at different levels of gamma irradiation in all genotypes. Maximum increase of 4.15 cm was noticed at 10 Kr in Punjab 91.

Table 12 Analysis of variance for root length in ten days old seedlings under laboratory conditions in three chickpea genotypes with different doses of gamma radiation separately and with GA_3 (1995-96).

Source of variation	D. F.	Sum of squares	Mean of squares	F-value
Replicates	3	1.64	0.54	1.10
Varieties (V)	2	261.28	130.64	266.61**
Error (a)	6	8.25	1.37	
Treatments (D)	19	2687.55	141.45	288.67**
(VxD) interaction	38	648.62	17.06	34.81**
Error (b)	171	85.10	0.49	
Total	**239**	**3692.44**		

** Indicate significance at 1% level of probability.

Table 13: Effect of different doses of gamma radiation separately and with GA_3 on root length of ten days old seedlings in three genotypes of chickpea.

Treatment	Noor 91	Punjab 91	C 141	Treatment means
Control				
Unsoaked	13.72A	15.87B	17.52 AB	15.70 B
Soaked	13.60 A	15.92 B	17.77 A	15.76 B
Gamma radiation				
10 Kr	12.17 BC	15.60 B	15.30 C	14.35 C
20 Kr	12.20 BC	11.55 FG	12.70 DE	12.15 E
30 Kr	10.15 D	10.85 GH	12.17 EF	11.05 F
40 Kr	8.37 E	9.65 HI	10.37 G	9.46 H
50 Kr	8.07 E	10.05 H	7.17 I	8.43 I
60 Kr	6.17 FG	10.05 H	6.07 I	7.43 J
70 Kr	5.25 GH	10.12 H	6.35 I	7.24 J
90 Kr	5.72 FG	5.47 K	4.62 J	5.27 K
110 Kr	4.92 H	5.05 K	3.27 K	4.40L
Gamma radiation + GA_3 (0.5 mM)				
10 Kr + GA_3	12.80 AB	19.75 A	16.42 BC	16.32 A
20 Kr + GA_3	10.85 CD	14.97 BC	15.57 C	13.80 C
30 Kr + GA_3	10.75 D	14.22 CD	13.90 D	12.95 D
40 Kr + GA_3	13.75 A	13.05 DE	12.05 EF	12.95 D
50 Kr + GA_3	10.02 D	12.10 EFG	12.07 EF	11.40 F
60 Kr + GA_3	7.05 EF	12.37 EF	11.27 FG	10.23 G
70 Kr + GA_3	7.10 EF	12.27 EF	8.70 H	7.70 IJ
90 Kr + GA_3	6.02 F	8.62 I	7.07 I	7.24 J
110 Kr + GA_3	5.32 GH	7.07 J	6.02 I	6.14 K
Means	**9.20 C**	**11.72 A**	**10.82 B**	

S.E. for variety mean =0.1308

S.E. for treatment mean=0.2020

S.E. for (VxD) interaction means=0.3500

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

iv: NUMBER OF ROOTS

The results of analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on the number of roots (Table 14) revealed indicates highly significant ($p<0.01$) differences within the treatments and genotypes. The interaction between genotype-treatment was also highly significant ($p<01$). It indicates highly inconsistent performance of genotypes across the various doses.

The results regarding the number of roots in the varieties indicated a maximum of 15.77 per plant in Punjab 91 followed by 11.88 and 10.57 per plant in Noor 91 and C 141, respectively (Table 15). There was a gradual decrease in number of roots with an increase in the radiation doses. The number of roots decreased with minimum at 110 Kr, 2.05, 2.95 and 6.80 in C 141, Noor 91 and Punjab 91, respectively.

In case of gibberellic acid treatment, a profound increase in the number of roots was observed at all the levels of gamma irradiation. Punjab 91 appears to be more responsive towards the gibberellic acid treatment and a maximum increase of 5.55 in the number of roots was observed at 10 Kr treatment.

Table 14: Analysis of variance for number of roots in ten days old seedlings under laboratory conditions in three chickpea genotypes with different doses of gamma radiation separately and with GA_3 (1995-96)

Source of variation	D. F.	Sum of squares	Mean of squares	F-value
Replicates	3	88.77	29.59	31.81
Varieties (V)	2	1169.05	584.52	628.51**
Error (a)	6	32.15	5.35	
Treatments (D)	19	6154.86	323.94	348.32**
(VxD) interaction	38	1195.65	31.46	33.82**
Error (b)	171	159.98	0.93	
Total	**239**	**8800.46**		

** indicate significance at 1% level of probability.

Table 15: **Effect of different doses of gamma radiation separately and with GA_3 on number of roots of ten days old seedlings in three genotypes of chickpea.**

Treatment	Noor 91	Punjab 91	C 141	Treatment means
Control				
Unsoaked	18.40 B	24.85 A	18.25 A	20.50 AB
Soaked	16.90 B	25.75 A	18.55 A	20.40 B
Gamma radiation				
10 Kr	18.60 B	20.60 B	17.35 A	18.85 CD
20 Kr	16.90 B	17.70 CD	13.10 BC	13.40 GH
30 Kr	13.30 C	15.95 D	11.70 C	13.65 G
40 Kr	9.70E FG	13.00 E	10.25 D	10.98 I
50 Kr	9.80 EF	12.55 E	7.05 EF	9.80 J
60 Kr	7.80 G	12.55 E	6.15 EFG	8.83 J
70 Kr	3.55 IJ	10.15 F	5.45 FG	6.38 KL
90 Kr	5.00 HI	9.35 FG	2.65 H	5.66 L
110 Kr	2.95 J	6.80 H	2.05 H	3.93 M
Gamma radiation+ GA_3 (0.5 mM)				
10 Kr + GA_3	21.25 A	26.15 A	16.90 A	21.43 A
20 Kr + GA_3	14.95 C	22.05 B	17.20 A	18.06 DE
30 Kr + GA_3	17.45 B	21.45 B	14.20 B	17.70 EF
40 Kr + GA_3	16.90 B	18.35 C	11.65 C	15.63 F
50 Kr + GA_3	13.80 C	14.00 E	10.80 D	12.86 H
60 Kr + GA_3	11.15 DE	13.25 E	9.75 D	11.38 I
70 Kr + GA_3	8.75 FG	13.25 E	7.40 E	9.80 J
90 Kr + GA_3	5.11 HI	10.00 F	6.45 EFG	7.18 K
110 Kr + GA_3	5.50 H	7.75 GH	4.65 G	5.96 L
Means	**11.88 B**	**15.77 A**	**10.57 B**	

S.E. for variety mean =0.2586
S.E. for treatment mean=0.2783
S.E. for (VxD) interaction means=0.4821
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

IV: MUTATION STUDIES IN M_1 GENERATION

Gamma irradiation is used extensively to induce extra-genetic variability. Plants in M_1 generation showed altered patterns of growth for various characters depending upon the extent of biological damage. To reduce the extensive physiological damage, gibberellic acid was used in this study. Change in radio sensitivity with gibberellic acid expressed in various plant characters is presented here.

i: PLANT HEIGHT (cm)

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on plant height in M_1 population of chickpea (Table 16) indicates highly significant ($p<0.01$) variation within treatments and genotypes. The genotype-treatment interaction was also highly significant ($p<0.01$). It reflects highly inconsistency in sensitivity of genotypes for this character across various treatments. On the average, maximum plant height of 97.00 cm was observed in C141 as compared to 93.91 and 86.87 cm in Punjab 91 and Noor 91, respectively. The results (Table17) revealed that the irradiation treatments significantly decreased the plant height as compared to control. However, the effect of gamma irradiation changed with the application of gibberellic acid and plant height increased significantly at 40 Kr, while decreased at 60 Kr.

A differential response of plant height among genotypes towards the mutagenic treatments was observed. Significant ($p<0.01$) increase in plant height at 60 Kr was noticed in Noor 91 as compared to control. However, at 40 and 50 Kr increase in plant height as compared to control was non-significant. With the application of gibberellic acid plant height increased significantly at all doses as compared to control. Punjab 91 exhibited an almost similar response to both mutagenic treatments. The control plants had the maximum plant height and was significantly ($p<0.01$) different from all other doses. In C 141 plant height decreased significantly ($p<0.01$) with both mutagenic treatments except at 40 Kr with gibberellic acid where non-significant difference as compared to control was recorded. Application of GA_3 changed the effect of irradiation and plant height decreased significantly at 50 and 60 Kr treatments.

Table 16: Analysis of variance for plant height in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	0.31	0.15	0.38
Varieties (V)	2	1293.03	646.51	1624.41**
Error (a)	4	1.59	0.39	-
Treatments (D)	7	382.59	54.65	96.39**
(VxD) interaction	14	671.40	95.91	169.16**
Error (b)	42	23.81	0.56	-
Total	71	**2372.75**		

** Indicate significance at 1% level of probability.

Table 17: Effect of different doses of gamma radiation separately and with GA_3 on plant height in M_1 population of three chickpea genotypes.

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	84.78C	98.43A	102.46 A	95.22 A
Soaked	84.85C	98.03A	102.81 A	95.23 A
Gamma radiation				
40 Kr	85.53 BC	90.75 CD	96.33 A	90.87 F
50 Kr	85.08 BC	95.81 B	98.81 B	93.23 CD
60 Kr	90.23 A	90.06 D	95.20 CD	91.83 E
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	89.00 A	91.00 CD	101.21 A	93.75 BC
50 Kr + GA_3	88.95 A	95.85 B	93.73 D	92.84 CD
60 Kr + GA_3	86.55 B	91.33 C	85.43 E	87.77 G
Mean	**86.87 C**	**93.91 B**	**97.00 A**	

S.E. for variety mean = 0.1274
S.E. for treatment mean = 0.2494
S.E. for (VxD) interaction means = 0.4320
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

Table 18 presents analysis of variance for the effect of different doses of gamma irradiation and with post mutagenic application of gibberellic acid on the number of primary branches in M_1 population of chickpea. The results show non-significant ($p>0.05$) differences within genotypes, while highly significant ($p<0.01$) differences among the treatments. The interaction between genotype-treatment was also highly significant. It reflects high variation in the performance of genotypes across the treatments. C141 exhibited maximum number of primary branches 6.62 per plant followed by 6.61 and 6.53 per plant in Punjab 91 and Noor 91, respectively (Table 19). It is evident from the results that the application of GA_3 had changed the effect of gamma irradiation and number of primary branches significantly ($p<0.01$) increased at 40 Kr and decrease at 60 Kr. Number of primary branches per plant decreased significantly at 60 Kr with both mutagenic treatments as compared with their respective controls.

In Noor 91 number of primary branches decreased at 40 Kr, while increased non-significantly at 50 and 60 Kr as compared with control. Application of GA_3 modulated the effect of gamma irradiation and number of primary branches increased and decreased significantly at 40 Kr and 50 and 60 Kr respectively. Number of primary branches in Punjab 91 increased non-significantly at 40 Kr and decreased significantly at 60 Kr as compared to control. In case of gibberellic acid application the number of primary branches decreased significantly ($p<0.01$) at 40 Kr. In C141 the number of primary branches decreased significantly ($p<0.01$) at 60 Kr with their respective controls in both mutagenic treatments. However, number of primary branches increased significantly at 60 Kr as compared to control.

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

It is obvious from the analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on the number of secondary branches (Table 20) in M_1 population of chickpea that differences among treatments and genotypes were highly significant. The interaction between genotype-treatments was also highly significant ($p<0.01$). The results indicate high fluctuation in the sensitivity of genotypes for this character across the treatments. Maximum number of secondary branches 20.68 were observed in C 141 as compared with 16.87 and 15.92 in Noor 91 and Punjab 91, respectively (Table 21).

Table 18: Analysis of variance for number of primary branches per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	0.0008	0.0004	0.0085
Varieties (V)	2	0.1292	0.0646	1.374
Error (a)	4	0.1880	0.047	
Treatments (D)	7	7.3966	1.0566	14.2977**
(VxD) interaction	14	14.0166	1.0011	13.5466**
Error (b)	42	3.1053	0.0739	-
Total	**71**	**24.8366**		

** Indicate significance at 1% level of probability.

Table 19: Effect of different doses of gamma radiation separately and with GA_3 on number of primary branches per plant in M_1 population of three chickpea genotypes.

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	6.45 BC	6.88 AB	6.78 BC	6.70 AB
Soaked	6.53 BC	7.08 A	6.56 BC	6.72 AB
Gamma radiation				
40 Kr	6.00 C	7.00 A	6.38 CD	6.46 BC
50 Kr	6.83 AB	6.88 AB	7.08 B	6.93 A
60 Kr	7.06 AB	6.03 C	5.63 D	6.24 C
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	7.26 A	5.96 C	7.80 A	7.01 A
50 Kr + GA_3	6.00 C	6.76 AB	7.16 B	6.65 ABC
60 Kr + GA_3	6.08 C	6.31 BC	5.58 D	5.99 D
Mean	**6.53 A**	**6.61 A**	**6.62 A**	

S.E. for variety mean = 0.0442
S.E. for treatment mean = 0.0906
S.E. for (VxD) interaction means = 0.1569
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Both two mutagenic treatments affected the number of secondary branches in a different way. Gamma irradiation significantly ($p<0.01$) reduced the number of secondary branches as compared to control and maximum reduction was observed at 40 Kr. Application of gibberellic acid increased the number of secondary branches significantly ($p<0.01$) at 40 Kr, while at 50 and 60 Kr the number of secondary branches decreased significantly.

Varieties exhibited differential response towards mutagenic treatments. In Noor 91 the number of secondary branches decreased significantly at 40 and 50 Kr with gamma irradiation. Application of GA_3 markedly changed the biological effects of gamma irradiation. Number of secondary branches increased significantly at 40 Kr, while decreased at 50 and 60 Kr. The number of secondary branches in Punjab 91 decreased significantly with both mutagenic treatments except at 50 Kr of gamma irradiation as compared with their respective untreated checks. C 141 exhibited a significant ($p<0.01$) decrease in the number of secondary branches with gamma irradiation at 40 and 60 Kr. The treatment of gibberellic acid significantly ($p<0.01$) increased the number of secondary branches at 40 Kr, while decreased at 50 and 60 Kr as compared with control.

iv: NUMBER OF PODS PER PLANT

The analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on number of pods per plant (Table 22) in M_1 population of chickpea reveals highly significant ($p<0.01$) differences within treatments and genotypes. The interaction between genotype-treatment interaction was also highly significant. It indicates that sensitivity of genotypes for this character was highly inconsistent across the treatments. On the average, maximum number of pods per plant 72.37 were obtained in C 141, followed by 62.58 and 52.28 in Punjab 91 and Noor 91, respectively (Table 23). The results show that the mutagenic treatments significantly ($p<0.01$) decreased the number of pods per plant as compared to their respective controls and minimum number of pods were obtained at 60 Kr with both treatments. However, significantly higher number of pods per plant obtained with the application of GA_3 at 40 and 60 Kr.

A differential response of varieties to the two mutagenic treatments was noticed. In Noor 91 number of pods per plant decreased significantly ($p<0.01$) at 40 and 50 Kr, while increased at 60 Kr with gamma irradiation as compared to control. Application of gibberellic acid modulated

Table 20: **Analysis of variance for number of secondary branches per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	4.45	2.22	4.62
Varieties (V)	2	304.20	152.10	316.87**
Error (a)	4	1.92	0.48	-
Treatments (D)	7	754.68	107.81	207.32**
(VxD) interaction	14	754.74	53.91	103.67**
Error (b)	42	22.21	0.52	-
Total	**71**	**1842.205**		

** Indicate significance at 1% level of probability.

Table 21: **Effect of different doses of gamma radiation separately and with GA_3 on number of secondary branches per plant in M_1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	18.35 BC	21.36 A	23.30 B	21.00 B
Soaked	17.46 BC	22.36 A.	22.06 BC	20.63 B
Gamma radiation				
40 Kr	11.56 E	12.90 BC	17.63 D	14.03 E
50 Kr	13.30 D	21.50 A	22.40 BC	19.06 C
60 Kr	18.61 B	11.58 C	20.80 C	17.00 D
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	28.58 A	11.16 D	26.66 A	22.13 A
50 Kr + GA_3	16.70 C	12.88 BC	19.00 D	16.20 D
60 Kr + GA_3	10.40 E	13.63 B	13.56 E	12.53 F
Mean	**16.87 B**	**15.92 C**	**20.68 A**	

S.E. for variety mean = 0.1414
S.E. for treatment mean = 0.2403
S.E. for (VxD) interaction means = 0.4163
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

the effects of gamma irradiation and the number of pods per plant increased at 40 and 50 Kr, while decreased significantly at 60 Kr. Punjab 91 exhibited a gradual and significant ($p<0.01$) decrease in number of pods per plant with an increase of gamma irradiation and minimum were obtained at 60 Kr. The application of gibberellic acid further decreased the number of pods per plant at 40 and 50 Kr, while increased at 60 Kr. In C 141 number of pods per plant decreased significantly at 40 and 60 Kr with gamma irradiation. However, the application of gibberellic acid markedly changed the biological effects of gamma irradiation and number of pods increased significantly ($p<0.01$) at 40 and 50 Kr as compared with their respective control.

v) NUMBER OF SEEDS PER POD

Table 24 exhibits highly significant ($p<0.01$) differences for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on number of seeds per pod in M_1 population of chickpea among the treatments and genotypes. The interaction between genotype-treatment was also highly significant. It reveals high variation in the sensitivity among genotypes for this character across the treatments. Punjab 91 exhibited the maximum number of seeds per pod 1.31 followed by 1.26 and 1.11 per pod in C 141 and Noor 91, respectively (Table 25). It is evident from the results that the number of seeds per pod affected with the two mutagenic treatments. Gamma radiation significantly ($p<0.01$) decreased the number of seeds per pod at all doses while, with gibberellic acid treatment significant ($p<0.01$) reduction occurred at 60 Kr as compared to control.

A differential response of varieties to the mutagenic treatments was observed. In Noor 91, the number of seeds per pod reduced significantly ($p<0.01$) at 40 and 50 Kr with gamma irradiation, while with the application of gibberellic acid non-significant ($p>0.01$) decrease at all levels of irradiation was observed as compared to their respective controls. Punjab 91 exhibited significant ($p<0.01$) decrease at all gamma irradiation doses, however with GA_3 at 60 Kr as compared to their controls. In C141 the increase or decrease in number of seeds per pod was non-significant ($p>0.01$) among the different mutagenic treatments as compared to their respective controls.

vi: 100-SEED WEIGHT PER PLANT (g)

The analysis of variance for the effect of different doses of gamma radiation with and without the application of gibberellic acid on 100-seed weight in M_1 population of chickpea (Table 26) indicates highly significant variation ($p<0.01$) within the treatments and genotypes.

Table 22: **Analysis of variance for number of pods per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	19.60	9.80	1.38
Varieties (V)	2	4843.13	2421.56	342.51**
Error (a)	4	28.27	7.07	-
Treatments (D)	7	2659.60	379.94	55.80**
(VxD) interaction	14	9757.67	696.97	102.34**
Error (b)	42	286.30	6.81	-
Total	**71**	**17595.09**		

** Indicate significance at 1% level of probability.

Table 23: **Effect of different doses of gamma radiation separately and with GA_3 on number of pods per plant in M1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	60.10 B	84.15 A	70.43 C	71.76 A
Soaked	57.00 BC	85.71 A	69.75 CD	70.82 A
Gamma radiation				
40 Kr	34.86 F	74.61 B	57.11 F	55.53 D
50 Kr	43.73 E	66.58 C	69.80 CD	60.03 C
60 Kr	66.45 A	47.56 DE	63.35 E	59.12 C
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	55.26 BC	46.90 DE	82.40 B	61.52 C
50 Kr + GA_3	52.50 CD	44.46 E	101.68 A	66.21 B
60 Kr + GA_3	48.40 DE	50.71 D	64.48 DE	54.53 D
Mean	**52.28 C**	**62.58 B**	**72.37 A**	

S.E. for variety mean = 0.5427
S.E. for treatment mean = 0.8698
S.E. for (VxD) interaction means = 1.5066

Means not followed by the same letter are statistically significant at by DMRT 1% level of probability.

Table 24: **Analysis of variance for number of seeds per pod in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	0.0048	0.0024	2.00
Varieties (V)	2	0.5497	0.2748	274.00**
Error (a)	4	0.0045	0.0011	-
Treatments (D)	7	0.7656	0.1091	7.26**
(VxD) interaction	14	0.7683	0.0548	3.60**
Error (b)	42	0.6336	0.0157	-
Total	**71**	**2.1565**		

** Indicate significance at 1% level of probability.

Table 25: **Effect of different doses of gamma radiation separately and with GA_3 on number of seeds per pod in M_1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	1.27 A	1.70 A	1.29 A	1.42 A
Soaked	1.24 A	1.49 AB	1.24 A	1.32 AB
Gamma radiation				
40 Kr	0.95 BC	1.25 BC	1.23 A	1.14 CD
50 Kr	0.82 C	1.29 BC	1.28 A	1.13 CD
60 Kr	1.10 AB	1.07 C	1.32 A	1.16 BCD
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	1.18 AB	1.38 B	1.31 A	1.29 AB
50 Kr + GA_3	1.15 AB	1.30 BC	1.29 A	1.25 BC
60 Kr + GA_3	1.16 AB	1.05 C	1.11 A	1.11 D
Mean	**1.11 C**	**1.31 A**	**1.26 B**	

S.E. for variety mean = 0.0067
S.E. for treatment mean = 0.0417
S.E. for (VxD) interaction means = 0.0723
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

The interaction between genotype-treatment was also highly significant. It reflects highly inconsistency in the performance of genotypes for this character among the treatments. On the average, maximum 100-seed weight 25.24g was obtained in C141 followed by 25.00 and 24.37g in Punjab 91 and Noor 91, respectively (Table 27). 100-seed weight decreased gradually and significantly ($p<0.01$) with an increase of irradiation dosages with both mutagenic treatments. However, significantly heavier seeds were produced with the application of GA_3 at various irradiation doses.

It is apparent from the results that the varieties show a varied response of 100-seed weight towards the mutagenic treatments. In Noor 91 100-seed weight decreased gradually and significantly ($p<0.01$) with an increase of gamma irradiation at 50 and 60 Kr with both mutagenic treatments as compared with their respective controls. Punjab 91 exhibited significant ($p<0.01$) decrease with an increase of gamma irradiation. Application of gibberellic acid modulated the effects of gamma irradiation and 100-seed weight increased significantly ($p<0.01$) at 50 and 60 Kr. In C 141 100-seed weight decreased significantly ($p<0.01$) at various irradiation dosages except at 40 Kr with application of gibberellic acid as compared to controls. However, heavier seeds were produced with the application of GA_3.

vii: BIOLOGICAL YIELD PER PLANT (g)

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on biological yield per plant in M_1 population of chickpea (Table 28) indicates highly significant differences ($p<0.01$) among treatments and genotypes. The interaction between genotype-treatment interaction was also highly significant. It shows considerable amount of variability in the performance of genotypes across the treatments. C141 exhibited maximum biological yield of 105.13 g per plant followed by 95.54 and 93.54 g in Punjab 91 and Noor 91, respectively (Table 29). It is also seen from the results that the effects of two mutagenic treatments on the biological yield were different from each other. Biological yield decreased significantly ($p<0.01$) with both mutagenic treatments as compared with their respective controls. The application of GA_3 increased the biological yield significantly at 40 Kr and decreased at 50 and 60 Kr. Genotypes varied in their response of biological yield towards the both mutagenic treatments.

Table 26: Analysis of variance for 100-seed weight per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D. F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.109	0.054	1.459
Varieties (V)	2	9.80	4.90	132.43**
Error (a)	4	0.148	0.037	-
Treatments (D)	7	30.420	4.345	188.91**
(VxD) interaction	14	1.700	0.121	5.26**
Error (b)	42	0.987	0.023	-
Total	**71**	**43.171**		

** Indicate significance at 1% level of probability.

Table 27: Effect of different doses of gamma radiation separately and with GA_3 on 100-seed weight per plant in M_1 population of three chickpea genotypes.

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	25.10 A	25.79 A	26.07 A	25.65 A
Soaked	25.02 A	25.84 A	26.04 A	25.63 A
Gamma radiation				
40 Kr	24.77 A	25.16 B	25.00 C	24.98 C
50 Kr	23.71 C	24.58 C	24.59 D	24.29 D
60 Kr	23.45 D	23.47 D	24.11 E	23.68 E
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	24.80 A	25.43 B	25.77 A	25.33 B
50 Kr + GA_3	24.38 B	25.13 B	25.39 B	24.97 C
60 Kr + GA_3	23.70 C	24.66 C	24.94 CD	24.43 D
Mean	**24.37 B**	**25.00 A**	**25.24 A**	

S.E. for variety mean = 0.0392
S.E. for treatment mean = 0.0505
S.E. for (VxD) interaction means = 0.0875
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 28: **Analysis of variance for biological yield per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	18.11	9.05	4.43
Varieties (V)	2	1840.27	920.13	451.04**
Error (a)	4	8.17	2.04	-
Treatments (D)	7	2809.00	401.28	152.57**
(VxD) interaction	14	2559.07	182.80	69.50**
Error (b)	42	110.697	2.63	-
Total	**71**	**7345.33**		

** Indicate significance at 1% level of probability.

Table 29: **Effect of different doses of gamma radiation separately and with GA_3 on biological yield per plant in M1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	96.54 C	106.96 A	112.93 AB	105.48 A
Soaked	96.03 C	108.45 A	110.20 B	104.89 A
Gamma radiation				
40 Kr	83.95 E	90.71 B	101.08 C	91.91 D
50 Kr	89.23 D	105.98 A	110.35 B	101.85 B
60 Kr	101.36 B	84.43 C	101.03 C	95.60 C
Gamma radiation+GA_3 (0.5 Mm)				
40 Kr + GA_3	105.75 A	84.11 C	115.60 A	101.82 B
50 Kr + GA_3	93.71 C	93.18 B	102.96 C	96.62 C
60 Kr + GA_3	81.80 F	90.51 B	86.88 D	86.40 E
Mean	**93.54 B**	**95.54 B**	**105.13 A**	

S.E. for variety mean = 0.2915
S.E. for treatment mean = 0.5405
S.E. for (VxD) interaction means = 0.9363

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

In Noor 91 biological yield decreased significantly ($P<0.01$) at 40 and 50 Kr with gamma irradiation as compared to control. Biological yield increased significantly at 40 Kr and decreased at 60 Kr with the treatment of gibberellic acid as compared to control. In Punjab 91 biological yield decreased significantly ($p<0.01$) at various doses of both mutagenic treatments except at 50 Kr of gamma irradiation. Effects of mutagens on the biological yield in C 141 were found to be different. Biological yield decreased significantly ($p<0.01$) at 40 and 60 Kr with gamma irradiation as compared to control. Application of gibberellic acid increased the biological yield at 40 Kr, while decreased at 50 and 60 Kr treatment.

viii: GRAIN YIELD PER PLANT (g)

Table 30 indicates highly significant ($p<0.01$) differences among the treatments and genotypes for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on grain yield per plant in M_1 population. The interaction between genotype-treatment was also highly significant. It shows high fluctuation in the performance of genotypes for this character across the treatments. C 141 exhibited the maximum grain yield of 23.11g per plant followed by 20.95 and 14.45g in Punjab 91 and Noor 91,respectively (Table 31). The results show that the grain yield responded differently towards the mutagenic treatments. Grain yield decreased significantly ($p<0.01$) with gamma irradiation as compared to control. However, grain yield increased significantly at 40 and 50 Kr and decreased at 60 Kr with the application of GA_3.

A differential response of varieties for grain yield was observed towards the two mutagenic treatments. In Noor 91grain yield decreased significantly ($p<0.01$) at 40 and 50 Kr with gamma irradiation as compared to control. Application of GA_3 changed the effects of gamma irradiation and biological yield increased significantly at 40 and 50 Kr, while decreased at 60 Kr. Punjab 91 exhibited a gradual and significant ($p<0.01$) decrease in grain yield with an increase of gamma radiation doses with both mutagenic treatments. However, more grain yield was obtained with gamma irradiation at 40 and 50 Kr. In C141 grain yield decreased significantly ($p<0.01$) at 40 Kr with gamma irradiation as compared to control. The treatment of gibberellic acid increased the grain yield significantly ($p<0.01$) at 40 and 50 Kr, while significant ($p<0.01$) decrease was observed at 60 Kr treatment as compared to control.

Table 30: **Analysis of variance for grain yield per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	1.85	0.92	0.578
Varieties (V)	2	975.05	487.52	308.55**
Error (a)	4	6.35	1.58	-
Treatments (D)	7	1005.41	143.63	107.02**
(VxD) interaction	14	1532.69	109.47	7.81**
Error (b)	42	56.40	1.342	
Total	**71**	**3577.7815**		

** Indicate significance at 1% level of probability.

Table 31: **Effect of different doses of gamma radiation separately and with GA_3 on grain yield per plant in M_1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	19.60 A	35.31 A	23.62 C	26.03 A
Soaked	17.66 AB	32.46 B	22.41 C	24.18 B
Gamma radiation				
40 Kr	8.47 E	23.25 C	17.64 D	16.45 D
50 Kr	8.84 E	20.54 D	21.34 C	16.91 D
60 Kr	17.03 AB	12.07 F	20.97 C	16.69 D
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	16.37 BC	16.32 E	27.59 B	20.09 C
50 Kr + GA_3	14.79 CD	14.32 EF	33.30 A	20.80 C
60 Kr + GA_3	13.31 D	13.34 F	18.03 D	14.89 E
Mean	**14.45 C**	**20.95 B**	**23.11 A**	

S.E. for variety mean = 0.2565
S.E. for treatment mean = 0.3861
S.E. for (VxD) interaction means = 0.6688
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

ix: HARVEST INDEX (%)

The analysis of variance of the data on harvest index (Table 32) indicates highly significant ($p<0.01$) variation among the treatments and genotypes for the effect of different doses of gamma radiation with and without the application of gibberellic acid in M_1 population of chickpea. The interaction between genotype-treatment was also highly significant. It reflects marked variationin the performance of genotype across the various doses. Maximum harvest index of 21.97 per plant was observed in C 141 as compared to 21.43 and 15.32 per plant in Punjab 91 and Noor 91, respectively (Table 33). It is apparent from the results that harvest index decreased significantly ($p<0.01$) at various doses of the two mutagenic treatments as compared to controls. However, significantly higher harvest index was obtained at 40 and 50 Kr with the application of GA_3.

The behaviour of harvest index of the genotypes was found to be different towards the two mutagenic treatments. Noor 91 exhibited a significant ($p<0.01$) decrease in harvest index at all the gamma irradiation treatments as compared to control. However, the application of gibberellic acid changed the effects of gamma irradiation and significantly ($P<0.01$) higher harvest index was observed at 40 and 50 Kr. In Punjab 91 harvest index gradually and significantly decreased with an increase in gamma irradiation. With the application of GA_3 harvest index decreased significantly at 40 and 50 Kr. C141 exhibited significant ($p<0.01$) decrease in harvest index at 40 Kr with gamma irradiation. However, harvest index increased significantly ($p>0.01$) at 40 and 50 Kr with gibberellic acid treatment as compared to control.

x: FLOWERING DAYS

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on number of days to 50% flowering in M_1 population of chickpea (Table 34) indicates highly significant ($p<0.01$) differences among the treatments and genotypes. The interaction between genotype-treatment was also highly significant for this character. It reveals high variation of flowering across the various treatments. Maximum time to 50% flowering 124.41 days taken by C 141 followed by 123.45 and 120.16 days in Noor 91 and Punjab 91, respectively (Table 35). It is evident from the results that the time taken to 50% flowering was changed with the type of mutagen. In both types of mutagenic treatments number of days to 50 % flowering increased gradually and significantly ($p<0.01$) with an increase in

Table 32: **Analysis of variance for harvest index per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	1.09	0.54	0.07
Varieties (V)	2	631.69	315.84	41.97**
Error (a)	4	30.10	7.52	-
Treatments (D)	7	574.62	82.08	186.54**
(VxD) interaction	14	1248.83	89.20	202.72**
Error (b)	42	18.62	0.44	-
Total	**71**	**2504.98**		

** Indicate significance at 1% level of probability.

Table 33: **Effect of different doses of gamma radiation separately and with GA_3 on harvest index per plant in M_1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	19.84 A	33.00 A	20.92 C	24.59 A
Soaked	18.38 AB	29.89 B	20.34 C	22.87 B
Gamma radiation				
40 Kr	10.08 D	25.63 C	17.46 D	17.72 E
50 Kr	9.89 D	19.37 D	19.36 C	16.21 F
60 Kr	16.86 BC	14.29 E	20.76 C	17.30 E
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	15.47 C	19.39 D	23.86 B	19.57 D
50 Kr + GA_3	15.78 C	15.36 E	32.33 A	21.16 C
60 Kr + GA_3	16.26 C	14.72 E	20.75 C	17.24 E
Mean	**15.32 B**	**21.43 A**	**21.97 A**	

S.E. for variety mean = 0.5597
S.E. for treatment mean = 0.2211
S.E. for (VxD) interaction means = 0.3829
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 34: **Analysis of variance for 50% flowering per plant in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	0.57	0.28	6.67
Varieties (V)	2	238.57	119.28	2773.95**
Error (a)	4	0.17	0.04	-
Treatments (D)	7	1225.70	175.10	486.38**
(VxD) interaction	14	61.42	4.38	12.16**
Error (b)	42	15.25	0.36	-
Total	**71**	**1541.70**		

** Indicate significance at 1% level of probability.

Table 35: **Effect of different doses of gamma radiation separately and with GA_3 on days to 50% flowering in M_1 population of three chickpea genotypes.**

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	119.33 E	114.33 F	119.66 E	117.77 G
Soaked	119.33 E	114.33 F	119.66 E	117.78 G
Gamma radiation				
40 Kr	124.33 C	123.66 C	125.33 C	124.44 C
50 Kr	127.33 B	126.66 B	128.33 B	127.44 B
60 Kr	129.33 A	128.66 A	131.66 A	129.88 A
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	120.66 E	116.33 E	122.33 D	119.77 F
50 Kr + GA_3	122.66 D	117.66 E	123.66 CD	121.33 E
60 Kr + GA_3	124.66 C	119.66 D	124.66 C	123.00 D
Mean	**123.45 B**	**120.16 C**	**124.41 A**	

S.E. for variety mean = 0.0408
S.E. for treatment mean = 0.2000
S.E. for (VxD) interaction means = 0.3464
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

irradiation dosages. Application of gibberellic acid, however decreased the number of days to 50% flowering significantly ($p<0.01$) at various doses of gamma irradiation.

The three varieties responded differently for time to 50% flowering to both mutagenic treatments. In Noor 91 number of days to 50% flowering increased gradually and significantly with an increase of irradiation with the two mutagenic treatments. Number of days to 50% flowering decreased significantly at different doses with the application of GA3. In Punjab 91 and C141 similar response of number of days to 50% flowering was observed with the two mutagenic treatments.

xi: MATURITY DAYS

The analysis of variance for the effect of different doses of gamma radiation with and without the application of gibberellic acid on maturity days (Table 36) indicated highly significant ($p<0.01$) differences among the treatments and genotypes. The genotype-treatment interaction was also highly significant. It shows high inconsistency in the performance of genotypes across the treatments. C 141 had taken the maximum time 173.91 days to maturity followed by 171.50 and 169.83 days in Punjab 91 and Noor 91, respectively (table 37). It is evident from the results that the maturity days increased significantly ($p>0.01$) and regularly with increasing gamma irradiation doses and maximum maturity days were recorded at 60 Kr treatment. Post mutagenic treatment with GA_3 decreased the number of days maturity significantly ($p<0.01$) at various levels of gamma irradiation.

The three genotypes responded in a different way to the both mutagenic treatments. In Noor 91 maturity days increased significantly ($p<0.01$) at various levels of gamma irradiation while, in Punjab 91 and C 141 the increase in number of days maturity were significant ($p<0.01$) at 40 Kr and non-significant ($p>0.01$) at 50 and 60 Kr treatments. Application of GA_3 decreased the number of days to maturity significantly ($p<0.01$) at different irradiation treatments in the three genotypes.

Table 36: Analysis of variance for days to maturity in M_1 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F	Sum of squares	Mean of squares	F-value
Replicates	2	2.58	1.29	6.14
Varieties (V)	2	202.33	101.16	481.70**
Error (a)	4	0.83	0.21	-
Treatments (D)	7	653.05	93.29	310.96**
(VxD) interaction	14	62.11	4.43	14.76**
Error (b)	42	12.58	0.30	
Total	**71**	**933.50**		

** Indicate significance at 1% level of probability.

Table 37: Effect of different doses of gamma radiation separately and with GA_3 on days to maturity in M_1 population of three chickpea genotypes.

Treatment	Noor 91	Punjab 91	C 141	Mean
Control				
Unsoaked	166.66 EF	169.33 C	170.66 D	168.88 F
Soaked	166.33 F	168.66 C	171.00 D	168.66 F
Gamma radiation				
40 Kr	170.33 C	171.66 B	174.33 B	172.11 C
50 Kr	171.66 B	175.33 A	179.66 A	175.55 B
60 Kr	177.66 A	176.33 A	178.66 A	177.55 A
Gamma radiation+GA_3 (0.5 mM)				
40 Kr + GA_3	167.66 DE	169.33 C	171.66 D	169.55 E
50 Kr + GA_3	168.00 D	169.66 C	172.00 C	171.00 D
60 Kr + GA_3	170.33 C	171.66 B	173.33 B	170.66 D
Mean	**169.83 C**	**171.50 B**	**173.91 A**	

S.E. for variety mean = 0.0935
S.E. for treatment mean = 0.1825
S.E. for (VxD) interaction means = 0.3162
Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

V: MUTATION STUDIES IN M_2 GENERATION

In this generation various variations were obtained due to the genetical changes preserved in the plants of M_1 generation and lasting throughout the developmental period. These genetic variations are manifested as induced variants, which provides the raw material for selection of desirable genotypes for crop improvement and also to widen the germ plasm pool. Data on various plant characters were recorded and expressed as follows.

i: PLANT HEIGHT (cm)

The analysis of variance for the effects of different doses of gamma irradiation separately and with the application of gibberellic acid on plant height in M_2 population of chickpea (Table 38) revealed highly significant ($p<0.01$) differences among treatments and genotypes. The genotype-treatment interaction was also highly significant ($p<0.01$). It indicates high fluctuation of induced variability in genotypes across different treatments. Maximum height per plant 96.09 cm was produced by C 141 as against 94.33 and 84.42 cm in Punjab 91 and Noor 91 respectively, (Table 39). The data regarding the main treatments on plant height revealed non-significant ($p>0.01$) decrease at 40 and 50 Kr and significant ($p<0.01$) increase at 60 Kr with both mutagenic treatments as compared to control.

With regard to genotype-treatment interaction, a differential behaviour was noted among varieties for this character. In Noor 91 gamma irradiation increased the plant height non-significantly ($p>0.01$) at various doses as compared to control. However, with the application of gibberellic acid non-significant decrease was noticed at 40 and 50 Kr treatments, while at 60 Kr significant ($p<0.01$) increase as compared to control was observed. There was a progressive decrease of plant height in Punjab 91 and significant ($p<0.01$) decrease at 60 Kr as compared to control was observed. The application of gibberellic acid decreased the plant height non-significantly ($p>0.01$) as compared to control. However, by comparing the two mutagenic treatments plant height increased significantly at 60 Kr with GA_3. In C141 both mutagenic treatments decreased the plant height significantly ($p<0.01$) except at 50 Kr with the treatment of gibberellic acid. GA_3 decreased the pant height significantly at 60 Kr by modulating the effects of gamma irradiation.

Table 38: Analysis of variance for plant height in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.05	0.02	0.07
Varieties (V)	2	1661.72	830.86	3195.61**
Error (a)	4	1.05	0.26	-
Treatment (D)	6	18.62	3.10	3.06*
(VxD) interaction	12	131.58	10.96	10.85**
Error (b)	36	36.49	1.01	
Total	**62**	**1849.51**		

*, ** indicate significance at 5 and 1% level of probability respectively.

Table 39: Effect of different doses of gamma radiation separately and with GA_3 on plant height in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	83.48 B	95.30 A	99.74 A	92.84 A
Gamma radiation				
40 Kr	84.40 AB	94.74 A	95.43 BC	91.53 ABC
50 Kr	85.60 AB	94.20 AB	96.70 BC	92.17 AB
60 Kr	84.80 AB	92.05 B	95.05 C	90.63 C
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	83.30 B	95.20 A	96.56 BC	91.70 ABC
50 Kr+GA_3	83.40 B	93.67 AB	97.60 AB	91.55 ABC
60 Kr+GA_3	86.00 A	95.16 A	91.58 D	90.91 BC
Mean	**84.42 C**	**94.33 B**	**96.09 A**	

S.E for variety mean=0.1112

S.E for treatment mean=0.3349

S.E for (VxD) interaction mean=0.5802

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

Table 40 presents analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on number of primary branches per plant in M_2 population of chickpea. The results show that the differences among genotypes, treatments and their interaction was highly significant ($p<0.01$). It reflects high inconsistency in the performance of genotypes for this character across the various doses. On the average, maximum number of primary branches 9.36 per plant was observed in Punjab 91, followed by 8.10 and 7.26 per plant in Noor 91 and C 141, respectively (Table 41). The data regarding the effects of main treatments on number of primary branches per plant revealed that both mutagenic treatments increased the number of primary branches. Number of primary branches per plant decreased significantly ($p<0.01$) at 50 and 60 Kr with the treatment of gibberellic acid. Maximum number of primary branches per plant observed at 40 Kr with gamma irradiation, while with the application of GA_3 at 60 Kr dose.

Different response of the varieties was observed with respect to different type of mutagenic application. In Noor 91 number of primary branches increased significantly ($p<0.01$) with both mutagenic treatments except at 60 Kr with GA_3 the increase was non-significant as compared to control. Application of GA_3 significantly decreased the number of primary branches at 50 and 60 Kr by changing the effects of radio sensitivity. Punjab 91 exhibited significant ($p<0.01$) increase in number of primary branches with different mutagenic treatments as compared to control. Maximum number of primary branches 10.86 and 11.95 per plant were recorded at 50 Kr treatment with gamma irradiation and gibberellic acid, respectively. In C141 the number of primary branches responded similarly and significant ($p<0.01$) increase was observed at 40 Kr with both mutagenic treatments.

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

It is obvious from the analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on the number of secondary branches per plant in M_2 population of chickpea (Table 42) that differences among genotypes and treatments were highly significant ($p<0.01$). The interaction between genotype-treatment was also highly significant ($p<0.01$). It reflects highly inconsistent performance of genotypes at different doses. Main genotypic effects revealed that C141 exhibited maximum number of

Table 40: Analysis of variance for number of primary branches in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.63	0.31	15.50
Varieties (V)	2	46.80	23.40	1170.00**
Error (a)	4	0.11	0.02	-
Treatment (D)	6	55.87	9.31	71.61**
(VxD) interaction	12	60.81	5.06	38.92**
Error (b)	36	4.80	0.13	
Total	**62**	**169.02**		

** indicate significance at 1% level of probability.

Table 41: Effect of different doses of gamma radiation separately and with GA_3 on number of primary branches in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	6.47 E	6.58 E	6.45 CD	6.50 E
Gamma radiation				
40 Kr	9.00 AB	7.77 D	8.15 B	8.30 C
50 Kr	9.25 A	10.86 B	7.95 B	9.35 A
60 Kr	9.67 A	10.80 B	6.30 CD	8.92 AB
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	8.05 BC	8.80 C	9.07 A	8.64 BC
50 Kr+GA_3	7.45 CD	11.95 A	6.90 C	8.76 BC
60 Kr+GA_3	6.80 DE	8.80 C	6.06 D	7.22 D
Mean	**8.10 B**	**9.36 A**	**7.26 C**	

S.E for variety mean=0.0308

S.E for treatment mean=0.1201

S.E for (VxD) interaction mean=0.2081

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

secondary branches 20.09 per plant followed by 19.60 and 16.17 per plant in Noor 91 and Punjab 91, respectively (Table 43). It is apparent from the results that number of secondary branches reduced significantly ($p< 0.01$) at various mutagenic treatments except at 40 Kr treatment with gibberellic acid as compared to control. Significantly higher number of secondary branches were obtained at 40 and 50 Kr with GA_3 treatment.

In Noor 91 the number of secondary branches increased significantly ($p< 0.01$) at 40 Kr with both mutagenic treatments as compared to control (Table 43). Application of GA_3 significantly increased the number of secondary branches at all levels of irradiation. In Punjab 91 significant ($p<0.01$) decrease in the number of secondary branches was noticed at all mutagenic treatments as compared to control. However, minimum number of secondary branches obtained at 60 Kr with gamma irradiation and at 40 Kr with the treatment of gibberellic acid. Number of secondary branches in C141 responded differentially to different mutagenic treatments. The number of secondary branches decreased significantly ($p<0.01$) with gamma radiation as compared to control. However, with gibberellic acid these were first increased significantly ($p<0.01$) at 40 Kr treatment and then decreased significantly at 50 and 60 Kr as compared to control.

iv: NUMBER OF PODS PER PLANT

The analysis of variance for the effect of different doses of gamma irradiation separately and with the treatment of gibberellic acid on number of pods per plant (Table 44) in M_2 population of chickpea reveals highly significant ($p<0.01$) differences among genotypes and treatments. The genotype-treatment interaction was also highly significant. It indicates highly inconsistent performance of genotypes across various treatments. Punjab 91 exhibited maximum number of pods 83.87 per plant followed by 75.41 and 72.86 per plant in Noor 91 and C 141, respectively (Table 45). It is evident from the results that number of pods responded differentially to the two mutagenic treatments. Number of pods increased significantly ($p<0.01$) with two mutagenic treatments as compared to control. GA3 treatment increased the number of pods significantly ($p<0.01$) at 50 Kr, while decreased at 40 and 60 Kr. Varietal response towards the two mutagenic treatments was found to be different. In

Table 42: **Analysis of variance for number of secondary branches in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	1.97	0.98	1.12
Varieties (V)	2	191.20	95.60	109.88**
Error (a)	4	3.51	0.87	-
Treatment (D)	6	258.81	43.13	139.12**
(VxD) interaction	12	324.85	27.07	87.32**
Error (b)	36	11.43	0.31	-
Total	**62**	**791.77**		

** indicate significance at 1% level of probability.

Table 43: **Effect of different doses of gamma radiation separately and with GA_3 on number of secondary branches in M_2 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	19.10 C	22.02 A	23.00 B	21.37 A
Gamma radiation				
40 Kr	20.95 B	16.32 B	19.00 C	18.75 B
50 Kr	17.36 DE	17.27 B	17.32 D	17.32 C
60 Kr	16.67 E	13.72 CD	20.20 C	16.86 C
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	26.20 A	12.85 D	26.11 A	21.72 A
50 Kr+GA_3	18.77 C	16.17 B	19.92 C	18.29 B
60 Kr+GA_3	18.12 CD	14.87 C	15.12 E	16.04 D
Mean	**19.60 A**	**16.17 B**	**20.09 A**	

S.E for variety mean=0.2035

S.E for treatment mean=0.1855

S.E for (VxD) interaction mean=0.3214

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Noor 91 mutagenic treatments increased the number of pods per plant significantly ($p<0.01$) as against the respective control. However, the number of pods increased significantly at 40 Kr and decreased at 60 Kr with the application of gibberellic acid (Table 45). In Punjab 91 number of pods per plant decreased significantly with gamma irradiation at 40 and 60 Kr, while with gibberellic acid significantly ($p<0.01$) decreased at 40 Kr and increased at 50 Kr as compared to control. GA_3 treatment modulated the effects of gamma irradiation and number of pods decreased significantly at 40 Kr and increased at 50 and 60 Kr. In C141 number of pods per plant increased significantly ($p<0.01$) at all irradiation treatments except at 40 Kr with GA_3 treatment. Number of pods decreased significantly with GA3 at 40 and 60 Kr, while increased at 50 Kr.

v: NUMBER OF SEEDS PER POD

Table 46 exhibits highly significant ($p<0.01$) differences within genotypes and treatments for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on the number of seeds per pod in M_2 population of chickpea. The genotype-treatment interaction was also highly significant ($p<0.01$). It reveals that sufficient variability induced in genotypes for this character across the various treatments. Maximum number of seeds 1.70 per pod were observed in Punjab 91 followed by 1.32 and 1.31 per pod in Noor 91 and C 141, respectively (Table 47). It is apparent from the results that the number of seeds responded differentially to both mutagenic treatments. There was found not any increase or decrease in the number of seeds with gamma irradiation as compared with control. However, the number of seeds per pod increased significantly ($p<0.01$) at all treatments with gibberellic acid and maximum seeds per pod were obtained at 50 Kr treatment.

Varieties did not perform uniformly to the two mutagenic treatments. In Noor 91 number of seeds increased non-significantly ($p>0.01$) as compared to control with gamma irradiation. While, the application of gibberellic acid increased the seeds per pod significantly ($p<0.01$) as compared to control at all irradiation dosages and maximum were found at 50 Kr treatment. In Punjab 91 gamma irradiation decreased the seeds per pod significantly ($P<0.01$) as compared to control at 50 and 60 Kr treatments. The application of gibberellic acid increased the seeds per pod non-significantly ($p>0.01$) as compared to control. C 141 was responded differently

Table 44: **Analysis of variance for number of pods/plant in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.06	0.03	0.01
Varieties (V)	2	1397.0	698.50	232.83**
Error (a)	4	12.03	3.00	-
Treatment (D)	6	719.41	119.90	114.19**
(VxD) interaction	12	1109.58	92.46	88.05**
Error (b)	36	38.13	1.05	
Total	**62**	**3276.21**		

** indicate significance at 1% level of probability.

Table 45: **Effect of different doses of gamma radiation separately and with GA_3 on number of pods per plant in M_2 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	60.52 E	84.67 B	68.71 D	71.30 D
Gamma radiation				
40 Kr	79.25 B	81.57 C	73.47 BC	78.10 B
50 Kr	76.95 C	85.85 B	71.60 C	78.13 B
60 Kr	81.27 AB	80.87 C	74.17 B	78.77 B
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	82.07 A	76.12 D	68.30 D	75.50 C
50 Kr+GA_3	76.62 C	91.17 A	82.26 A	83.35 A
60 Kr+GA_3	71.22 D	86.87 B	71.45 C	76.51 C
Mean	**75.41 B**	**83.87 A**	**72.86 C**	

S.E for variety mean=0.3779

S.E for treatment mean=0.3415

S.E for (VxD) interaction mean=0.5916

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 46: Analysis of variance for number of seeds/pod in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.0003	0.0001	1.00
Varieties (V)	2	2.1405	1.0702	10702.00**
Error (a)	4	0.0004	0.0001	-
Treatment (D)	6	0.0556	0.0092	23.00**
(VxD) interaction	12	0.0182	0.0015	3.75**
Error (b)	36	0.0149	0.0004	
Total	**62**	**2.2299**		

** indicate significance at 1% level of probability.

Table 47: Effect of different doses of gamma radiation separately and with GA_3 on number of seeds per pod in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	1.27 C	1.70 A	1.28 B	1.42 C
Gamma radiation				
40 Kr	1.29 C	1.71 A	1.27 B	1.42 C
50 Kr	1.28 C	1.68 B	1.31 B	1.42 C
60 Kr	1.29 C	1.69 B	1.27 B	1.42 C
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	1.35 B	1.73 A	1.35 A	1.47 B
50 Kr+GA_3	1.39 A	1.73 A	1.35 A	1.49 A
60 Kr+GA_3	1.37 A	1.70 A	1.34 A	1.47 B
Mean	**1.32 B**	**1.70 A**	**1.31 B**	

S.E for variety mean=0.0021

S.E for treatment mean=0.0066

S.E for (VxD) interaction mean=0.0115

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

to the two mutagenic treatments. Any appreciable change with gamma irradiation in number of seeds per pod significantly ($p<0.01$) at all irradiation dosages.

vi: 100-SEED WEIGHT (g)

The analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on 100-seed weight per plant in M_2 population of chickpea (Table 48) indicates highly significant ($p<0.01$) variation within genotypes and treatments. Variety-treatment interaction was also highly significant ($p<0.01$). It reflects that a marked variability is induced for this character across the different treatments. Maximum 100-seed weight 26.19 g per plant was observed in C141, followed by 25.72 and 25.37 g per plant in Punjab 91 and Noor 91, respectively (Table 49).

It is evident from the results that the 100-seed weight was differentially responded to the both mutagen treatments. Gamma irradiation decreased the 100-seed weight significantly ($p<0.01$) at 60 Kr as compared to control. Application of gibberellic acid increased the 100-seed weight at all irradiation dosages. There was significant ($p<0.01$) increase in 100-seed weight at 40 Kr, while at 50 and 60 Kr doses the increase in 100-seed weight was non-significant ($p>0.01$) as compared to control.

The varieties responded differentially for 100-seed weight to the two mutagenic treatments. In Noor 91 the 100-seed weight decreased non-significantly ($p>0.01$) at all gamma irradiation dosages as compared to control. However, with the application of GA_3 100-seed weight increased significantly ($p<0.01$) at 40 Kr treatment. 100-seed weight in Punjab 91 increased non-significantly ($p>0.01$) at 40 and 50 Kr while, it was decreased significantly ($p<0.01$) at 60 Kr with gamma irradiation as compared to control. Application of gibberellic acid decreased the 100-seed weight at 40 and 60 Kr doses, while at 50 Kr treatment it was increased as compared to control. However, the decrease or increase in 100-seed weight was non-significant ($p>0.01$) as compared to control. Gamma irradiation in C141 decreased the 100-seed weight at 40 and 60 Kr but significantly ($p<0.01$) at 60 Kr treatment. However, the increase in 100-seed weight at 50 Kr treatment was non-significant ($p<0.01$) as compared to control. 100-seed weight increased significantly ($p<0.01$) with GA_3 at 40 and 60 Kr treatment, while the increase at 50 Kr was non-significant as compared to control.

Table 48: **Analysis of variance for 100-seed weight in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.03	0.01	0.25
Varieties (V)	2	7.11	3.55	88.75**
Error (a)	4	0.17	0.04	-
Treatment (D)	6	5.00	0.83	20.75**
(VxD) interaction	12	3.54	0.29	7.25**
Error (b)	36	1.70	0.04	
Total	**62**	**17.55**		

** indicate significance at 1% level of probability.

Table 49: **Effect of different doses of gamma radiation separately and with GA_3 on 100-seed weight in M_2 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	25.22 B	25.82 AB	26.03 B	25.70 B
Gamma radiation				
40 Kr	25.10 B	25.95 AB	25.97 B	25.67 B
50 Kr	25.20 B	25.86 AB	26.07 B	25.71 B
60 Kr	25.07 B	25.17 C	25.42 C	25.22 C
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	26.30 A	25.70 AB	26.67 A	26.22 A
50 Kr+GA_3	25.36 B	26.00 A	26.40 AB	25.92 B
60 Kr+GA_3	25.32 B	25.52 BC	26.75 A	25.86 B
Mean	**25.37 C**	**25.72 B**	**26.19 A**	

S.E for variety mean=0.0436

S.E for treatment mean=0.0666

S.E for (VxD) interaction mean=0.1154

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

vii: BIOLOGICAL YIELD PER PLANT (g)

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on biological yield per plant in M_2 population of chickpea (Table 50) indicates highly significant ($p< 0.01$) differences among genotypes and treatments. The genotype-treatment interaction was also highly significant ($p<0.01$). It indicates highly inconsistent performance of genotypes across various treatments. Genotype Punjab 91 exhibited the maximum biological yield 99.00 g per plant as compared with 97.94 and 89.21 g per plant in C141 and Noor 91, respectively (Table 51). It is apparent from the results that the different mutagenic treatments decreased the biological yield significantly ($P<0.01$) as compared to control. Application of gibberellic acid decreased the biological yield gradually with an increase in gamma irradiation dosages, however, with gamma irradiation the response was inconsistent.

A differential response of genotypes for biological yield (Table 51) was observed across the different mutagenic treatments. In Noor 91 the biological yield decreased non-significantly with gamma irradiation, while significant ($p<0.01$) decrease was observed with gibberellic acid at 50 and 60 Kr treatments as compared to control. Punjab 91 exhibited significant decrease in biological yield with gamma radiation at 40 and 60 Kr treatments. Whereas, a regular and non-significant ($p>0.01$) decrease in biological yield was observed across the various treatments with gibberellic acid as compared to control. GA_3 treatment changed the effects of gamma irradiation and biological yield increased at 40 Kr. In C141 biological yield decreased significantly ($p<0.01$) and consistently across the two mutagenic treatments as compared to control.

viii: GRAIN YIELD PER PLANT (g)

Table 52 indicates highly significant differences within treatments and genotypes for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on grain yield per plant in M_2 population of chickpea. The interaction between genotype and treatment was also highly significant ($p<0.01$). It reflects the highly inconsistent performance of genotypes for this character. Maximum grain yield of 37.00 g per plant was observed in Punjab 91 followed by 25.42 and 25.21 g per plant in Noor 91 and C 141, respectively (Table 53).

Table 50: Analysis of variance for biological yield in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	3.61	1.80	1.87
Varieties (V)	2	1213.04	606.52	631.80**
Error (a)	4	3.85	0.96	-
Treatment (D)	6	1203.80	200.63	163.11**
(VxD) interaction	12	562.32	46.86	38.09**
Error (b)	36	44.60	1.23	
Total	**62**	**3031.22**		

** indicate significance at 1% level of probability.

Table 51: Effect of different doses of gamma radiation separately and with GA_3 on biological yield in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	94.71 A	105.48 A	112.57 A	104.25 A
Gamma radiation				
40 Kr	89.22 AB	91.30 C	98.77 B	93.10 CD
50 Kr	88.37 AB	101.60 A	96.15 B	95.37 BC
60 Kr	88.87 AB	93.75 BC	92.75 BC	91.80 C
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	93.02 AB	102.88 A	99.20 B	98.37 B
50 Kr+GA_3	84.90 B	100.57 AB	98.57 B	94.68 BCD
60 Kr+GA_3	85.37 B	97.45 ABC	87.58 C	90.13 D
Mean	**89.21 B**	**99.00 A**	**97.94 A**	

S.E for variety mean=0.2138

S.E for treatment mean=0.3696

S.E for (VxD) interaction mean=0.6403

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

It is seen from the results that the grain yield increased significantly ($p<0.01$) across the different mutagenic treatments of gamma irradiation and with gibberellic acid as compared to control. Maximum increase in grain yield was observed at 50 Kr treatment with gibberellic acid.

A differential response of varieties across the various mutagenic treatments was observed. In Noor 91 grain yield per plant increased significantly ($p<0.01$) with both mutagenic treatments. However, with the application of gibberellic acid significantly more grain yield was recorded at 40 and 50 Kr. Grain yield in Punjab 91 decreased significantly ($p<0.01$) with gamma irradiation at 60 Kr treatment as compared to control. Application of gibberellic acid changed the effect of gamma irradiation and significant ($p<0.01$) decrease and increase in grain yield was observed at 40 Kr and 50 Kr treatment, respectively as compared to control. In C141 gamma irradiation had non-significant ($p>0.01$) effects on grain yield, while with gibberellic acid at 50 and 60 Kr significant ($p<0.01$) increase was observed as compared to control.

ix: HARVEST INDEX (%)

The analysis of variance for the effect of different doses of gamma radiation separately and with the treatment of gibberellic acid on harvest index per plant in M_2 population of chickpea (Table 54) indicates highly significant ($p<0.01$) variation among the treatments and genotypes. Genotype-treatment interaction was also highly significant ($p<0.01$). This indicates that genotypes responded differently for this character across the various treatments. Punjab 91 exhibited a maximum harvest index of 37.43 per plant followed by 25.94 and 25.57 per plant in C141 and Noor 91, respectively (Table 55). It is evident from the results that the mutagenic treatment increased the harvest index significantly ($p<0.01$) as compared to control, but the effects were inconsistent in the two treatments. Maximum harvest index of 34.15 per plant was observed with gibberellic acid at 50 Kr treatment against 25.86 per plant in control.

Varieties varied in their response to various treatments. In Noor 91 harvest index increased significantly ($p<0.01$) across all the mutagenic treatments as compared to control. However, significantly more harvest index was observed at 40 and 50 Kr with the application of gibberellic acid. In Punjab 91 harvest index increased non-significantly ($p>0.01$) with

Table 52: Analysis of variance for grain yield in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	1.17	0.58	7.25
Varieties (V)	2	1912.95	956.47	11955.87**
Error (a)	4	0.35	0.08	-
Treatment (D)	6	154.45	25.74	59.86**
(VxD) interaction	12	175.44	4.87	11.32**
Error (b)	36	15.58	0.43	-
Total	**62**	**2259.94**		

** indicate significance at 1% level of probability.

Table53: Effect of different doses of gamma radiation separately and with GA_3 on grain yield in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	19.80 D	37.56 BC	23.35 C	26.90 D
Gamma radiation				
40 Kr	25.96 BC	36.27 C	24.42 BC	28.85 BC
50 Kr	24.93 C	37.50 BC	24.63 BC	29.02 BC
60 Kr	26.45 B	34.60 D	24.04 BC	28..36 C
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	29.27 A	33.92 D	24.75 BC	29.31 B
50 Kr+GA_3	26.76 B	41.26 A	29.50 A	32.50 A
60 Kr+GA_3	24.88 C	37.91 B	25.77 B	29.52 B
Mean	**25.42 B**	**37.00 A**	**25.21 B**	

S.E for variety mean=0.0617

S.E for treatment mean=0.2185

S.E for (VxD) interaction mean=0.3785

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

gamma radiation except at 40 Kr. Application of gibberellic acid decreased the harvest index significantly ($p<0.01$) at 40 Kr, while increased significantly ($p<0.01$) at 50 and 60 Kr treatment as compared to control. C 141 exhibited a significant ($p < 0.01$) increase with both mutagenic treatments. However, significantly more harvest index was obtained at 50 and 60 Kr with the treatment of gibberellic acid.

x: FLOWERING DAYS

The analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on days to 50% flowering in M_2 population of chickpea (Table 56) indicates highly significant ($p<0.01$) differences within the genotypes and treatments. The interaction between genotype-treatment was also highly significant ($p<0.01$). It reflects highly inconsistent performance of genotypes for this character. Maximum time taken to 50% flowering was 119.85 days in C141 followed by 119.35 and 114.00 days in Noor 91 and Punjab 91, respectively (Table 57). It is apparent from the results that the mutagenic effect on time to 50% flowering was different across the two mutagenic treatments. A significant ($p<0.01$) increase in number of days to 50% flowering was observed with gamma irradiation at 40 and 60 Kr treatment as compared to control. However, the time taken to 50% flowering with gibberellic acid treatments was significantly ($P<0.01$) less as compared to control at 40 and 50 Kr treatments.

The response of varieties towards the two mutagenic treatments varied. In Noor 91 the effect of gamma irradiation on time to 50% flowering was erratic and less time to 50% flowering was observed at 50 Kr treatment as compared to control. The application of gibberellic acid significantly decreased the time to 50% flowering as compared to control at 40 and 50 Kr treatments. In Punjab 91 similar response to the two mutagenic treatments was observed. Days taken to 50% flowering were affected non-significantly ($p>0.01$) across the various mutagenic treatments except at 60 Kr with GA_3 treatment a significant ($p<0.01$) decrease as compared to control was observed. In C141 the days to 50% flowering were significantly ($p<0.01$) increased at 40 and 60 Kr treatments with gamma irradiation as compared to control. However, the application of gibberellic acid decreased the time to 50% flowering at 40 and 50 Kr treatments as compared to control.

Table 54: Analysis of variance for harvest index in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation	D.F.	Sum of squares	Mean of squares	F-value
Replicates	2	0.27	0.13	0.30
Varieties (V)	2	1521.85	760.92	1811.71**
Error (a)	4	1.70	0.42	-
Treatment (D)	6	358.23	59.70	87.79**
(VxD) interaction	12	171.12	14.26	20.97**
Error (b)	36	24.63	0.68	
Total	**62**	**2077.80**		

** indicate significance at 1% level of probability.

Table 55: Effect of different doses of gamma radiation separately and with GA_3 on harvest index in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	20.90 C	35.61 C	21.06 C	25.86 E
Gamma radiation				
40 Kr	29.00 B	39.72 AB	24.72 B	31.15 C
50 Kr	28.22 B	36.90 C	25.60 B	30.24 CD
60 Kr	29.80 AB	36.91 C	25.91 B	30.87 CD
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	31.46 A	32.97 D	24.95 B	29.80 D
50 Kr+GA_3	31.50 A	41.02 A	29.93 A	34.15 A
60 Kr+GA_3	29.14 B	38.90 B	29.43 A	32.50 B
Mean	**25.57 B**	**37.43 A**	**25.94 B**	

S.E for variety mean=0.1414

S.E for treatment mean=0.2748

S.E for (VxD) interaction mean=0.4760

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

xi: MATURITY DAYS

Table 58 indicates highly significant differences within treatments and genotypes. The genotype-treatment interaction was also highly significant ($p<0.01$). Maximum time taken to maturity was 168.92 days in C141 followed by 167.07 and 165.14 days in Punjab 91 and Noor 91, respectively (Table 59). It is apparent from the results that the maturity was responded differentially to the two mutagenic treatments. The effects of gamma radiation at all levels on maturity was non-significant, while the number of days to maturity with gibberellic acid at 40 and 50 Kr treatment were significantly ($p<0.01$) less as compared to control.

A differential response of maturity among various treatments was observed for the varieties. In Noor 91 the time taken to crop maturity was non-significant ($p>0.01$) at various mutagenic treatments except at 40 Kr treatment with gibberellic acid where a significant ($p<0.01$) decrease was observed as compared to control. In Punjab 91 significant ($p<0.01$) decrease in time taken to maturity was observed at 40 Kr treatment with gamma irradiation, while with gibberellic acid at 40 and 50 Kr treatment as compared to control. C141 exhibited significant ($p<0.01$) decrease in time to maturity at 50 Kr treatment with gamma irradiation, while with gibberellic acid significant ($p<0.01$) decrease in days to maturity was found at 40 and 60 Kr treatment as compared to control.

Table 56: Analysis of variance for days to 50% flowering in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.

Source of variation		Sum of squares	Mean of squares	F-value
Replicates	2	2.88	1.44	144.00**
Varieties (V)	2	442.78	221.39	22139.00**
Error (a)	4	0.04	0.01	-
Treatment (D)	6	68.43	11.40	23.75**
(VxD) interaction	12	85.21	7.10	14.79**
Error (b)	36	17.58	0.48	-
Total	**62**	**616.92**		

** indicate significance at 1% level of probability.

Table 57: Effect of different doses of gamma radiation separately and with GA_3 on days to 50% flowering in M_2 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	120.00 A	114.50 A	119.50 B	118.00 B
Gamma radiation				
40 Kr	120.50 A	114.00 A	122.50 A	119.00 A
50 Kr	118.50 B	115.00 A	119.50 B	117.66 B
60 Kr	120.00 A	114.50 A	123.50 A	119.33 A
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	118.00 B	114.00 A	117.50 C	116.50 C
50 Kr+GA_3	118.50 B	114.50 A	116.50 C	116.50 C
60 Kr+GA_3	120.00 A	111.50 B	120.00 B	117.16 B
Mean	**119.35 B**	**114.00 C**	**119.85 A**	

S.E for variety mean=0.0218

S.E for treatment mean=0.2309

S.E for (VxD) interaction mean=0.4000

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 58: **Analysis of variance for days to maturity in M_2 populations of three chickpea genotypes under different radiation doses separately and with GA_3.**

Source of variation		Sum of squares	Mean of squares	F-value
Replicates	2	1.56	0.78	0.72
Varieties (V)	2	150.54	75.27	70.34**
Error (a)	4	4.29	1.07	-
Treatment (D)	6	106.90	17.81	27.82**
(VxD) interaction	12	220.95	18.41	28.76**
Error (b)	36	23.11	0.64	
Total	62	507.35		

** indicate significance at 1% level of probability.

Table 59: **Effect of different doses of gamma radiation separately and with GA_3 on days to maturity in M_2 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	166.50 A	169.50 A	170.50 A	168.83 A
Gamma radiation				
40 Kr	165.00 A	165.50 BC	172.50 A	167.66 AB
50 Kr	167.50 A	168.50 AB	166.50 BC	167.50 AB
60 Kr	164.00 A	169.50 A	169.50 AB	167.66 AB
Gamma radiation +GA_3(0.5mM)				
40 Kr+GA_3	161.50 B	164.50 BC	167.00 BC	164.33 C
50 Kr+GA_3	164.00 A	164.00 C	171.50 A	166.50 B
60 Kr+GA_3	167.50 A	168.00 AB	165.00 C	166.83 AB
Mean	**165.14 C**	**167.07 B**	**168.92 A**	

S.E for variety mean=0.2257

S.E for treatment mean=0.2666

S.E for (VxD) interaction mean=0.4618

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

VI: MUTATION STUDIES IN M_3 GENERATION

i: PLANT HEIGHT (cm)

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on plant height in M_3 population of chickpea (Table 60) indicates highly significant ($p<0.01$) differences among the treatments and genotypes. The interaction between genotype-treatment was also highly significant ($p<0.01$). It indicates that a sufficient variability is induced across the various doses. Maximum plant height 87.25 cm was produced by C141 as against 84.60 and 83.60 cm in Noor 91 and Punjab 91, respectively (Table 61). The data regarding the various irradiation doses on plant height revealed that there was a significant ($P<0.01$) decrease in plant height with both mutagenic treatments, however, the effects at different levels varied. Minimum plant height was noticed at 40 Kr with gamma irradiation, while with gibberellic acid treatment at 60 Kr dosage.

The three varieties responded differentially to the two mutagenic treatments. In Noor 91 plant height decreased non-significantly ($p<0.01$) at various levels of irradiation with both mutagenic treatments. In Punjab 91 and C 141 plant height decreased significantly ($p<0.01$) as compared to control with various mutagenic treatments. In Noor 91 and Punjab 91 the differences within the both treatments were non-significant, while in C141 plant height decreased significantly with GA_3 at 40 and 50 Kr.

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

The analysis of variance for the effect of different doses of gamma radiation with and without the application of gibberellic acid on number of primary branches per plant in M_3 population of chickpea (Table 62) indicated highly significant ($p < 0.01$) variation within the treatments. The interaction between genotype-treatment was also highly significant ($p<0.01$). It reflects highly inconsistent performance of genotypes across the various treatments. However, the difference due to genotypes was not enough to reach the level of significance ($p>0.05$). On the average, maximum number of primary branches 7.19 per plant was observed in Punjab 91, followed by 6.98 and 6.96 per plant in C141 and Noor 91, respectively (Table 63). The data regarding the number of primary branches per plant

Table 60: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on plant height in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	49.24	24.62	36.74**
Variety (V)	2	149.76	74.88	111.76**
Error I	4	2.70	0.67	
Treatment (D)	6	739.49	123.24	43.24**
(VxD)	12	479.23	39.93	14.01**
Error II	36	102.94	2.85	
Total	**62**	**1523.38**		

** indicate significance at 1% level of probability.

Table 61: Effect of different doses of gamma radiation separately and with GA_3 on plant height in M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	85.16 A	94.33 A	99.83 A	93.11 A
Gamma radiation				
40 Kr	85.52 A	77.93 D	88.15 B	83.87 BCD
50 Kr	84.18 A	83.64 BC	88.58 B	85.46 B
60 Kr	82.53 A	83.56 BC	83.08 C	83.06 CD
Gamma radiation + GA_3 (0.5 mM)				
40 Kr+GA_3	83.20 A	80.25 CD	81.90 C	81.78 D
50 Kr+GA_3	85.25 A	85.22 B	83.64 C	84.70 BC
60 Kr+GA_3	86.37 A	80.25 CD	85.58 C	84.06 BCD
Mean	**84.60 B**	**83.60 B**	**87.25 A**	

S.E for variety mean=0.1786

S.E for treatment mean=0.5627

S.E for (VxD) interaction mean=0.9746

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 62 **Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on number of primary branches in M_3 generation of three chickpea genotypes.**

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	0.0445	0.0222	0.1742
Variety (V)	2	0.7082	0.3541	2.7790
Error I	4	0.5108	0.1274	
Treatment (D)	6	4.1240	0.6873	5.6707**
(VxD)	12	6.0331	0.5694	4.6980**
Error II	36	4.3632	0.1212	
Total	**62**	**16.5839**		

** indicate significance at 1% level of probability.

Table 63: Effect of different doses of gamma radiation separately and with GA_3 on number of primary branches M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	7.17 AB	7.20 A	7.33 AB	7.23 AB
Gamma radiation				
40 Kr	6.37 B	7.15 A	6.89 BC	6.80 B
50 Kr	7.24 A	7.31 A	7.15 BC	7.12 AB
60 Kr	6.67 AB	7.92 A	6.54 C	7.04 B
Gamma Radiatioon +GA_3 (0.5mM)				
40 Kr+GA_3	6.72 AB	7.17 A	6.40 C	6.76 B
50 Kr+GA_3	7.08 AB	6.43 B	6.96 BC	6.82 B
60 Kr+GA_3	7.48 A	7.19 A	7.95 A	7.54 A
Mean	**6.96 A**	**7.19 A**	**6.98 A**	

S.E for variety mean=0.0778

S.E for treatment mean=0.1160

S.E for (VxD) interaction mean=0.2009

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

revealed that both mutagenic treatments decreased the number of primary branches except at 60 Kr with GA_3 treatment where the number of primary branches increased as compared to control. However, this decrease or increase in number of primary branches was non-significant ($p>0.01$).

It is apparent from the table 63 that the three varieties responded differentially to the two mutagenic treatments. In Noor 91 behaviour of the number of primary branches with various gamma irradiation was inconsistent and a non-significant decrease or increase was observed with both mutagenic treatments. The number of primary branches in Punjab 91 decreased significantly ($p<0.01$) at 50 Kr with GA_3 treatment, while at other doses the increase or decrease was non-significant ($p>0.01$) as compared to control. In C141 the number of primary branches decreased significantly ($p<0.01$) at 60 Kr of gamma irradiation and at 40 Kr with the application of GA_3 as compared to control. The number of primary branches increased significantly ($p<0.01$) at 60 Kr with the application of GA_3.

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

Table 64 presents analysis of variance for the effect of different doses of gamma radiation with and without the application of gibberellic acid on the number of secondary branches per plant is M_3 population of chickpea. The results indicate significant ($p<0.05$) difference among the genotypes and treatments. However, genotype-treatment interaction was highly significant ($p<0.01$). It shows that a sufficient variability is created across various doses in the three genotypes. Noor 91 exhibited maximum number of secondary branches 19.19 per plant followed by 18.84 and 17.88 per plant in Punjab 91 and C141, respectively (Table 65). It is seen from the results that the two mutagenic treatments affected the number of secondary branches per plant in a different way. Gamma irradiation reduced the number of secondary branches significantly ($p<0.01$) at 40 and 60 Kr dosages and non-significantly ($p>0.01$) at 50 Kr as compared to control. Application of gibberellic acid decreased the number of secondary branches per plant non-significantly ($p>0.01$) as compared to control.

It is seen from table 65 that the genotypes Noor 91 and Punjab 91 responded indentically towards the two mutagenic treatments and the increase or decrease in the number of secondary branches was non-significant as compared to the untreated check ($p<0.01$). However, in C141 significant ($p<0.01$) decrease in the number of secondary branches was

Table 64: **Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on number of secondary branches in M_3 generation of three chickpea genotypes.**

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	7.20	3.60	1.23
Variety (V)	2	43.04	21.52	7.36*
Error I	4	11.70	2.92	
Treatment (D)	6	66.76	11.12	4.29**
(VxD)	12	70.69	5.89	2.27*
Error II	36	93.56	2.54	
Total	**62**	**292.98**		

*,** indicate significance at 5 and 1% level of probability.

Table 65: **Effect of different doses of gamma radiation separately and with GA_3 on number of secondary branches in M_3 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	21.33 A	18.37 A	22.13 A	20.61 A
Gamma radiation				
40 Kr	18.73 A	17.94 A	15.04 C	17.24 C
50 Kr	21.71 A	18.45 A	19.70 AB	19.95 AB
60 Kr	19.51 A	18.04 A	17.08 BC	18.21 BC
Gamma radiation +GA_3 (0.5 mM)				
40 Kr+GA_3	19.81 A	19.11 A	16.63 BC	18.52 ABC
50 Kr+GA_3	18.86 A	19.60 A	17.79 BC	18.75 ABC
60 Kr+GA_3	19.42 A	20.40 A	16.83 BC	18.88 ABC
Mean	**19.19 A**	**18.84 A**	**17.88 A**	

S.E for variety mean=0.3728

S.E for treatment mean=0.5312

S.E for (VxD) interaction mean=0.9201

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

observed with the two treatments except at 50 Kr of gamma irradiation where the decrease was non-significant ($p<0.01$) as compared to control.

iv: NUMBER OF PODS PER PLANT

Table 66 exhibits highly significant differences ($p<0.01$) for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on number of pods per plant in M_3 population of chickpea within the treatments and genotypes. The interaction between genotype-treatment was also highly significant. It reflects highly significant variation among genotypes for this character. Maximum number of pods 86.58 per plant was produced by variety Punjab 91 as against 82.91 and 76.78 per plant in Noor 91 and C141, respectively (Table 67). The data regarding the pods per plant revealed that the effects at various doses of two mutagenic treatments were inconsistent and non-significant increase or decrease ($p>0.01$) was observed as compared to control. However, the number of pods per plant decreased significantly ($p<0.01$) at 50 Kr with the application of gibberellic acid.

The response of three varieties with regards to pods per plant was differential with the two mutagenic treatments. In Noor 91 the number of pods increased significantly ($p<0.01$) at all levels of irradiation in both treatments. Application of GA_3 significantly ($p<0.01$) increased the number of pods per at 60 Kr dose. In Punjab 91 the number of pods decreased significantly at 40 and 50 Kr, while increased non-significantly at 60 Kr dose of gamma irradiation as compared to control. Application of GA_3 significantly decreased the number of pods at 50 Kr. C141 exhibited a decrease in the number of pods per plant at all levels of irradiation in two mutagenic treatments. However, number of pods per plant decreased significantly ($p<0.01$) at 60 Kr of gamma irradiation and at 40 Kr with the application of gibberellic acid as compared to control. However, significantly more number of pods per plant obtained at 60 Kr with the treatment of GA_3.

v: NUMBER OF SEEDS PER POD

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on number of seeds per pods in M_3 population of chickpea (Table 68) reveals highly significantly ($p<0.01$) differences among treatments and genotypes. The interaction between genotype-treatment was also highly

Table 66: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on number of pods per plant in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	4.09	2.04	0.82
Variety (V)	2	1029.96	514.98	208.49**
Error I	4	9.89	2.47	
Treatment (D)	6	216.26	36.04	3.86**
(VxD)	12	775.69	64.64	6.92**
Error II	36	335.90	9.33	
Total	**62**	**2371.82**		

** indicate significance at 1% level of probability.

Table 67: Effect of different doses of gamma radiation separately and with GA_3 on number of pods per plant in M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	74.67 C	89.46 A	82.46 A	82.20 AB
Gamma radiation				
40 Kr	83.08 AB	86.05 AB	74.60 BC	81.24 AB
50 Kr	89.66 A	89.41 A	77.10 AB	85.39 A
60 Kr	77.56 BC	90.48 A	72.94 C	80.33 B
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	86.07 A	86.58 AB	73.38 C	82.01 AB
50 Kr+GA_3	82.44 AB	80.77 B	75.61 ABC	79.61 B
60 Kr+GA_3	86.93 A	83.31 AB	81.39 AB	83.87 AB
Mean	**82.91 B**	**86.58 A**	**76.78 C**	

S.E for variety mean=0.3429

S.E for treatment mean=1.0181

S.E for (VxD) interaction mean=1.7635

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

significant ($p<0.01$). It indicates highly inconsistent performance of genotypes across the different doses. On the average, maximum number of seeds 1.63 per plant were observed in Punjab 91, followed by 1.49 and 1.44 per pod in C141 and Noor 91, respectively (Table 69). The data regarding the number of seeds per pod revealed that both mutagenic treatments increased the number of seeds per pod significantly ($p<0.01$) at various levels of irradiation as compared to control. However, significantly more number of seeds per pod obtained at 40 and 50 Kr with the application of gibberellic acid.

Generally, the three varieties responded differently at various levels of irradiation with the two mutagenic treatments. In Noor 91 the number of seeds per pod increased significantly ($p<0.01$) with both mutagenic treatments as compared to control. Treatment of GA_3 significantly increased the number of seeds per pod at 50 Kr by changing the effects of gamma irradiation. In Punjab 91, the number of seeds per pod decreased significantly ($p<0.01$) at 40 and 60 Kr of gamma irradiation, while with gibberellic acid the increase or decrease in number of seeds per pod at various doses was non-significant ($p>0.01$) as compared to control. Application of GA_3 increased the number of seeds per pod significantly ($p<0.01$) at 40 and 60 Kr. C141 exhibited significant ($p<0.01$) increase in the number of seeds per pod at various levels of gamma irradiation as compared to control. However, significantly ($p<0.01$) more number of seeds per pod were recorded with gibberellic acid at 60 Kr dose.

vi:100 SEED WEIGHT PER PLANT (g)

Table 70 presents analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on 100-seed weight in M_3 population of chickpea. The results show highly significant ($p<0.01$) differences within treatments, while for genotypes it was non-significant ($p>0.05$). The interaction between genotype-treatment was also highly significant ($p<0.01$). It reveals highly inconsistent performance of genotypes for this character. Maximum 100-seed weight of 26.07 g was produced by C141 as against 25.97 and 25.58 g in Punjab 91 and Noor 91, respectively (Table 71). It is seen from the results (Table 71) that 100-seed weight increased at various levels of irradiation in two mutagenic treatments. However, heavier seeds were produced with the application of gibberellic acid at all levels of irradiation and significant increase was noticed at 40 Kr treatment as compared to control.

Table 68: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on number of seeds per pod in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	0.0048	0.0024	4.00
Variety (V)	2	0.4181	0.2090	348.33**
Error I	4	0.0027	0.0006	
Treatment (D)	6	0.2004	0.0334	30.36**
(VxD)	12	0.1688	0.0140	12.72**
Error II	36	0.0396	0.0011	
Total	**62**	**0.8346**		

** indicate significance at 1% level of probability.

Table 69: Effect of different doses of gamma radiation separately and with GA_3 on number of seeds per pod M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	1.27 D	1.65 A	1.29 D	1.40 E
Gamma radiation				
40 Kr	1.44 BC	1.56 C	1.51 C	1.50 D
50 Kr	1.39 C	1.66 A	1.50 C	1.51 CD
60 Kr	1.54 A	1.58 BC	1.52 C	1.55 AB
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	1.44 BC	1.68 A	1.51 C	1.54 BC
50 Kr+GA_3	1.53 A	1.64 AB	1.54 BC	1.57 AB
60 Kr+GA_3	1.49 AB	1.67 A	1.60 A	1.58 A
Mean	**1.44 C**	**1.63 A**	**1.49 B**	

S.E for variety mean=0.0053

S.E for treatment mean=0.0110

S.E for (VxD) interaction mean=0.0191

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Modulation of radio sensitivity with gibberellic acid was observed in 100-seed weight in the three genotypes. 100-seed weight increased at various levels of gamma irradiation in Noor 91 and Punjab 91 except at 60 Kr dose in Noor 91, where smaller seeds were produced as compared to control. This increase in 100-seed weight was irregular and also non-significant ($p>0.01$) as compared to control. However, the application of gibberellic acid increased the 100-seed weight non-significantly ($p>0.01$) at various irradiation dosages. In C141 weight of 100-seeds decreased at 40 and 50 Kr, while increased at 60 Kr dose non-significantly as compared to control. Application of gibberellic acid increased the weight of 100-seeds significantly ($p<0.01$) at 40 Kr dose, while the increase at other doses was non-significant ($P<0.01$) as compared to control.

vii: BIOLOGICAL YIELD PER PLANT (g)

It is obvious from the analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on biological yield per plant (Table 72) in M_3 population of chickpea that differences within treatments were highly significant. The results show that the variability due to genotypes was non-significant ($p>0.05$). The interaction between genotype-treatment was highly significant ($p<0.01$). On the average, maximum amount of biological yield 37.64 g per plant was produced by C141, followed by 86.33 and 86.29 g. in Noor 91 and Punjab 91, respectively (Table 73). It is evident from table 73 that the biological yield decreased significantly ($p<0.01$) at various levels of gamma irradiation across the two mutagenic treatments as compared to control.

The main effect of genotype-dose was highly significant ($p<0.01$). In Noor 91, gamma irradiation decreased the biological yield per plant non-significantly ($p>0.01$) at 40 and 50 Kr, while significantly at 60 Kr as compared to control. However, this decrease with gibberellic acid was significant ($p<0.01$) at all the treatments. Biological yield per plant decreased significantly ($p<0.01$) with both mutagenic treatments in Punjab 91 and C 141 as compared to control. However, non-significant differences were recorded at various doses in both mutagenic treatments.

Table 70: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on 100-seed weight in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	0.35	0.17	0.68
Variety (V)	2	2.84	1.42	5.68
Error I	4	1.02	0.25	
Treatment (D)	6	2.74	0.45	5.00**
(VxD)	12	2.91	0.24	2.66**
Error II	36	3.35	0.09	
Total	**62**	**13.23**		

** indicate significance at 1% level of probability.

Table 71: Effect of different doses of gamma radiation separately and with GA_3 on 100-seed weight in M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	25.42 AB	25.53 B	25.97 BC	25.64 BC
Gamma raidation				
40 Kr	25.49 AB	25.96 AB	25.90 BC	25.78 BC
50 Kr	25.55 AB	26.18 AB	25.83 BC	25.85 ABC
60 Kr	25.19 B	25.56 B	26.06 ABC	25.60 C
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	25.81 AB	26.24 AB	26.68 A	26.24 A
50 Kr+GA_3	26.11 A	26.33 A	25.65 C	26.03 AB
60 Kr+GA_3	25.50 AB	26.00 AB	26.42 AB	25.97 ABC
Mean	**25.58 A**	**25.97 A**	**26.07 A**	

S.E for variety mean=0.1091

S.E for treatment mean=0.1000

S.E for (VxD) interaction mean=0.1732

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 72: **Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on biological yield in M_3 generation of three chickpea genotypes.**

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	9.86	4.93	1.74
Variety (V)	2	24.83	12.41	4.38
Error I	4	11.32	2.83	
Treatment (D)	6	2382.07	397.01	30.44**
(VxD)	12	567.20	47.26	3.62**
Error II	36	469.45	13.04	
Total	**62**	**3464.75**		

** indicate significance at 1% level of probability.

Table 73: **Effect of different doses of gamma radiation separately and with GA_3 on biological yield in M_3 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	93.40 A	99.70 A	111.60 A	101.56 A
Gamma radiation				
40 Kr	85.25 AB	82.85 B	84.46 B	84.19 B
50 Kr	88.48 AB	84.55 B	86.72 B	86.92 B
60 Kr	85.10 B	84.14 B	81.00 B	83.41B
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	84.12 B	83.88 B	82.36 B	83.45 B
50 Kr+GA_3	83.33 B	85.12 B	83.76 B	84.07 B
60 Kr+GA_3	84.62 B	83.82 B	82.60 B	83.68 B
Mean	**86.33 A**	**86.29 A**	**87.64 A**	

S.E for variety mean=0.3670

S.E for treatment mean=1.2036

S.E for (VxD) interaction mean=2.0848

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

viii: GRAIN YIELD PER PLANT (g)

The analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on grain yield per plant (Table 74) in M_3 population chickpea reveals highly significant ($p<0.01$) differences among the treatments and genotypes. The interaction between genotype-treatment was also highly significant for this character. It reflects highly inconsistent performance of genotypes across the various treatments. Maximum grain yield 36.83 g per plant was produced in genotype Punjab 91, followed by 30.63 and 29.95 g in Noor 91 and C141, respectively (Table 75). It is apparent from the results that grain yield per plant increased significantly at various levels of irradiation in two mutagenic treatments except at 40 Kr with gamma irradiation as compared to control. However, significantly ($p<0.01$) more grain yield was obtained at 60 Kr with gibberellic acid.

In Noor 91 grain yield per plant increased significantly ($p<0.01$) at various levels of gamma irradiation in the two mutagenic treatments. In Punjab 91 grain yield per plant decreased at 40 and 60 Kr, while increased at 50 Kr non-significantly ($p>0.01$) as compared to control. Application of gibberellic acid decreased the grain yield significantly at 50 Kr dose. In C141 grain yield per plant increased at various levels of gamma irradiation non-significantly ($p<0.01$) as compared to control. Application of gibberellic acid increased the grain yield significantly ($p<0.01$) at 60 Kr as compared to control.

ix: HARVEST INDEX (%)

Table 76 exhibits highly significant ($p<0.01$) differences for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on harvest index percentage in M_3 population of chickpea. However, the interaction between genotype-treatment was non-significant ($p>0.05$). On the average, maximum harvest index 42.68 percent exhibited by Punjab 91 as against the 35.68 and 34.70 percent in Noor 91 and C141, respectively (Table 77). It is seen from the results that harvest index increased significantly ($p<0.01$) as compared to untreated check at various level of irradiation in two mutagenic treatments. However, more percentage of harvest index was observed with the treatment of gibberellic acid.

Table 74: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on grain yield in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	8.90	4.45	4.15
Variety (V)	2	604.87	302.43	282.64**
Error I	4	4.31	1.07	
Treatment (D)	6	128.15	21.35	9.04**
(VxD)	12	164.70	13.72	5.81**
Error II	36	85.24	2.36	
Total	**62**	**996.18**		

** indicate significance at 1% level of probability.

Table 75: Effect of different doses of gamma radiation separately and with GA_3 on grain yield in M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	24.10 B	37.92 AB	27.58 B	29.80 C
Gamma radiation				
40 Kr	30.53 A	34.97 B	29.33 B	31.61 BC
50 Kr	31.82 A	38.95 A	29.85 B	33.54 AB
60 Kr	30.15 A	36.66 AB	29.02 B	31.94 B
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	31.97 A	38.16 AB	29.54 B	33.22 AB
50 Kr+GA_3	32.80 A	35.20 B	29.87 B	32.62 AB
60 Kr+GA_3	33.03 A	36.18 AB	34.46 A	34.56 A
Mean	**30.63 B**	**36.83 A**	**29.95 B**	

S.E for variety mean=0.2257

S.E for treatment mean=0.5120

S.E for (VxD) interaction mean=0.8869

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

Table 76: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on harvest index in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	18.48	9.24	2.67
Variety (V)	2	838.81	419.40	121.21**
Error I	4	13.86	3.46	
Treatment (D)	6	796.07	132.67	17.05**
(VxD)	12	192.13	16.01	2.05
Error II	36	280.18	7.78	
Total	**62**	**2139.56**		

** indicate significance at 1% level of probability.

Table 77: Effect of different doses of gamma radiation separately and with GA_3 on harvest index in M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	25.85 B	37.88 B	24.71 C	29.48 B
Gamma radiationl				
40 Kr	35.78 A	42.25 AB	34.83 B	37.62 A
50 Kr	36.01 A	46.21 A	34.07 B	38.76 A
60 Kr	35.51 A	43.72 AB	35.81 AB	38.35 A
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	38.06 A	45.57 A	35.94 AB	39.86 A
50 Kr+GA_3	39.40 A	41.37 AB	35.74 AB	38.83 A
60 Kr+GA_3	39.12 A	43.16 AB	41.79 A	41.36 A
Mean	**35.68 B**	**42.88 A**	**34.70 B**	

S.E for variety mean=0.4059

S.E for treatment mean=0.9297

S.E for (VxD) interaction mean=1.6103

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

In Noor 91 and C141 harvest index increased significantly ($p<0.01$) at various levels of irradiation in two mutagenic treatments. Whereas, in Punjab 91 harvest index increased significantly ($p<0.01$) at 50 Kr of gamma irradiation and at 40 Kr with gibberellic acid as compared to control.

x: FLOWERING DAYS

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on days to 50% flowering in M_3 population of chickpea (Table 78) indicates highly significant ($p<0.01$) differences within genotypes and treatments. The interaction between genotype-treatment was also highly significant ($p<0.01$). It reveals that sufficient variability exists among the genotypes for this character. On the average, maximum time 119.43 days to 50% flowering were taken by Noor 91, followed by 118.78 and 113.77 days in C141 and Punjab 91, respectively (Table 79). It is seen from the table 79 that time taken to 50% flowering decreased at 40 Kr, while increased at 50 and 60 Kr of gamma irradiation significantly ($p<0.01$) as compared to control. Application of gibberellic acid at various levels of irradiation delayed 50% flowering days non-significantly ($p>0.01$) as compared to control.

Modulation of radio sensitivity with gibberellic acid was observed either in positive or negative direction at various levels of irradiation in all the genotypes. In Noor 91 days to 50% flowering increased at various levels of irradiation in the two mutagenic treatments except at 60 Kr of gibberellic acid as compared to control. In Punjab 91, days to 50% flowering increased at various levels of irradiation except at 40 Kr dose of gamma irradiation and with gibberellic acid treatment as compared to control. By comparing the various intensities of irradiation in the two mutagenic treatments on days to 50% flowering, significant ($p<0.01$) differences were observed at 40 and 60 Kr doses. C141 exhibited a linear increase in number of days to 50% flowering with an increase of gamma irradiation dosages. However, significant ($p<0.01$) difference as compared to control was found at 50 and 60 Kr doses of gamma irradiation. Application of gibberellic acid changed the effect of gamma irradiation and significant ($p<0.01$) earliness in 50% flowering was observed at 50 Kr.

Table 78: **Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on days to 50% flowering in M_3 generation of three chickpea genotypes.**

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	1.25	0.62	7.75
Variety (V)	2	402.95	201.47	2518.37**
Error I	4	0.35	0.08	
Treatment (D)	6	28.67	4.77	59.62**
(VxD)	12	23.60	1.96	24.50**
Error II	36	5.22	0.14	
Total	**62**	**462.05**		

** indicate significance at 1% level of probability.

Table 79: **Effect of different doses of gamma radiation separately and with GA_3 on days to 50% flowering in M_3 population of three chickpea genotypes.**

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	118.67 D	113.50 BC	118.33 D	116.83 C
Gamma radiation				
40 Kr	118.97 CD	112.06 D	118.17 D	116.40 D
50 Kr	120.03 AB	114.22 B	119.20 BC	117.82 D
60 Kr	120.46 AB	115.33 A	120.12 A	118.64 A
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	120.60 A	112.94 C	118.41 CD	117.32 C
50 Kr+GA_3	119.71 BC	114.33 B	117.94 D	117.32 C
60 Kr+GA_3	117.60 E	114.04 B	119.33 AB	116.99 C
Mean	**119.43 A**	**113.77 C**	**118.78 B**	

S.E for variety mean=0.0617

S.E for treatment mean=0.1247

S.E for (VxD) interaction mean=0.2160

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

xi: MATURITY DAYS

The analysis of variance for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on days to maturity (Table 80) in M_3 generation of chickpea indicates highly significant differences among genotypes and treatments. The interaction between genotype-treatment treatment was also highly significant ($p<0.01$). Noor 91 exhibited earliness in maturity with minimum 166.67 days, followed by 168.63 and 169.75 days in Punjab 91 and C141, respectively (Table 81). Main mutagenic treatments indicated that gamma irradiation decreased the number of days to maturity significantly ($p<0.01$) at 40 Kr as compared to control. However, the application of gibberellic acid decreased the days to maturity significantly ($p<0.01$) at 40 and 50 Kr doses as compared to control.

The interaction of varieties and the treatments revealed that sufficient variability was induced for this character. In Noor 91, all levels of gamma irradiation significantly ($p<0.01$) increased the days to maturity as compared to control. However, the gibberellic acid treatment increased the days to maturity at 60 Kr, while at 40 and 50 Kr doses statistically no difference in days to maturity was observed as compared to control. In Punjab 91 days to maturity decreased significantly ($p<0.01$) at various levels of irradiation except at 50 Kr of gamma irradiation and at 60 Kr with gibberellic acid where it was non-significant as compared to control. On comparing the two mutagenic treatments, significant ($p<0.01$) difference in days to maturity was found at 50 Kr where earliness in maturity was noted with GA_3. Days to maturity decreased at all levels of irradiation significantly ($p<0.01$) in C141 as compared to control.

Table 80: Analysis of variance for the effect of different doses of gamma radiation separately and with the application of gibberellic acid on days to maturity in M_3 generation of three chickpea genotypes.

Source of variation	D.F.	Sum of squares	Mean squares	F-value
Replication	2	1.17	0.58	3.41
Variety (V)	2	101.80	50.90	299.41**
Error I	4	0.68	0.17	
Treatment (D)	6	35.54	5.92	25.73**
(VxD)	12	39.50	3.29	14.30**
Error II	36	8.38	0.23	
Total	**62**	**187.10**		

** indicate significance at 1% level of probability.

Table 81: Effect of different doses of gamma radiation separately and with GA_3 on days to maturity in M_3 population of three chickpea genotypes.

Treatments	Noor 91	Punjab 91	C 141	Mean
Control	165.33 C	170.33 A	171.00 A	168.89 A
Gamma radiation				
40 Kr	166.89 B	167.83 C	168.75 C	167.82 B
50 Kr	166.93 B	169.20 AB	170.33 B	168.82 A
60 Kr	167.87 B	168.67 BC	169.75 BC	168.76 A
Gamma radiation +GA_3 (0.5mM)				
40 Kr+GA_3	165.33 C	168.44 BC	169.75 BC	167.84 B
50 Kr+GA_3	165.32 C	166.49 D	169.22 BC	167.01 C
60 Kr+GA_3	169.05 A	169.45 AB	169.64 BC	169.32 A
Mean	**166.67 C**	**168.63 B**	**169.75 A**	

S.E for variety mean=0.0899

S.E for treatment mean=0.1598

S.E for (VxD) interaction mean=0.2768

Means not followed by the same letter are statistically significant by DMRT at 1% level of probability.

VII: CHLOROPHYLL AND MORPHOLOGICAL MUTATION SPECTRUM IN M_2 GENERATION

Several types of chlorophyll and morphological mutants arised in M_2 population. The frequency of occurrence of these mutations in treated population is considered an appropriate measure for determining the effectiveness of a mutagen. Different types of chlorophyll and morphological mutants obtained through the seed treatment with gamma irradiation and post mutagenic application of GA_3 are described below:

A: CHLOROPHYLL MUTATIONS

i: MUTATION FREQUENCY

The frequencies of chlorophyll mutations in terms of percentage of M_2 population basis as well as on M_2 family basis are presented in table 82. The trend of the mutation frequency was different in the two methods of mutagenic treatment. The frequency of chlorophyll mutations on the family basis decreased with an increase of gamma irradiation in the three genotypes. However, with the post mutagenic treatment of GA_3 such trend was not observed. Noor 91 exhibited the highest mutation frequency on M_2 family basis (21.20%) and M_2 population basis (1.22%). The effective dose of gamma irradiation to achieve the saturation effect i.e. the highest mutation frequency varied among the genotypes. The highest mutation frequencies were obtained following 40 Kr dose in Noor 91 and C 141 genotype, however, in Punjab 91 saturation effect was attained at 40 Kr with GA_3 treatment.

ii: SINGLE AND MULTIPLE MUTATIONS

Relative frequencies of families segregating for single and multiple chlorophyll mutation type are presented in table 84. Most of the progenies segregated for one chlorophyll mutation type. The proportion of segregation families ranged from 97.22 to 100% for one type and from 2.23 to 78% for two types. Induction of multiple chlorophyll mutations appeared independent of higher or lower side effect of mutagen.

Table 82: Frequency of chlorophyll mutants in M_2 generation of three chickpea genotypes planted at BARI, Chakwal, 1996-97.

Genotype	Frequency on M_2 family basis				Frequency on M_2 population basis		
	Treatment	Total families	Segregating families	%	Total plants	Mutants	%
Noor 91	Control	10	0	0	265	0	0
	40 Kr	250	53	21.20	5438	57	1.04
	50 Kr	250	47	18.80	5613	69	1.22
	60 Kr	250	36	14.40	5140	45	0.87
	40 Kr+GA_3	250	48	19.20	5529	54	0.97
	50 Kr+GA_3	250	51	20.40	5430	43	0.79
	60 Kr+GA_3	250	45	18.00	5260	53	1.00
Punjab 91	Control	10	0	0	274	0	00
	40 Kr	250	45	18.00	5381	33	0.61
	50 Kr	250	43	17.20	5470	41	0.74
	60 Kr	250	34	13.60	5290	28	0.52
	40 Kr+GA_3	250	47	18.80	5476	30	0.54
	50 Kr+GA_3	250	42	16.80	5310	36	0.67
	60 Kr+GA_3	250	43	17.20	5332	35	0.65
C 141	Control	10	0	0	268	0	00
	40 Kr	250	25	10.00	5511	22	0.39
	50 Kr	250	21	8.40	5476	28	0.51
	60 Kr	250	17	6.80	5380	27	0.50
	40 Kr+GA_3	250	23	9.20	5490	20	0.36
	50 Kr+GA_3	250	20	8.00	5481	24	0.43
	60 Kr+GA_3	250	22	8.80	5415	34	0.62

iii: SPECTRUM OF CHLOROPHYLL MUTANTS

A wide spectrum of chlorophyll mutations was observed which included *albina, xantha, chlorina* and *viridis* (Table 86). The different characteristics of these mutants were as follow:

1. **Albina:** These were characterized by yellowish leaves with white tips. The yellow pigment became more intense in color from the tip to the base. These mutants survived for 15-25 days and were found in all treatments of gamma radiation and also in post irradiation with GA_3 population.

2. **Xantha:** These mutants were light yellowish-green, carotenoids predominate, while chlorophyll did not form. Although these were the most frequent types of mutants in almost all the treatments yet they failed to survive for long.

3. **Chlorina:** These were characterized by greenish-yellow leaves. Most of these seedlings died within 15 days and in few cases *chlorina* mutants did survive but were late in flowering.

4. **Viridis:** This type was light green to yellowish-green in color. All the *viridis* mutants survived and grew vigorously, but had few branches, very weak stems and a low number of seeds and pods per plant.

All these chlorophyll mutation did not show any association with a specific genotype. On the overall basis of three genotypes, the mutant can be arranged in the following order: *Xantha chlorina albina viridis.*

B: MORPHOLOGICAL MUTATIONS

i: MUTATION FREQUENCY

Besides the chlorophyll mutation, many morphological mutations were observed in all the treatments. Table 83 shows the frequency of these mutants. The data show that such mutations occurred more frequently in the simultaneous application of gamma radiation and GA_3 than in gamma irradiation separately except in Punjab 91, where more morphological mutations occurred with gamma irradiation. A dose dependent increase in the frequency of morphological mutations was observed with gamma irradiation and with simultaneous application of GA_3, both on the percentage of segregation of M_2 families and population basis. Viable mutation frequency ranged from 4.80 to 17.60% on progeny basis and 0.36 to 1.02% on population basis.

Table 83: Frequency of morphological mutants in M_2 generation of three chickpea genotypes planted at BARI, Chakwal, 1996-97.

Genotype	Frequency on M_2 family basis				Frequency on M_2 population basis		
	Treatment	Total families	Segregating families	%	Total plants	Mutants	%
Noor 91	Control	10	0	0.00	265	0.00	0.00
	40 Kr	250	17	6.80	5438	23	0.42
	50 Kr	250	20	8.00	5613	27	0.48
	60 Kr	250	29	11.60	5140	40	0.77
	40 Kr+GA_3	250	21	8.40	5529	28	0.50
	50 Kr+GA_3	250	25	10.00	5430	30	0.55
	60 Kr+GA_3	250	27	10.80	5260	29	0.55
Punjab 91	Control	10	0	0.00	274	0	0.00
	40 Kr	250	41	16.40	5381	55	1.02
	50 Kr	250	37	14.80	5470	56	1.02
	60 Kr	250	44	17.60	5290	55	1.03
	40 Kr+GA_3	250	35	14.00	5476	47	0.85
	50 Kr+GA_3	250	39	15.60	5310	54	1.01
	60 Kr+GA_3	250	42	16.80	5332	54	1.01
C 141	Control	10	0	0.00	268	0	0.00
	40 Kr	250	12	4.80	5511	20	0.36
	50 Kr	250	17	6.80	5476	28	0.51
	60 Kr	250	22	8.80	5380	37	0.68
	40 Kr+GA_3	250	16	6.40	5490	25	0.45
	50 Kr+GA_3	250	21	8.40	5481	34	0.62
	60 Kr+GA_3	250	22	8.80	5415	33	0.60

Table 84: Relative frequency of M_2 families segregating for varying number of mutation types

a) **Chlorophyll mutations**

Treatment	Genotypes					
	Noor 91		Punjab 91		C 141	
	Type 1	Type 2	Type 1	Type 2	Type 2	Type 2
Control	0.00	0.00	0.00	0.00	0.00	0.00
40 Kr	100.00	0.00	97.77	2.23	100.00	0.00
50 Kr	100.00	0.00	100.00	0.00	100.00	0.00
60 Kr	97.22	2.78	100.00	0.00	100.00	0.00
40 Kr+GA_3	100.00	10.00	100.00	0.00	100.00	0.00
50 Kr+GA_3	100.00	0.00	100.00	0.00	100.00	0.00
60 Kr+GA_3	100.00	0.00	97.67	2.33	100.00	0.00

b) **Morphological mutations**

Treatment	Genotypes								
	Noor 91			Punjab 91			C 141		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40 Kr	94.33	3.77	1.88	97.77	2.23	0.00	100.00	0.00	0.00
50 Kr	95.74	4.26	0.00	90.69	6.97	2.32	95.23	4.77	0.00
60 Kr	94.44	2.77	2.77	88.23	8.82	2.94	88.23	11.77	0.00
40 Kr+GA_3	97.91	2.09	0.00	100.00	0.00	0.00	86.95	8.69	4.34
50 Kr+GA_3	94.11	3.92	1.96	95.23	4.77	0.00	100.00	0.00	0.00
60 Kr+GA_3	93.33	6.67	0.00	100.00	0.00	0.00	100.00	0.00	0.00

Table 85: Correlation between appearance of different types of chlorophyll mutants and morphological mutants in M_2 generation of three chickpea genotypes.

1	*Albina*	1.000					
2	*Xantha*	0.740	1.000				
3	*Chlorina*	0.836*	0.922**	1.000			
4	*Viridis*	0.482	-0.331	0.644	1.000		
5	Total chlorophyll	0.918**	0.945**	0.947**	-0.423	1.000	
6	Total morphological	-0.034	-0.388-	-0.285	0.237	-0.013	1.000
		1	2	3	4	5	6

d.f = 4

From the table 86 it could be concluded that the proportion of morphological mutations were lesser than that of chlorophyll mutation (645:709). The correlation worked out between the appearances of different types of chlorophyll mutants and morphological mutants in M_2 population are presented in table 85. A non-significant negative correlation between appearance of *albina, xantha, viridis, chlorina* mutant and of morphological mutants was observed. The correlation has shown that the appearance of morphological mutants is less than the chlorophyll mutants appeared in the M_2 population.

ii: SINGLE AND MULTIPLE MUTATIONS

Relative frequencies of families segregating for single and multiple morphological mutation types are presented in table 84. Most of the families segregated for one type of mutation. The proportion of segregating families ranged from 86.95 to 100% for one type, 2.09 to 11.77 % for two types of morphological mutants, respectively. Noor 91 variety segregated for two and three types with both types of treatment while in C 141 and Punjab 91 genotype progenies segregated only for two types. Gamma irradiation of 60 Kr treatment was most effective to induce multiple mutations in all the genotypes. However, no relationship was observed between frequency of families segregating for two or more types with an increase in mutagen treatment.

iii: SPECTRUM OF MORPHOLOGICAL MUTANTS

The mutagenic treatments induced mutations affecting plant height, growth habit, branching and stem structure, stem and foliage color, leaf type, flowering and maturity, seed and pod type (Table 86). There were differences in mutation spectrum between the genotypes.

A: PLANT HEIGHT MUTANTS

These could be further categorized into stunted, dwarf, semi-dwarf, compact and tall.

1. **Stunted:** Several miniature plants having reduced height with shorter internodes were grouped under this category. All these mutants produced flowers and fruits and set seeds also. Most of these mutants were segregated in Noor 91 and only a few were noticed in Punjab 91. In C 141 no such mutants were segregated. Gamma irradiation of 60 Kr

produced the most stunted seedling mutants, followed by the combined treatment of 60 Kr with GA_3.

2. **Dwarfs:** Dwarf mutants were observed in M_2 population of various mutagenic treatments. Dwarfs were more frequently induced by combined treatments of gamma irradiation with GA_3 than by gamma irradiation alone. In some of these mutants, the leaves had small and narrow leaflets while others had normal leaves. Seed yield in the dwarfs was reduced. These dwarf mutants were found in all the three genotypes.
3. **Semi-dwarfs:** The internodal length in the semi-dwarfs was greater than the dwarfs, but it was conspicuously shorter than the control. There was no apparent reduction in seed yield among the semi-dwarfs. These were more frequent in the combined treatment of gamma irradiation followed by GA_3.
4. **Compacts:** A simple bushy mutant was noticed in Punjab 91. This mutant had similar morphology to the dwarf plants but it had dense growth with increased secondary and tertiary branches, which resulted in a bushy appearance.

B: GROWTH HABIT MUTANTS

These mutants were grouped into erect, spreading, bushy and prostrate type of branching system:

1. **Erect**: These mutants were characterized by the emergence of primary branching in almost vertical direction. These mutants had more primary branches as compared to their respective controls. These mutants were recorded for all the treatments and genotypes, but they were more frequent in Punjab 91.
2. **Spreading**: Reduced number of primary branches in combination with large number of secondary branches were the mutant characters for this group of plants. No spreading mutants were segregated in C 141 with gamma irradiation, however, they did appeared in combined treatment of gamma radiation and GA_3.
3. **Bushy**: These mutants were characterized by having large number of primary branches with a small number of secondary branches. These branches were stiff and stout in texture.
4. **Prostrate**: Only a single prostrate mutant was recorded in Noor 91 with 50 Kr treatment of gamma irradiation.

Table 86: Spectrum of chlorophyll and morphological mutation in M_2 generation of three chickpea genotypes treated with different dosed of gamma irradiation alone and with gibberellic acid (GA_3) planted at BARI, Chakwal, 1996-97.

Mutational group	Mutational classes							
	Gamma irradiation				Gamma irradiation +GA_3			
	Noor 91	Punjab 91	C 141	Total	Noor 91	Punjab 91	C 141	Total
Chlorophyll								
Albina	46	35	10	91	51	21	13	85
Xantha	61	21	23	105	43	30	22	95
Chlorina	38	18	15	91	37	26	19	92
Viridis	26	28	29	83	19	24	24	67
Total	**171**	**102**	**77**	**370**	**150**	**101**	**78**	**339**
Plant height								
Miniature	5	4	0	9	4	6	0	10
Dwarf	3	7	4	14	7	5	8	20
Semi-dwarf	1	3	1	5	3	2	4	9
Compact	0	1	0	1	0	0	0	0
Tall	0	13	0	13	2	19	2	23
Others	0	4	0	4	0	3	0	3
Growth habit								
Erect	11	30	19	50	13	26	15	54
Spreading	7	3	0	10	6	7	3	16
Bushy	3	2	8	13	1	3	11	15
Prostrate	1	0	0	1	0	0	0	0
Branching and stem structure								
Basal	0	5	0	5	0	3	0	3
Umbrella	0	21	1	22	0	17	4	21
Fasciated	7	0	0	7	5	0	1	6
Others	3	0	0	3	1	2	0	3
Plant and foliage color								
Light green	11	0	0	11	8	1	0	9
Dark green	2	0	0	2	3	0	11	14
Violet green	0	3	17	20	0	1	20	21
Leaf								
Tiny	0	4	0	4	0	2	0	2
Narrow	7	20	1	28	4	25	0	29
Small	2	6	3	11	3	10	1	14
Large	0	4	0	4	0	0	0	0
Long lax	4	2	1	7	3	0	0	3
Flower								
Male sterile	0	3	0	3	0	0	0	0
Pink	0	3	0	3	0	0	0	0
Open type	0	3	0	3	0	0	0	0
Gynoecium slender	0	3	0	3	0	0	0	0
Flowering and maturity								
Early	2	7	1	10	7	10	4	21
Late	10	5	0	15	7	10	0	17
Not flowering	0	2	15	17	0	0	9	9

Mutational group	Mutational classes							
	Gamma irradiation				Gamma irradiation +GA_3			
	Noor 91	Punjab 91	C 141	Total	Noor 91	Punjab 91	C 141	Total
Pod and seed								
Large pod	0	4	0	4	0	3	0	3
Long pod	1	0	0	1	4	0	0	4
Variegated seed	0	0	15	15	0	0	0	0
Round seed	10	0	0	10	6	0	0	6
Bold seed	0	4	0	4	0	0	0	0
Total	**261**	**268**	**162**	**691**	**237**	**256**	**170**	**663**

C: BRANCHING AND STEM STRUCTURE MUTANTS

Based on branching and stem structure, the following types of mutants were observed:

1. **Basal**: Basal mutants were recorded only in Punjab 91 in treatment of gamma irradiation and also with GA_3. In these mutants all the primary branches were originated at the most basal area of the stem.
2. **Umbrella**: These mutants were characterized by the origination of primary branches at a distance in the air. Such mutants were recorded more frequently in Punjab 91 and these were not segregated in Noor 91 at all levels of treatments.
3. **Fasciated**: These mutants were observed in Noor 91 with treatments of gamma irradiation and a single plant was also noticed in C 141 at a dose of 60 Kr with GA_3. Such mutants were characterized by the origination of a number of peduncles from each node as against s single peduncle in case of the control.

D: PLANT COLOR AND OTHER MUTANTS

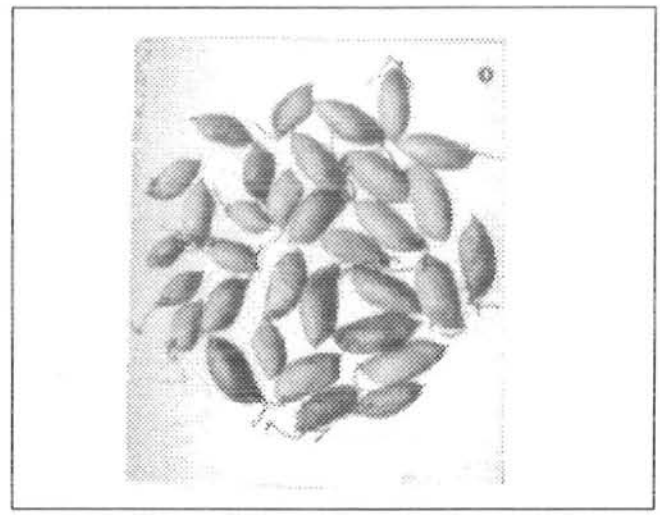
Besides lethal chlorophyll mutants of various categories, some plants markedly different from the normal green color were obtained:

1. **Light green**: These otherwise normal looking plants had a light green color and were markedly different from the normal bright green color plants. Light green mutants were recorded in Noor 91 at all levels of gamma irradiation treatment separately and with GA_3. They had not been observed in C 141 with both types of treatments. However, a single mutant for light green color was observed at 40 Kr with GA_3 in Punjab 91.

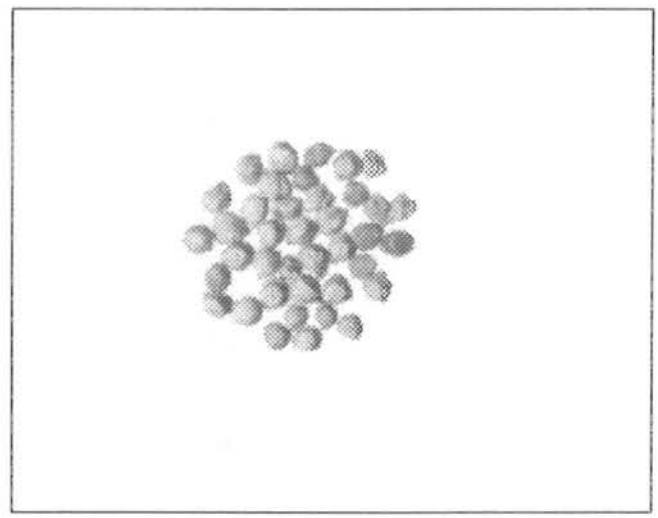
2. **Dark green**: These mutants were segregated in Noor 91 with gamma irradiation alone and with GA_3. In C 141, such mutants were observed only in combined treatment of gamma irradiation and GA_3. However, in Punjab 91 these mutants were not recorded.
3. **Purple pigmented**: A varying degree of anthocyanin pigmentation was observed in some plants. Such mutants were frequently observed in C 141 with the treatment of gamma irradiation alone and with GA_3. These were characterized by having a few numbers of primary branches and low in grain and pod setting. These mutants were not observed in Punjab 91 in both types of treatments.
4. **Leaf mutants**: Mutants showing alterations in leaf characteristics were induced. Five types of leaf mutants were found, namely tiny, narrow, small, large (Fig. 38) and long lax. It becomes evident from the data that the genotype Punjab 91 was sensitive towards leaf mutants. Tiny and large leafed mutants were only recorded in Punjab 91 and also the narrow leafed mutants were more frequent in this genotype. The small-leaf mutants characterized by either reduction in the number and size of leaflets or reduction in both of these.
5. **Flower**: Three mutants with modification in flower parts were recorded in Punjab 91 at a dose of 50 Kr. These mutants were characterized by having polyandrous while sterile stamens, flower open and pink, gynoecium was malformed with protruding beak. The ovary was also protruded out of the flower (Fig 39). Such type of mutants were not recorded in other two genotypes.
6. **Maturity mutants**: The number of days from sowing to first flower in variety Noor 91 ranged from 115-122; in variety Punjab 91 from 110-117 and from 115-123 in genotype C 141. Early and late flowering plants flowering outside the range in respective controls were included in their respective groups. Late flowering had not been recorded in genotype C 141.
7. **Sterile mutants**: Sterile mutants were scored in individual treatment of gamma radiation and with combination of GA_3 in variety C 141 and two mutants were recorded at the highest dose of 60 Kr individually in genotype Punjab 91. Sterile mutants were not observed in genotype Noor 91.
8. **Pod mutants**: Two categories of pod mutants were recorded. The three seeded long pods (instead of normal two seeded ones) were induced by the 40 Kr individually and in

combination with GA_3 in genotype Noor 91, while the large pods of one seeded were recorded in 40 Kr with GA_3.dose in variety Punjab 91. Variant in pod size is shown in Fig 35-37.

9. **Seed mutants**: Among the different seed mutants, variation in color, shape and size of the seed were noticed. A variegated seed mutant which was recorded in genotype C 141 of M_1 generation at a dose of 40 Kr gamma irradiation individually was bred true in M_2 generation (Fig. 40). Four mutants of bold seeds were segregated in genotype Punjab 91 at a dose of 40 Kr with GA_3 (Fig. 38). Round seeded mutants were only recorded in genotype Noor 91. Variation in seed size induced through gamma irradiation is shown in Fig 39-41.

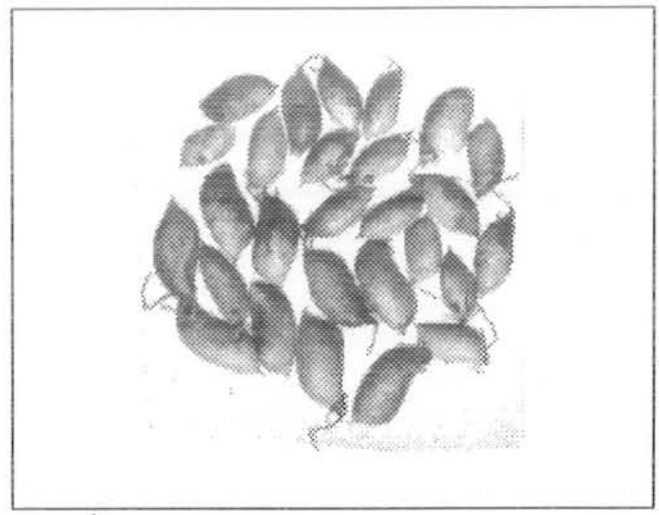


A

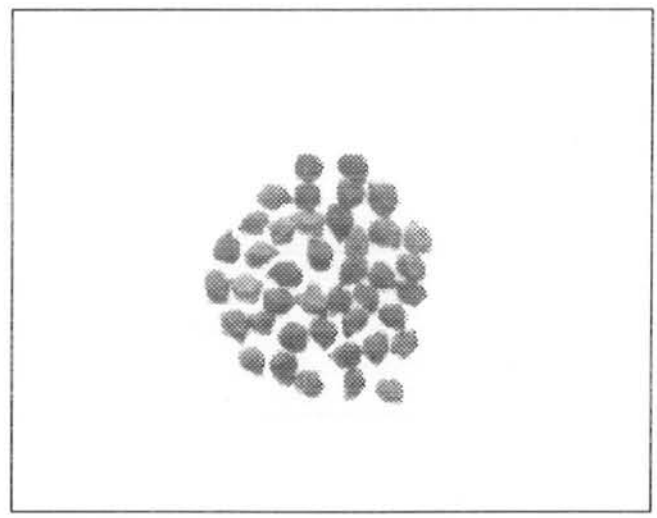


B

Fig. 35: Photograph showing variation in A) pod size and B) seed size in Noor 91 induced through gamma irradiation.

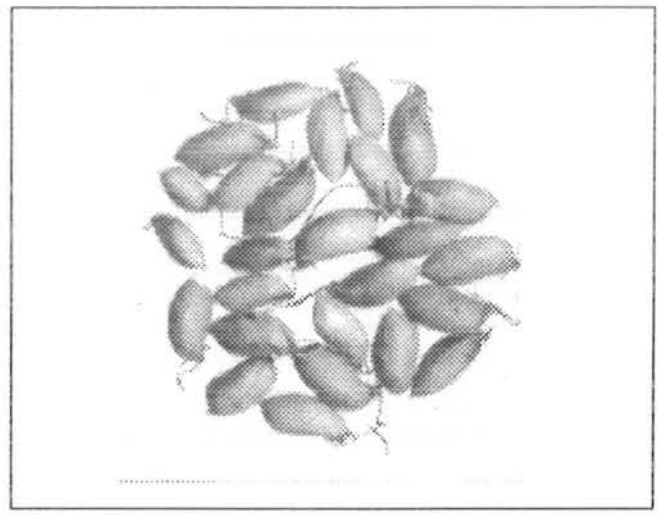


A

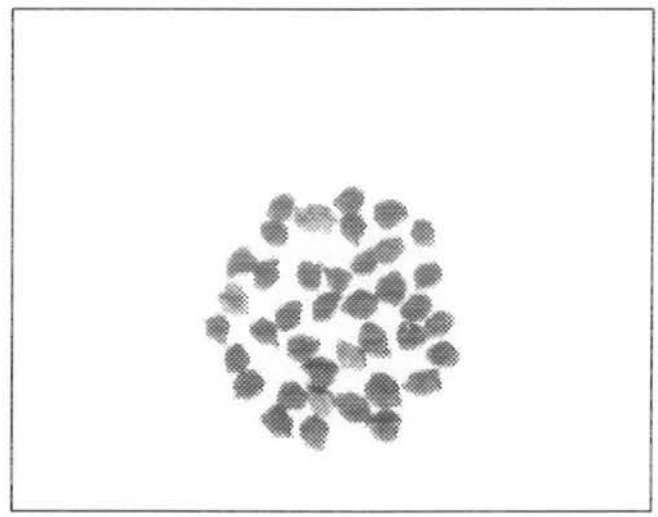


B

Fig. 36: Photograph showing variation in A) pod size and B) seed size in Punjab 91 induced through gamma irradiation.

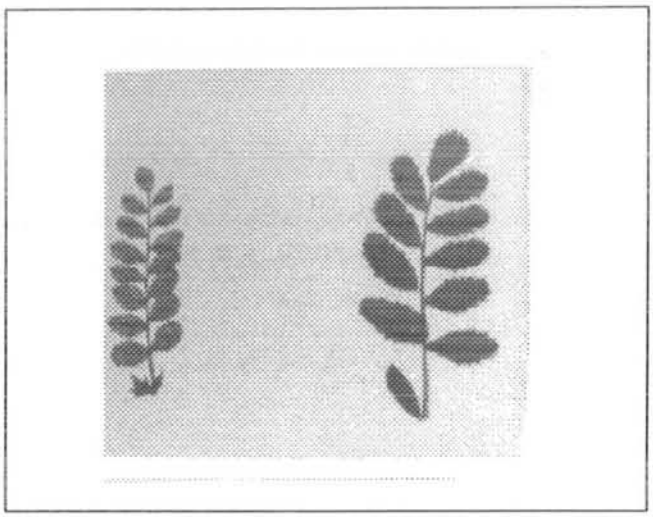


A

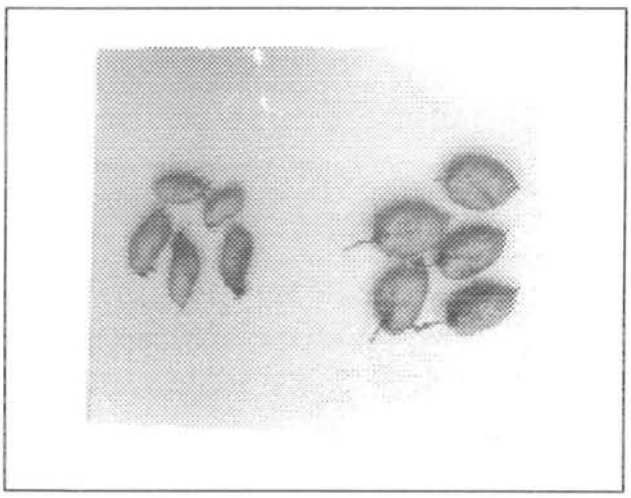


B

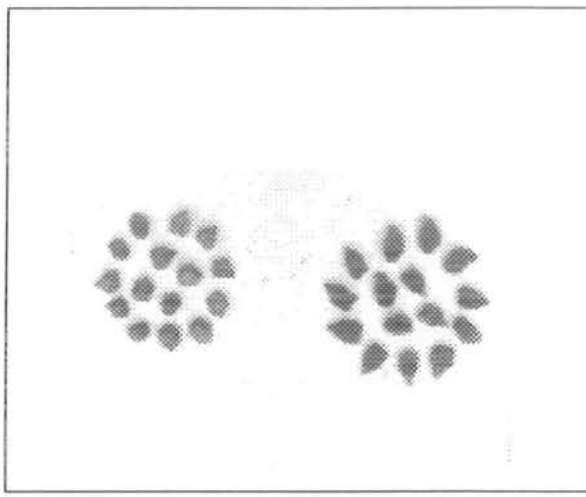
Fig. 37: Photograph showing variation in A) pod size and B) seed size in C141 induced through gamma irradiation.



i A ii



i B ii



i C ii

Fig. 38: Photograph showing i) normal ii) enlarged A) leaf B) pods and C) seed, induced through gamma irradiation in Punjab 91

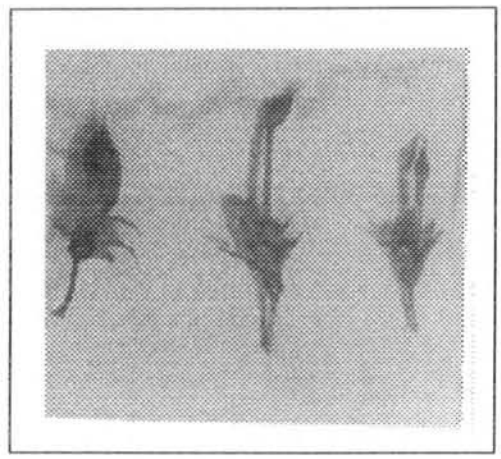
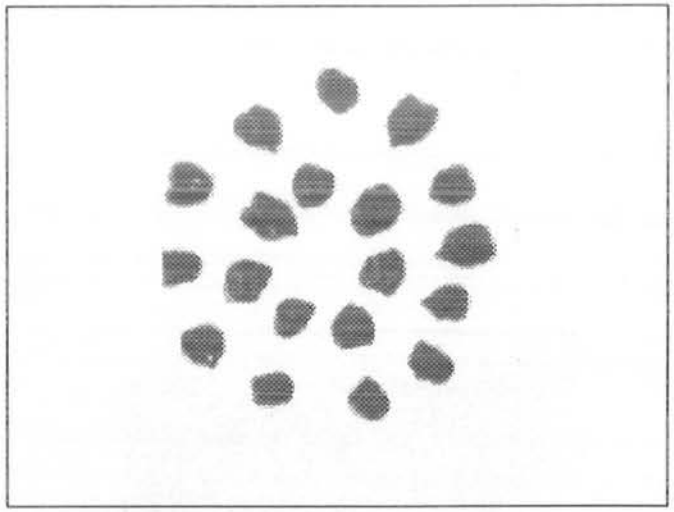
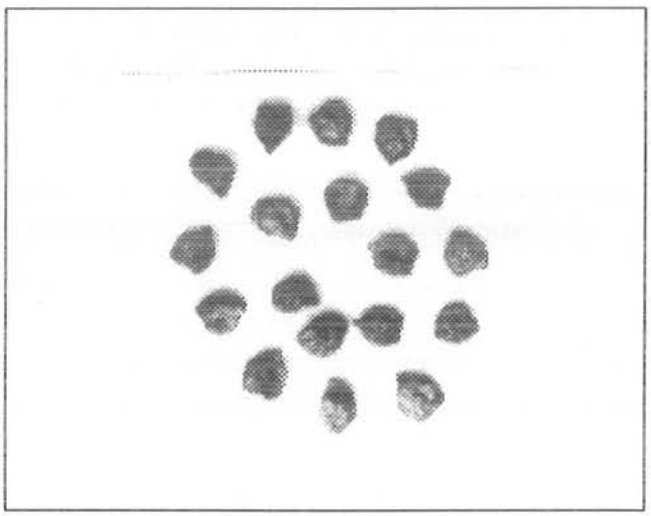


Fig. 39: Photograph showing protruded ovary with development stages induced through gamma irradiation in Punjab 91.



A



B

Fig. 40: Photograph showing variation in A) non-irradiated seeds and B) variegated seeds induced through gamma irradiation in C141.

VIII: HERITABILITY AND GENETIC ADVANCE

I: M_1 GENERATION

The estimates of phenotypic and genotypic coefficient of variability (Table 87) revealed that the magnitude of phenotypic coefficient of variability (PCV) were higher than genotypic coefficient of variability (GCV) for all the characters except for seeds per pod with the combined treatment in Noor 91 which was higher and negative.

It is revealed from the results that the application of gibberellic acid modulated the effects of gamma irradiation to a considerable extent in the three genotypes. Coefficient of variability of plant height decreased with gibberellic acid in Noor 91 and Punjab 91. However, in C 141 genotype more variations were recorded with the combined treatment. Gibberellic acid induced more variations for number of primary and secondary branches in genotypes Noor 91 and C141, while in Punjab 91 the case was reversed. Variability decreased with the post mutagenic application of gibberellic acid for rest of the characters in Noor 91 and Punjab 91.However, more variations were recorded for biological yield in Noor 91 with the combined treatment. In C141 GA_3 treatment increased the coefficient of variability for pods per plant, seeds per pod, biological yield, grain yield and harvest index while, for 100-seed weight, days to flowering and maturity the variability decreased.

In the present studies all the characters in M_1 population showed high heritability values in the three varieties. Grain yield in Noor 91 had the lowest heritability (24.44%) with the combined treatment. It was further observed (Table 87) that low genetic advance was associated with low genotypic coefficient of variability as in plant height, 100-seed weight and days to flowering and maturity. Heritability and genetic advance were considered as zero for the negative genetic variance.

II: M_2 GENERATION

The estimates of coefficient of variability (Table 88) revealed that magnitude of PCV was higher than GCV for all the characters in the three genotypes. However, negative GCV values obtained for seeds per pod, 100-seed weight, biological yield grain yield and harvest index with gamma irradiation in Noor 91. In C141 negative GCV values observed for plant height, seeds per pod, grain yield and harvest index with gamma irradiation and for seeds

Table 89: **Estimates of phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV) heritability (h^2) and genetic advance in M_3 generation of three chickpea genotypes *(Cicer arietinum)* after gamma irradiation separately and with the application of gibberellic acid (Parenthesis).**

Noor 91								
Characters	**PCV**		**GCV**		**h^2%**		**G.A% of mean**	
Plant height	2.06	(2.50)	0.54	(-3.17)	34.70	(-185.81)	1.44	(0.00)
Primary branches	5.79	(7.07)	5.40	(2.58)	86.96	(60.47)	10.39	(8.81)
Secondary branches	7.03	(7.09)	-40.4	(-5.36)	-202.25	(-88.62)	0.00	(0.00)
Pods per plant	6.58	(3.61)	5.30	(3.48)	64.91	(93.03)	8.80	(6.92)
Seeds/ pod	4.76	(3.14)	4.06	(1.98)	72.34	(40.95)	10.30	(0.98)
100-seed weight	1.21	(1.50)	1.20	(1.48)	98.84	(99.12)	2.47	(3.04)
Biological yield	3.77	(3.48)	-2.66	(-3.91)	-49.68	(-83.69)	0.00	(0.00)
Grain yield	4.38	(3.00)	-2.61	(1.28)	-35.51	(18.43)	0.00	(1.13)
Harvest index	4.80	(5.48)	-5.15	(-4.74)	-114.75	(-74.80)	0.00	(0.00)
Days to flowering	0.64	(1.09)	0.18	(-2.49)	90.49	(-518.26)	1.12	(0.00)
Days to maturity	0.37	(1.07)	-0.42	(1.05)	-2.39	(95.94)	0.00	(2.12)

Punjab 91								
Characters	**PCV**		**GCV**		**h^2%**		**G.A% of mean**	
Plant height	3.65	(3.52)	3.36	(3.19)	84.72	(81.92)	6.38	(5.95)
Primary branches	4.93	(7.37)	0.17	(-1.18)	85.25	(-2.45)	8.66	(0.00)
Secondary branches	6.83	(7.93)	-5.37	(-7.63)	-61.88	(-92.95)	0.00	(0.00)
Pods per plant	3.80	(3.68)	-2.33	(3.39)	-37.72	(84.39)	0.00	(6.44)
Seeds/ pod	3.26	(1.84)	2.19	(-0.76)	44.44	(-17.20)	3.00	(0.00)
100-seed weight	1.37	(1.27)	0.64	(-0.93)	21.56	(-53.98)	0.61	(0.00)
Biological yield	3.45	(2.96)	-3.36	(-2.18)	-94.92	(-54.29)	0.00	(0.00)
Grain yield	6.48	(5.03)	-2.66	(4.41)	-16.91	(76.89)	0.00	(7.97)
Harvest index	8.64	(5.96)	7.66	(4.63)	78.61	(60.27)	14.00	(7.40)
Days to flowering	1.22	(0.59)	1.18	(0.55)	93.84	(86.91)	2.37	(1.06)
Days to maturity	0.41	(0.75)	0.33	(0.70)	66.60	(88.11)	0.56	(1.36)

C 141								
Characters	**PCV**		**GCV**		**h^2%**		**G.A% of mean**	
Plant height	3.35	(2.72)	2.38	(1.24)	50.82	(20.66)	3.50	(1.16)
Primary branches	3.57	(9.88)	2.00	(8.00)	31.27	(65.48)	2.30	(13.33)
Secondary branches	13.24	(7.11)	8.23	(-6.12)	38.67	(-74.02)	10.55	(0.00)
Pods per plant	3.90	(5.20)	-2.37	(3.26)	-37.05	(39.22)	0.00	(4.20)
Seeds/ pod	2.07	(3.03)	-1.88	(1.94)	-82.65	(40.90)	0.00	(3.96)
100-seed weight	1.17	(2.00)	1.16	(1.04)	98.07	(99.52)	12.37	(4.00)
Biological yield	4.49	(3.30)	-0.53	(-3.00)	-1.42	(-82.77)	0.00	(0.00)
Grain yield	4.46	(7.71)	-4.58	(6.55)	-105.61	(72.20)	0.00	(11.47)
Harvest index	6.29	(9.53)	-6.00	(4.13)	-90.81	(18.78)	0.00	(3.68)
Days to flowering	0.72	(0.51)	-0.74	(0.49)	-107.07	(92.05)	0.00	(0.98)
Days to maturity	0.41	(0.24)	0.38	(-0.14)	86.04	(-34.52)	0.74	(0.00)

IX:CORRELATION STUDIES

Phenotypic and genotypic correlations between yield and it components as well as among the components themselves were worked out in M_1, M_2 and M_3 generation of three chickpea genotypes on the variability created through the use of gamma irradiation separately and with gibberellic acid and are presented in Table 90-98 . The genotypic correlations in most of the cases were higher than the corresponding phenotypic correlations. The higher magnitude of genotypic correlation than that of phenotypic, indicated the masking effect of environment (Asawa et al. 1981)

I: M_1 GENERATION

CORRELATION BETWEEN YIELD AND ITS COMPONENTS

1. Plant height Vs grain yield per plant

Plant height was found to be positively correlated with grain yield at genotypic and phenotypic levels in the three genotypes, however, this association was significant in the case of Punjab 91 but non-significant in genotypes Noor 91 and C 141. The Association of plant height with grain yield was found to be higher in C 141 as compared to Noor 91.

2. Primary branches Vs grain yield per plant

The correlation between primary branches and grain yield at the genotypic and phenotypic levels in the three genotypes was positive. This association was significant at the genotypic levels in Punjab 91 (0.7595) and in C 141 (0.7821), but non-significant in the case of Noor 91 (0.3299).

3. Secondary branches Vs grain yield per plant

Secondary branches and grain yield was positively correlated at the genotypic and phenotypic levels in three genotypes. The association was significant in Punjab 91, but non-significant for Noor 91 and C 141. However, more association was observed in Noor 91 than with C 141.

4. Number of pods Vs grain yield per plant

Number of pods and grain yield was positively and highly significantly correlated both at genotypic and phenotypic levels in the three genotypes. However, maximum association was observed in C 141, followed by Punjab 91 and Noor 91.

5. Seeds per pod Vs grain yield per plant

Seeds per pod were positively and highly significantly correlated with grain yield per plant at genotypic and phenotypic levels in Noor 91 and Punjab 91, however, in case of genotype C 141, this association was non-significant.

6. 100-Seed weight Vs grain yield per plant

100-seeds weight was positively correlated with grain yield at genotypic and phenotypic levels in the three genotypes. This association was significant in Punjab 91, but non-significant in Noor 91 and C 141. However, association of 100-seed weight with grain yield was greater in C 141 as compared to Noor 91.

7. Biological yield Vs grain yield per plant

Biological yield was positively correlated with grain yield at genotypic and phenotypic levels in the three genotypes. This association was significant at genotypic (0.8068) and phenotypic (0.8009) levels in Punjab 91, while only significant association was found at the genotypic (0.715) level in Noor 91.Whereas, non-significant association was observed in C 141 at both the genotypic and phenotypic levels.

8. Harvest index Vs grain yield per plant

The correlation between harvest index and grain yield was positive in the three genotypes. This association was highly significant both at the genotypic and phenotypic levels in the genotypes Noor 91 and Punjab 91, whereas, it was highly significant at the genotypic and only significant at the phenotypic levels in C 141.

9. Days to 50% flowering Vs grain yield per plant

Days to 50% flowering were negatively associated with grain yield in the three genotypes. However, this association was non-significant and maximum was exhibited by Punjab 91, followed by Noor 91 and C 141 genotype.

10. Days to maturity Vs grain yield per plant

The correlation between days to maturity and grain yield was negative at both the genotypic and phenotypic levels in all the genotypes. However, this association was non-significant and maximum was found in Punjab 91 followed by Noor 91 and C141 genotype.

Table 90: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_1 generation of chickpea variety Noor 91.

Variables	Plant height	Pri. Branch	Sec. Branch	Pods / plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harves Index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
Primary branches	.3933 .3420	1 1									
Secondary branches	.4911 .4606	.8051* .6228	1 1								
Pods per plant	.4482 .4546	.5678 .4440	.5760 .5446	1 1							
Seeds per pod	.1467 .1410	-.0418 -.0089	.4485 .4307	.6834 .6329	1 1						
100-seed weight	-.4588 -.4004	-.2189 -.1302	.3454 .3195	-.0790 -.0884	.4542 .4547	1 1					
Biological yield	.5301 .5323	.8206* .7301*	.9456** .9151**	.7764* .7586*	.4556 .4354	.1856 .1654	1 1				
Grain yield	.2459 .2683	.3299 .2846	.6069 .5832	.9093** .9058**	.9101** .8783**	.2960 .2848	.7155* .7043	1 1			
Harvest index	.1230 .1454	.0905 .0815	.3858 .3637	.8315* .8261*	.9523** .9187**	.2633 .2564	.4946 .4790	.9602** .9588**	1 1		
Days to flowering	.3793 .3384	.1855 .1195	-.3938 -.3889	-.1148 -.1250	-.6971 -.6659	-.9319** -.8829**	-.2342 -.2443	-.5055 -.4969	-.5210 -.5040	1 1	
Days to maturity	.4909 .4695	.3470 .2455	-.2109 -.2034	.1437 .1413	-.4795 -.4580	.8692** .8325*	-.0079 -.0104	-.2419 -.2310	-.2868 -.2745	.9470** .9259**	1 1

Table 91: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_1 generation of chickpea variety Punjab 91

Variables	Plant height	Pri. Branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.7555*	1									
	.6233	1									
Secondary branches	.8655**	.7483*	1								
	.8365**	.6236	1								
Pods per plant	.6228	.8357**	.8010*	1							
	.6115	.6917	.7979*	1							
Seeds per pod	.7781*	.5330	.6446	.6849	1						
	.7655*	.4544	.6396	.6784	1						
100-seed weight	.6189	.5755	.4506	.5848	.8273*	1					
	.5969	.4742	.4444	.5772	.8026*	1					
Biological yield	.9182**	.8412**	.9843**	.7990*	.6699	.5197	1				
	.8997**	.7316*	.9768**	.7945*	.6635	.5112	1				
Grain yield	.7414*	.7595*	.7986*	.9521**	.8666**	.7340*	.8068*	1			
	.7304*	.6270	.7965*	.9474**	.8659**	.7182*	.8009*	1			
Harvest index	.6224	.7086*	.6797	.9348**	.8579**	.7558*	.6889	.9822**	1		
	.6154	.5691	.6793	.9293**	.8568**	.7393*	.6824	.9821**	1		
Days to flowering	-.5512	-.2335	-.2830	-.3100	-.7036	-.8866**	-.3396	-.5329	-.5356	1	
	-.5449	-.2137	-.2796	-.3101	-.6976	-.8684**	-.3398	-.5308	-.5320	1	
Days to maturity	-.4447	-.2981	-.1522	-.2949	-.6518	-.9160**	-.2373	-.4921	-.5289	.9709**	1
	-.4284	-.2590	-.1388	-.2825	-.6270	-.8998**	-.2235	-.4696	-.5062	.9460**	1

Table 92: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_1 generation of chickpea variety C 141.

Variables	Plant height	Pri. Branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.6146	1									
	.5848	1									
Secondary branches	.8810**	.7317*	1								
	.8644**	.7099*	1								
Pods per plant	.0572	.6469	.2572	1							
	.0653	.6213	.2596	1							
Seeds per pod	.7406*	.5791	.8490**	.4065	1						
	.5909	.5161	.7322*	.3184	1						
100-seed weight	.5415	.5329	.3989	.3491	.0451	1					
	.5378	.5119	.3939	.3433	.0225	1					
Biological yield	.9563**	.7976*	.9608**	.2666	.8306*	.5167	1				
	.9420**	.7640*	.9429**	.2773	.6657	.5068	1				
Grain yield	.3508	.7821*	.5768	.9834**	.6390	.4792	.5509	1			
	.3094	.6799	.4941	.8688**	.5393	.4110	.4928	1			
Harvest Index	-.1435	.4205	.0559	.9687**	.2787	.2192	.8998**	.0505	1		
	-.1468	.4155	.0513	.9437**	.2768	.2148	.7995*	.0300	1		
Days to flowering	-.4181	-.4290	-.2430	-.2851	.1341	-.9862**	-.3717	-.3790	-.1720	1	
	-.4145	-.4033	-.2278	-.2727	.1397	-.9788**	-.2884	.3585	-.1689	1	
Days to maturity	-.2119	-.3096	-.1031	-.3744	.1747	-.9175**	-.4314	-.1864	-.3463	.9333**	1
	-.2111	-.2927	-.0902	-.3571	.1520	-.8963**	-.3668	-.1655	-.3382	.9157**	1

II: M_2 GENERATION

CORRELATION BETWEEN YIELD AND ITS COMPONENTS

1. Plant height Vs grain yield per plant

Plant height and grain yield per plant was negatively and non-significantly correlated at the genotypic and phenotypic levels in the varieties Noor 91 and C141, while it was positively and non-significantly associated at both the levels in the case of Punjab 91.

2. Primary branches Vs grain yield per plant

Grain yield per plant was positively and non-significantly correlated at the genotypic and phenotypic levels in case of Noor 91 and Punjab 91, while in C141, it was negative and non-significant at both the levels.

3. Secondary branches Vs grain yield per plant

Secondary branches and grain yield per plant was positively and non-significantly correlated in Noor 91 at the genotypic and phenotypic levels, while it was negatively and non-significantly correlated at both the levels in Punjab 91 and C141 genotypes.

4. Number of pods Vs grain yield per plant

Grain yield per plant was positively correlated with number of pods at the genotypic and phenotypic levels in the three varieties. However, this correlation was highly significant at the genotypic and phenotypic levels in Noor 91. In case of Punjab 91, it was non-significant at both levels, but in the case of C141 the correlation at both the levels was significant.

5. Seeds per pod Vs grain yield per plant

Seeds per pod and grain yield per plant was positively but non-significantly correlated at genotypic and phenotypic levels in the three varieties. However, maximum correlation was found in C141, followed by Noor 91 and Punjab 91 genotype.

6. 100-seed weight Vs grain yield per plant

100-seed weight and grain yield per plant was positively but non-significantly correlated at both the levels in the case of variety Noor 91 and C141, while in the case of Punjab 91, the correlation was negative and non-significant at the genotypic and phenotypic level.

7. Biological yield Vs grain yield per plant

Grain yield per plant was negatively but non-significantly correlated with biological yield at the genotypic and phenotypic levels in the case of Noor 91 and C141 , while in the case of Punjab 91 it was positive and non-significant at both the levels.

8. Harvest index Vs grain yield per plant

Harvest index and grain yield per plant was positively but highly significantly correlated at the genotypic and phenotypic level in Noor 91, while in Punjab 91 it was positive and non-signficant, however a positive and significant correlation was observed in C141 at both levels.

9. Days to 50% flowering Vs grain yield per plant

Grain yield per plant and days to 50% flowering was negatively and non-significantly correlated in the case of Noor 91 and C141 at the genotypic and phenotypic levels, while in the case of Punjab 91 the correlation was positive and non-significant at both the levels.

10. Days to maturity Vs grain yield per plant

Days to maturity and grain yield per plant were negatively and non-significantly correlated in Noor 91, while a positive and non-significant correlation was found in Punjab 91 and C141 at both the levels.

Table 93: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_2 generation of chickpea variety Noor 91

Variables	Plant height	Prl. branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.3031	1									
	.2013	1									
Secondary branches	-.6710	-.1273	1								
	-.4591	-.1227	1								
Pods per plant	.0703	.7685*	.2916	1							
	.0237	.7581*	.2727	1							
Seeds per pod	-.0369	-.4000	.2339	.2261	1						
	-.1393	-.3816	.1995	.2406	1						
100-seed weight	-.6068	-.2102	.9076**	.2888	.4354	1					
	-.2758	-.2006	.8165*	.2886	.4050	1					
Biological yield	-.6229	-.1226	.4960	-.3037	-.6128	.3824	1				
	-.3728	-.0679	.4889	-.2924	-.5904	.2804	1				
Grain yield	-.0624	.4760	.4922	.9281**	.5238	.5600	-.3177	1			
	-.1071	.4646	.4486	.9237**	.5397	.5452	-.3137	1			
Harvest index	.1235	.4399	.2680	.8875**	.6464	.3604	.9550**	-.5838	1		
	.0137	.4088	.2193	.8752*	.6569	.3711	.9499**	-.5937	1		
Days to flowering	.3951	-.0499	-.4651	-.3653	-.3736	-.7406	-.5397	-.0569	-.4457	1	
	.2156	.0106	-.3430	-.3275	-.3416	-.6145	-.4699	.0986	-.4348	1	
Days to maturity	.7683*	.2247*	-.7034	-.6091	-.2899	-.6975	-.7161	-.2750	-.5381	-.4410	1
	.6198	.2012	-.6771	-.5900	-.3010	-.6087	-.6902	-.2181	-.5233	-.3776	1

Table 94: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_2 generation of chickpea variety Punjab 91

Variables	Plant height	Pri. branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	-.2068	1									
	-.2054	1									
Secondary branches	.4709	-.1791	1								
	.3832	-.1471	1								
Pods per plant	.2105	.7145	-.4560	1							
	.2130	.6945	-.4616	1							
Seeds per pod	.2165	-.2421	.5476	-.4467	1						
	.2215	-.2382	.3547	-.4039	1						
100-seed weight	-.4385	-.6300	-.1479	-.7160	.4265	1					
	-.4317	-.6249	-.1657	-.6935	.4300	1					
Biological yield	.9215**	-.1563	.3910	.2893	.1614	-.5355	1				
	.9203**	-.1509	.3236	.2876	.1617	-.5308	1				
Grain yield	.1692	.4086	-.2067	.5411	.4434	-.0659	.2075	1			
	.1750	.3646	-.3554	.5473	.4785	-.0285	.2006	1			
Harvest index	-.8678*	.3219	-.4666	-.0805	.0333	.5106	-.9149**	.2033	1		
	-.8577*	.3129	-.4442	-.0642	.0518	.5127	-.9083**	.2209	1		
Days to flowering	-.2384	.0569	-.5324	-.0485	.6964	.4153	-.1304	.6809	.4037	1	
	-.1462	.0529	-.0672	-.1223	.3323	.2333	-.0814	.2304	.1853	1	
Days to maturity	.2584	.5113	.1380	.5994	.2086	-.5272	.4609	.7105	-.1485	-.0624	1
	.2321	.5065	.1455	.5322	.1669	-.5070	.4383	.5589	-.1576	-.0049	1

Table 95: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_2 generation of chickpea variety C 141

Variables	Plant height	Pri. branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.2240	1									
	.1902	1									
Secondary branches	.6280	.5035	1								
	.6387	.4190	1								
Pods per plant	-.0093	-.2534	-.3281	1							
	.0409	-.2606	-.2712	1							
Seeds per pod	-.1827	.2128	.0484	.1652	1						
	-.1204	.2186	.0382	.1955	1						
100-seed weight	-.2906	.3090	-.0144	-.1446	1.0699	1					
	-.1808	.1081	.0461	-.0441	.6434	1					
Biological yield	.9294**	.1335	.6362	-.2348	-.3044	-.1618	1				
	.8689*	.1418	.6184	-.2251	-.2037	-.1693	1				
Grain yield	-.0809	-.1038	-.2383	.8407*	.6811	.4328	-.2722	1			
	-.0151	-.1258	-.1684	.8424*	.6572	.4310	-.2418	1			
Harvest index	-.6419	-.2280	-.5750	.6644	.6332	.4084	.8077*	-.7792	1		
	-.5312	-.2400	-.5038	.6844	.5793	.4233	.8197*	-.7440	1		
Days to flowering	-.3962	-.2762	-.3224	-.1808	-.9131**	-.7794*	-.6287	-.2423	-.2679	1	
	-.3141	-.2204	-.2925	-.1799	-.7748*	-.6482	-.5931	-.2422	-.2644	1	
Days to maturity	.5459	-.0195	.2593	.5131	-.5069	-.4845	.1770	.5312	-.2374	.1659	1
	.4679	.0294	.2184	.4190	-.4440	-.5038	.0695	.4998	-.2795	.1803	1

III: M_3 GENERATION

CORRELATION BETWEEN YIELD AND ITS COMPONENTS

1. Plant height Vs grain yield per plant

Plant height and grain yield per plant was negatively and non-significantly correlated at genotypic level and positively at phenotypic level in Noor 91, while in case of Punjab 91, it was positive and non-significant. A negative and non-significant correlation was seen in the variety C141.

2. Primary branches Vs grain yield per plant

Grain yield per plant was negatively and non-significantly correlated with primary branches at the genotypic level and positively correlated at phenotypic level in the case of Noor 91, while in

the case of Punjab 91 and C141, a positive and non-significant correlation was observed at both levels.

3. Secondary branches Vs grain yield per plant

Secondary branches were negatively and non-significantly correlated with grain yield per plant at the genotypic and phenotypic level in Noor 91 and C141, while in case of Punjab 91, it was positive at genotypic level and negative at phenotypic level.

4. Number of pods Vs grain yield per plant

Number of pods and grain yield per plant were positively and significantly correlated at the genotypic and phenotypic level in variety Noor 91, while a non-significant correlation was found in Punjab 91 and C141.

5. Seeds per pod Vs grain yield per plant

Grain yield per plant was positively and non-significantly correlated with seeds per pod at the phenotypic and genotypic level in the case of varieties Noor 91 and Punjab 91, while in the case of C141, positive and significant correlation was seen at the genotypic level.

6. 100-seed weight Vs grain yield per plant

100-seed weight and grain yield per plant was positively and non-significantly correlated at the genotypic and phenotypic level in varieties Noor 91 and C141, while in case of Punjab 91, negative and non-significant at genotypic and positive and non-significant correlation was seen at the phenotypic level.

7. Biological yield Vs grain yield per plant

Biological yield was negatively and non-significantly correlated with grain yield per plant at the genotypic and phenotypic level in the case of Noor 91 and C141, however, it was positive and highly significant at genotypic and non-significant at phenotypic level in the variety Punjab 91.

8. Harvest index Vs grain yield per plant

Harvest index and grain yield per plant was positively and non-significantly correlated at the genotypic, while highly significant at the phenotypic level in the case of Noor 91. It was significant at genotypic and phenotypic level in variety C141, but in the case of Punjab 91, negative and significant at the genotypic level and non-significant at phenotypic level correlation was observed.

9. Days to 50% flowering Vs grain yield per plant

Days to 50% flowering were positively and non-significantly correlated with grain yield per plant at genotypic and phenotypic level in the three genotypes.

10. Days to maturity Vs grain yield per plant

Grain yield per plant and days to maturity were positively and non-significantly correlated at genotypic and phenotypic level in Noor 91 but significantly at genotypic level in Punjab 91, however, it was negative and non-significant at both levels in C141.

Table 96: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_3 generation of chickpea variety Noor 91

Variables	Plant height	Pri. branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest Index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.4638	1									
	.4257	1									
Secondary branches	-.2075	.7120	1								
	-.1575	.3405	1								
Pods per plant	.1275	.2346	.2408	1							
	.1606	.2443	-.1464	1							
Seeds per pod	.1922	-.4438	-1.3070	-.1276	1						
	-.0446	-.0821	-.1002	.1681	1						
100-seed weight	.2691	.2166	-.6782	.5122	-.0551	1					
	.1760	.0441	-.0295	.1645	-.0658	1					
Biological yield	.3031	.6485	1.1418	-.5781	-1.1187	-.5625	1				
	-.0558	.0144	.6536	-.3265	.0262	-.2926	1				
Grain yield	-.0600	-.0290	-.7014	.7902*	.3798	.5728	-1.1005	1			
	.1106	.1455	-.3770	.7949*	.2331	.3031	-.6656	1			
Harvest index	-.1126	-.1590	-.7830*	.7223	.5666	.5845	-1.0502	1.0084	1		
	.1166	.1176	-.5201	.6841	.1365	.3323	-.8475*	.9582**	1		
Days to flowering	-1.2151	-.5549	.1575	.0126	-.0635	.1313	-.3459	.1539	.1889	1	
	-.5906	-.4211	.0333	.0551	-.0304	.1961	-.1092	.1522	.1355	1	
Days to maturity	.2704	.2996	-.1979	.2546	.4581	-.6233	-.3698	.3566	.3451	-.4653	1
	.0764	.1159	-.1846	.2935	.2090	-.5245	-.1324	.3506	.2930	-.3567	1

Table 97: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_3 generation of chickpea variety Punjab 91

Variables	Plant height	Pri. branch	Sec. Branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.0958	1									
	.0169	1									
Secondary branches	.2654	1.0064	1								
	-.1585	-.3673	1								
Pods per plant	.3586	1.1051	-1.5312	1							
	.2835	.5270	-.5534	1							
Seeds per pod	.2784	-.5320	1.6716	-.3975	1						
	.2572	-.3814	.3637	-.1907	1						
100-seed weight	-.6911	-.8638*	2.3805*	-1.0225*	.5633	1					
	-.2796	-.5139	-.0121	-.3495	.4369	1					
Biological yield	.7374	1.5428	-2.5710	1.3575	-.0104	-1.8497	1				
	.3121	.0458	.0146	.4224	-.0952	-.1910	1				
Grain yield	.3740	.6921	.2259	.5546	.5474	-.4450	.9385**	1			
	.2812	.1167	-.2519	.7060	.5043	.2137	.3622	1			
Harvest index	-.1431	-.6.1545	9.2949	-4.0503	1.1926	5.3186	-.8052*	-1.9105*	1		
	-.0262	-.0511	-.0763	-.3410	.2023	.1181	.9483**	-.2674	1		
Days to flowering	.2948	.3316	.5389	.2532	.1282	-.3061	-.3293	.2837	1.4807	1	
	.2535	.2869	.0113	.0445	.0669	-.1261	-.1260	.0274	.1362	1	
Days to maturity	.4483	.7001	-.0770	.7088	.2012	.8481*	1.4049*	.7994*	-3.7357*	.0446	1
	.4113	.3637	-.1440	.5318	.1064	-.3728	.7112	.4818	-.5818	.0531	1

Table 98: Genotypic (rg) and phenotypic (rp) correlation coefficient among yield and yield components in M_3 generation of chickpea variety C 141

Variables	Plant height	Pri. branch	Sec. branch	Pods/ plant	Seeds/ pod	100-seed weight	Biol. yield	Grain yield	Harvest Index	Flow. Days	Matu. Days
	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp	rg/rp
Plant height	1										
	1										
Primary branches	.4329	1									
	.3325	1									
Secondary branches	.8286*	.3510	1								
	.6775	.0174	1								
Pods per plant	.7929*	1.0234*	.7519	1							
	.6451	.6789	.4875	1							
Seeds per pod	-.8947**	.0214	-.2923	-.0001	1						
	-.8281*	.0256	-.7390	-.3032	1						
100-seed weight	-.3055	-.0299	-.2923	-.0001	.2019	1					
	-.1773	.0695	-.2311	-.0005	.1209	1					
Biological yield	1.0006	.3106	.9634**	.8135*	-.9770**	-.1234	1				
	.8815**	.3247	.6664	.5643	-.8573*	-.2589	1				
Grain yield	.4731	.7113	-.4274	.1457	.7697*	.3816	-.5134	1			
	-.3089	.5402	-.3654	.4429	.6949	.3174	-.4137	1			
Harvest Index	-.9169**	.1372	-.8757*	-.4909	1.0277	.2657	-.9146**	.8166*	1		
	-.7201	.0633	-.6057	-.1272	.9034**	.3814	-.8764*	.7913*	1		
Days to flowering	-.2760	.0191	-.0878	-.0610	.3475	.2832	-.3657	.3597	.4429		
	-.2003	-.0247	.0167	-.1136	.2352	.2394	-.3283	.1749	.3379		
Days to maturity	.7607*	.1407	1.1017	.7228	-.8426*	.1517	.8489*	-.3938	-.7524	.2437	1
	.5264	-.0114	.6906	.2881	-.6224	-.0330	.6032	-.3703	-.5868	.0838	1

X: PATH COEFFICIENT STUDIES

When cause and effect relationship in a system of variables is established, path analysis can be applied to partition correlations into direct and indirect effects through alternate pathways by using genotypic correlations and substituting them on the path equations. Simultaneous solution of these equations give path values, when the grain yield per plant is considered as resultant variable and plant height, primary branches, secondary branches, number of pods, seeds per pod, 100-seed weight, biological yield, harvest index, days to 50% flowering and days to maturity as causal variables. The results of path analysis of M_1, M_2 and M_3 generation are given in tables 99-107. The overall results obtained are discussed as follows:

I: M_1 GENERATION

1. Plant height Vs grain yield per plant

The direct effect of plant height on grain yield per plant was negative (-0.0139) in Noor 91 genotype. The influence of plant height via primary branches, secondary branches, 100-seed weight and days to 50% flowering were observed to be also negative. The indirect effect of remaining characters were positive (Table 99).

In the case of Punjab 91 variety, the direct effect of plant height on grain yield was negative (-0.0308). The indirect effect of plant height via primary branches, secondary branches, number of pods, seeds per pod and 100-seed weight were observed to be also negative but low. The indirect influence of plant height via biological yield (0.3464), days to 50% flowering (0.0732) and days to maturity (0.0008) were low and positive. However, a moderate positive (0.5921) effect of harvest index on grain yield was recorded (Table 100).

In the case of C141 genotype, the direct effect of plant height on grain yield was positive (0.4946). It has also positive genotypic correlation with grain yield. The high positive association of plant height with grain yield was mainly due to its indirect positive influence via primary branches (0.0078), secondary branches (0.6123) number of pods (0.0703), 100-seed weight (1.1772) and harvest index (0.0387) (Table 101). The indirect effects through rest of the characters were negative.

2. **Primary branches Vs grain yield per plant**

A negative direct effect (-0.0136) of number of primary branches on the grain yield of chickpea genotype Noor 91 was recorded. It occurred owing to its negative indirect effect via plant height (-0.0055), secondary branches (-0.0003), seeds per pod (-0.0017), 100-seed weight (-0.0169) and days to flowering (-0.0111). But it has positive genotypic association with grain yield. The positive indirect effect of number of pods (0.1058), biological yield (0.1943), harvest index (0.0561) and days to maturity (0.0228) shifted the genotypic correlation towards positive direction (Table 99).

In the case of variety Punjab 91, the direct effect of number of primary branches on the grain yield was negative (-0.0050) and low, but it has strong positive genotypic association with grain yield. The direct negative effect of primary branches could be attributed by the indirect effects via plant height (-0.0233), secondary branches (-0.0469), number of pods (-0.0729), seeds per pod (-0.0091) and 100-seed weight (-0.1067). However, the positive indirect effect of biological yield (0.3174), harvest index (0.6742), days to 50% flowering (0.0310) and days to maturity (0.0005) which shifted the genotypic correlation towards the positive direction (Table 100).

A positive direct effect (0.0127) of number of primary branches on the grain yield of chickpea genotype C141 was recorded. The magnitude of direct effect was however, lower than the genotypic correlation value (0.7821). The positive indirect effect of primary branches via plant height (0.3040), secondary branches (0.5085), number of pods (0.7945), and 100-seed weight (1.1585) had shifted the genotypic correlation towards the positive trend. But negative indirect effect via seeds per pod (-0.0266), biological yield (-1.0363), harvest index (-0.1134), days to 50% flowering (-0.7319) and days to maturity (-0.0878) had reduced the direct effect of number of primary branches on the grain yield (Table 101).

3. **Secondary branches Vs grain yield per plant**

The direct effect of number of secondary branches on the grain yield in variety Noor 91 was low and negative (-0.0003) but it has strong positive genotypic association (0.6069) with grain yield. This negative direct effect of secondary branches could be due to the indirect effect via plant height (-0.0068) primary branches (-0.0109) and days to maturity (-0.0138). However, the positive indirect effect of number of pods (0.1073), seeds per pod (0.0183),

100-seed weight (0.0267), biological yield (0.2238), harvest index (0.2392) and days to 50% flowering (0.0235) had shifted the genotypic correlation towards the positive direction (Table 99).

The results showed that number of primary branches had positive and strong association with grain yield in variety Punjab 91 at genotypic level (0.7986). The partitioning of genotypic correlation revealed a negative direct effect of secondary branches (-0.0626) on the grain yield. The higher genotypic correlation value of number of secondary branches with grain yield was due to its positive indirect effect via biological yield (0.3713), harvest index (0.6467), following days (0.0367) and maturity days (0.0003). The influence of secondary branches via plant height (-0.0267), primary branches (-0.0037), number of pods (-0.0699), seeds per pod (-0.0110) and 100-seed weight (-0.0836) were observed to be negative which contributed in the negative direct effect of secondary branches on grain yield (Table 100).

The path coefficient analysis in C141 genotype revealed that secondary branches had positive relationship (0.5768) with grain yield at genotypic level. This positive association had also exerted positive direct effect on the grain yield (0.6950). The positive indirect effect of secondary branches via plant height (0.4357), primary branches (0.0093), number of pods (0.3158) and 100-seed weight (0.8672) had shifted the genotypic correlation towards the positive direction. But negative indirect effect via seeds per pod (-0.0391), biological yield (-1.2483), harvest index (-0.0151), days to 50% flowering (-0.4146) and days to maturity (-0.0292) had reduced the direct effect of number of secondary branches on the grain yield (Table 101).

4. **Number of pods per plant Vs grain yield per plant**

The results showed that number of pods in genotype Noor 91 had positive and strong association (0.9093) with grain yield at genotypic level. The partitioning of genotypic correlation revealed a positive direct effect of pods (0.1862) on the grain yield. The magnitude of direct effect was however, lower than the genotypic correlation. The positive indirect effect of number of pods via seeds per pod (0.0279), biological yield (0.1838), harvest index (0.5154), days to 50% flowering (0.0069) and days to maturity (0.0094) had shifted the positive genotypic correlation towards the more positive direction. But negative indirect effect via plant height (-0.0062), primary branches (-0.0077), secondary branches (-

0.0002) and 100-seed weight (-0.0061) had decreased the direct effect of number of pods on the grain yield (Table 99).

The results showed that number of pods in genotype Punjab 91 had positive and strong association (0.9521) with grain yield. However, the direct effect of number of pods on grain yield was low and negative (-0.0872). The higher positive genotypic correlation value of number of pods with grain yield was due to its positive indirect effect via biological yield (0.3015), harvest index (0.8894), days to 50% flowering (0.0412) and days to maturity (0.0005). The influence of number of pods via plant height (-0.0192), primary branches (-0.0041), secondary branches (-0.0502), seeds per pod (-0.0117) and 100-seed weight (-0.1084) were observed to be negative which contributed in the negative direct effect of number of pods on grain yield (Table 100).

The path coefficient analysis revealed in variety C141, that number of pods had strong direct influence (1.2282) on seed yield. The genotypic correlation between this pair of characters was also positive and high (0.9834). A positive indirect effect of number of pods on grain yield was observed via plant height (0.0283), primary branches (0.0082), secondary branches (0.1787) and 100-seed weight (0.7589) which had promoted the genotypic relationship towards positive direction (Table 101).

5. **Seeds per pod Vs grain yield per plant**

The genotypic correlation coefficient in variety Noor 91 between seeds per pod and grain yield was strongly positive (0.9101). The direct effect of this character on grain yield was also positive (0.0408), while indirect effect via plant height (-0.0020), secondary branches (-0.0002) and days to maturity (-0.0312) was negative. These indirect negative effects were counter balanced by positive indirect effects via primary branches (0.0006), pods per pod (0.1273), 100-seed weight (0.0351), biological yield (0.1079), harvest index (0.5903) and days to 50% flowering (0.0416) and by virtue of these positive indirect effects a strong positive genotypic correlation between this pair of character was established (Table 99).

In the case of Punjab 91, the direct effect of number of seeds per pod on grain yield was negative (-0.0170), but it was masked by indirect positive effect via rest of component characters like biological yield (0.2528), harvest index (0.8162), days to 50% flowering

(0.0934) and days to maturity (0.0012). Positive and strong genotypic correlation between this pair of characters (0.8666) was due to the largest indirect effect via biological yield and harvest index (Table 100).

The direct effect of seeds per pod in C141 on grain yield was negative but low (-0.0460), while the genotypic correlation obtained between this pair of characters was positive and higher order value (0.6390). The influence of seeds per pod via biological yield (-1.0792) and harvest index (-0.0751) was observed to be negative and strong which shifted the direct effect of seeds per pod on grain yield in negative direction. The higher positive genotypic correlation between this pair of characters was due to its positive indirect effect via plant height (0.3663), primary branches (0.0073), secondary branches (0.5900), pods per plant (0.4992), 100-seed weight (0.0980), days to 50% flowering (0.2288) and days to maturity (0.0495) (Table 101).

6. **100-seed weight Vs grain yield per plant**

100-seed weight had positive direct effect on grain yield (0.0773) and genotypic correlation between this pair of characters was also positive (0.2960) in variety Noor-91. The reduction in direct effect was mainly due to negative indirect effect via secondary branches (-0.0001), pods per plant (-0.0147) and days to maturity (-0.0570). Other characters like plant height (0.0064), primary branches (0.0030), seeds per pod (0.0186), biological yield (0.0439), harvest index (0.1632) and days to 50% flowering (0.0556) contributed positively towards the improvement of grain yield (Table 99).

In the case of Punjab 91 genotype the path coefficient revealed that 100-seed weight had negative direct effect (-0.1854) on grain yield. But the 100-seed weight showed a positive and strong association with yield at genotypic level (0.7340). Virtually positive genotypic association was due to the largest positive indirect influence via harvest index (0.7191), biological yield (0.1961) and days to 50% flowering (0.1177). The negative direct effect of 100-seed weight on grain yield obtained due to the negative indirect effect via plant height (-0.0191), primary branches (-0.0029), secondary branches (-0.0282), number of pods (-0.0510) and seeds per pod (-0.0141) (Table 100).

The path coefficient analysis in C141 genotype revealed that 100-seed weight had highest positive direct effect (2.1739) on grain yield and the association between this pair of

characters was moderate and positive (0.4792) at the genotypic level. The moderate genotypic correlation between 100-seed weight and grain yield was due to its higher indirect negative effects via days to 50% flowering (-1.6827), biological yield (-0.6713), days to maturity (-0.2600) and harvest index (-0.0591) inspite of highest positive direct effect of 100-seed weight on grain yield (Table 101).

7. **Biological yield Vs grain yield per plant**

Path coefficient analysis in genotype Noor 91 revealed that biological yield had positive direct effect on grain yield (0.2367) and correlation between this pair of characters was positive and of higher value at genotypic level (0.7155). The reduction in direct effect was mainly due to negative indirect effect via plant height (-0.0074), primary branches (-0.0111), secondary branches (-0.0003) and days to maturity (-0.0005). Others characters like pods per plant (0.1446, seeds per pod (0.0186), 100-seed weight (0.0143) harvest index (0.3065) and days to 50% flowering (0.0140) contributed positively towards the improvement of grain yield (Table 99).

In variety Punjab 91 biological yield had positive direct effect on grain yield (0.3773) and a strong positive correlation between this pair was also observed (0.8068) at the genotypic level. This strong positive association was due to the largest indirect effect via harvest index (0.6555) and days to 50% flowering (0.0451). The reduction in direct effect occurred via rest of component characters like plant height (-0.0283), primary branches (-0.0042) secondary branches (-0.0617), pods per plant (-0.0697), seeds per pod (-0.0114) and 100-seed weight (-0.0964) (Table 100).

In the case of genotype C141, biological yield had strong negative effect (-1.2992) on grain yield. It occurred owing to the higher indirect effect via days to 50% flowering (-0.6467), days to maturity (-0.0528), seeds per pod (-0.0382) and harvest index (-0.0136). Positive and moderate genotypic (0.5509) correlation was observed between this pair of characters. This positive correlation was due to the largest indirect effect via secondary branches (0.6677), plant height (0.4730) and pods per plant (0.3274) (Table 101).

8. **Harvest index Vs grain yield per plant**

Path coefficient analysis in variety Noor 91 revealed that harvest index ranked first in respect of highest direct positive effect (0.6198) on grain yield. Its utility is more beneficial

because of its strong genotypic positive association (0.9602) with grain yield. The indirect effect via pods per plant (0.1548) seeds per pod (0.0389), 100-seed weight (0.0203), biological yield (0.1171) and days to 50% flowering (0.0311) was also positive, while plant height (-0.0017), primary branches (-0.0012), secondary branches (-0.0001) and days to maturity (-0.0188) had a negative indirect effect on grain yield (Table 99).

In variety Punjab 91, the results of path coefficient revealed a strong positive direct effect (0.9514) of harvest index on grain yield and also a strong positive genotypic correlation (0.9822) was recorded between this pair of characters. The indirect effect via biological yield (0.2599), days to 50% flowering (0.0711) and days to maturity (0.0010) was positive while the rest of the component characters had a negative indirect effect (Table 100).

Harvest index had negative direct effect (-0.2697) on grain yield, which was contributed by indirect effect via plant height (-0.0710), seeds per pod (-0.0128), biological yield (-0.0656), days to 50% flowering (-0.2934) and days to maturity (-0.0982). A strong positive genotypic association (0.8998) was recorded between harvest index and grain yield. The highest indirect positive effect was reflected via pods per plant (1.1897) followed by 100-seed weight (0.4765) (Table 101).

9. **Days to 50% flowering Vs grain yield per plant**

The genotypic correlation coefficient in genotype Noor 91 was negative between days to 50% flowering and grain yield (-0.5055). The direct effect of this character on grain yield was also negative (-0.0597), while indirect effect via secondary branches (0.0001) and days to maturity (0.0621) was positive. However, the indirect effect of the rest of the component characters was negative which nullified the positive indirect effect (Table 99). In the case of Punjab 91, days to 50% flowering had negative direct effect (-0.1328) on grain yield and the genotypic correlation coefficient between this pair was also negative (-0.5329). The influence of days to 50% flowering via biological yield (-0.1281), harvest index (-0.5095) and days to maturity (-0.0018) was also found to be negative and contributed in the genotypic correlation. However, rest of all the characters had positive indirect effect which had reduced the negative direct effect of days to 50% flowering on grain yield (Table 100). Days to 50% flowering in C141 genotype had a strong positive direct effect (1.7063) on grain yield, while the association between this pair of characters was negative (-0.3717) at the genotypic level.

This negative correlation was due to the highest indirect negative effect via 100-seed weight (-2.1438) followed by pods per plant (-0.3502) and plant height (-0.2068) irrespective of the higher positive direct effect between this pair of characters (Table 101). The positive direct effect was enhanced due to the indirect effect via biological yield (0.4924), days to maturity (0.2645) and harvest index (0.0464).

10. **Days to maturity Vs grain yield per plant**

The results of path coefficient analysis of variety Noor 91 (Table 99) revealed that days to maturity had a positive direct effect (0.0656) on grain yield while the genotypic correlation between these two characters was negative (-0.2419). The indirect influence of days to maturity via secondary branches (0.0001) and pods per plant (0.0268) was positive, while the rest of all the component characters had negative indirect effect by virtue of which negative genotypic correlation was recorded between these two characters. In the case of variety Punjab 91, the genotypic correlation coefficient between days to maturity and grain yield was negative (-0.4921). The direct effect of this character on grain yield was also negative (-0.0018), while the indirect effect via plant height (0.0137), primary branches (0.0015), secondary branches (0.0095), pods per plant (0.0257), seed per pod (0.0111) and 100-seed weight (0.1699) was positive. The indirect positive effect of most of the characters was counter balanced by negative indirect effect via harvest index (-0.5032), days to 50% flowering (-0.1289) and biological yield (-0.0895) (Table 100).

The direct effect of days to maturity in C141 genotype was positive (0.2834) on grain yield while the genotypic association between these two characters was negative (-0.4314). The negative association was attributed due to the indirect negative effects via plant height (-0.1048), primary branches (-0.0039), secondary branches (-0.0717), pods per plant (-0.4599), seeds per pod (-0.0080) and 100-seed weight (-1.9945). Virtually, direct positive effect was due to the larger positive indirect influence via days to 50% flowering (1.5924), biological yield (0.2421) and harvest index (0.0934). (Table 101)

Table 99: Noor 91 M_1 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variable	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harv. Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(-.0139)	-.0053	-.0002	.0835	.0060	-.0355	.1255	.0763	-.0226	.0322	.2459
Pri. Branches	-.0055	(-.0136)	-.0003	.1058	-.0017	-.0169	.1943	.0561	-.0111	.0228	.3299
Sec. Branches	-.0068	-.0109	(-.0003)	.1073	.0183	.0267	.2238	.2392	.0235	-.0138	.6069
Pods/ Plant	-.0062	-.0077	-.0002	(.1862)	.0279	-.0061	.1838	.5154	.0069	.0094	.9093
Seeds/ Pod	-.002	.0006	-.0002	.1273	(.0408)	.0351	.1079	.5903	.0416	-.0312	.9101
100-Seed Weight	.0064	.003	-.0001	-.0147	.0186	(.0773)	.0439	.1632	.0556	-.057	.2960
Biol. Yield	-.0074	-.0111	-.0003	.1446	.0186	.0143	(.23367)	.3065	.014	-.0005	.7155
Harvest Index	-.0017	-.0012	-.0001	.1548	.0389	.0203	.1171	(.6198)	.0311	-.0188	.9602
Flow. Days	-.0053	-.0025	.0001	-.0214	-.0285	-.0270	-.0554	-.323	(-.0597)	.0621	-.5055
Maturity Days	-.0068	-.0047	.0001	.0268	-.0194	-.0672	-.0019	-.1778	-.565	(.0656)	-.2419

Table 100: Punjab 91 M_1 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield. The last column shows genotype correlation of independent variables with grain yield)

Variables	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harves Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(-.0308)	-.0037	-.0542	-.0543	-.0133	-.1148	.3464	.5921	.0732	.0008	.7414
Pri. Branches	-.0233	(-.005)	-.0469	-.0729	-.0091	-.1067	.3174	.6742	.031	.0005	.7595
Sec. Branches	-.0267	-.0037	(-.0626)	-.0699	-.011	-.0836	.3713	.6467	.0376	.0003	.7986
Pods/ Plant	-.0192	-.0041	-.0502	(.0872)	-.0107	-.1084	.3015	.8894	.0412	.0005	.9521
Seeds/ Pod	-.024	-.0026	-.0404	-.0597	(-.017)	-.1534	.2528	.8162	.0934	.0012	.8666
100-Seed Weight	-.0191	-.0029	-.0282	-.051	-.0141	(-.1854)	.1961	.7191	.1177	.0017	.734
Biol. Yield	-.0283	-.0042	-.0617	-.0697	-.0114	-.0964	(.3773)	.6555	.0451	.0004	.8068
Harvest Index	-.0192	-.0035	-.0426	-.0815	-.0146	-.1401	.2599	(.9514)	.0711	.001	.9822
Flow. Days	.017	.0012	.0177	.027	.012	.1644	-.1281	-.5095	(-.1328)	-.0018	-.5329
Matu. Days	.0137	.0015	.0095	.0257	.0111	.1699	-.0895	-.5032	-.1289	(-.0018)	-.4921

Table 101: C 141 M_1 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variables	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harvest Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(-.4946)	.0078	.6123	.0703	-.0341	1.1772	-1.2424	.0387	-.7135	-.06	.3508
Pri. Branches	.304	(.0127)	.5085	.7945	-.0266	1.1585	-1.0363	-.1134	-.7319	-.0878	.7821
Sec. Branches	.4357	.0093	(.695)	.3158	-.0391	.8672	-1.2483	-.0151	-.4146	-.292	.5768
Pods/ Plant	.0283	.0082	.1787	(1.2282)	-.0187	.7589	-.3464	-.2612	-.4865	-.1061	.9834
Seeds/ Pod	.3663	.0073	.59	.4992	(-.046)	.098	-1.0792	-.0751	-.2288	.0495	.639
100-Seed Weight	.2678	.0068	.2772	.4288	-.0021	(2.1739)	-.6713	-.0591	-1.6827	-.2600	.4792
Biol. Yield	.473	.0101	.6677	.3274	-.382	1.1232	(1.2992)	-.0136	-.6467	-.0528	.5509
Harvest Index	-.071	.0053	.0389	1.1897	-.128	.4765	-.0656	(-.2697)	-.2934	-.0982	.8998
Flow. Days	-.2068	-.0054	-.1688	-.3502	-.0062	-2.1438	.4924	.0464	(1.7063)	.2645	-.3717
Maturity Days	-.1048	-.0039	-.0717	-.4599	-.008	-1.9945	.2421	.0934	1.5924	(.2834)	-.4314

II: M_2 GENERATION

1. Plant height Vs grain yield per plant

In variety Noor 91 the genotypic correlation coefficient of plant height with grain yield was negative (-0.0624), while the direct effect of plant height on grain yield was positve (0.0167). It also indirectly affected the grain yield via secondary branches (0.0127), pods per plant (0.0113), harvest index (0.1292), days to 50% flowering (0.0019) and days to maturity (0.0330). The negative indirect path via other traits had shifted the genotypic correlation towards negative direction. The highest negative effect was found through biological yield (-0.0015) (Table 102)

Plant height had a negative direct effect (-1.5219) in Punjab 91 genotype on the grain yield. However, the genotypic correlation between these two characters was positive (0.1692) and was low in value. The indirect positive influence of plant height via harvest index (4.6831), pods per plant (0.8498), seeds per pod (0.4533), secondary branch (0.2498), days to maturity (0.0266), and day to 50% flowering (0.0200) had shifted the genotypic correlation towards the positive value. The positive indirect effects of the above said characters were counter balanced by the strong negative effects via biological yield (-3.2952), 100-seed weight (-1.1681) and primary branches (-0.1282). (Table 103)

In the case of C141 variety, the genotypic correlation coefficient of plant height with grain yield was negative (-0.0809). The direct effect of plant height on the grain yield was also negative (-0.0472). Yet it had positive indirect effect via biological yield (0.4033), days to 50% flowering (0.0267), secondary branches (0.0129) and primary branches (9.0107). The negative indirect path via other traits had shifted the genotypic correlation towards the negative direction. The highest negative indirect effect was found through harvest index (-0.0129), 100-seed weight (-0.0270) seeds per pod (-0.0214), days to maturity (-0.0210) and pods per plant (-0.0050) (Table 104).

2. Primary branches Vs grain yield per plant

Number of primary branches in Noor 91, had positive and strong genotypic correlation (0.4760) with a direct negative path towards grain yield (-0.0363). Although direct contribution of primary branches towards yield was negative, but its high and positive indirect path via harvest index (0.4600) and pods per plant (0.1232) had promoted the genotypic relationship on positive trend (Table 102).

In the case of Punjab 91, the primary branches, which had high positive genotypic correlation (0.4086) also exerted positive direct effect (0.6199) on the grain yield. The maximum positive indirect effect of primary branches was observed via pods per plant (2.8845), followed by biological yield (0.5590), plant height (0.3147) and days to maturity (0.0527) shifting the correlation towards positive direction. While indirect effect via harvest index (-1.7374), 100-seed weight (-1.6783), seeds per pod (-0.5069), secondary branches (-0.0950) and days to 50% flowering (-0.0048) reduced the direct effect (Table 103).

In variety C141, primary branches had positive direct effect on grain yield (0.0478), while the genotypic correlation between the two characters was negative (-0.1038). Negative indirect path via harvest index (-0.1467), pods per plant (-0.1357) and plant height (-0.0106) had promoted the genotypic association towards the negative direction. The indirect influence of primary branches via secondary branches (0.0103), seeds per pod (0.0250), 100-seed weight (0.0287), biological yield (0.0579), days to flowering (0.0186) and days to maturity (0.0008) was found to be positive (Table 104).

3. Secondary branches Vs grain yield per plant

Path coefficient analysis is genotype Noor 91 revealed that secondary branches had positive genotypic correlation (0.4922) with grain yield. But it had negative direct contribution towards grain yield (-0.0189). The positive indirect path via harvest index (0.2803), biological yield (0.1902), pods per plant (0.0468) had shifted the genotypic correlation towards the positive direction. The indirect influence of secondary branches via plant height (-0.112), days to 50% flowering (-0.0023) and days to maturity (-0.0302) was observed to be negative (Table 102).

The results given in table 103 revealed that secondary branches in genotype Punjab 91 had negative genotypic correlation (-0.2067) with a direct positive path (0.5306) towards grain yield. The highest indirect effect (2.5179) was computed through harvest index followed by seeds per pod (1.1464), days to 50% flowering (0.0446) and days to maturity (0.0142). Where as indirect negative effect observed via rest of the traits. The highest negative effect was found via pods per plant (-1.8407) followed by biological yield (-1.3980), plant height (-0.7166), 100-seed weight (-0.3940) and primary branches (-0.1110).

In the case of C141 variety, the secondary branches had direct positive influence (0.0205) towards the grain yield. While it had negative genotypic correlation (-0.2383) with grain yield. Although direct contribution of secondary branches towards yield was positive, but its high and negative indirect path via harvest index (-0.3699), pods per plant (-0.1756), plant height (-0.0296), days to maturity (-0.0100) and 100-seed weight (-0.0013) had promoted the genotypic association on negative direction (Table 104).

4. Number of pods per plant via grain yield per plant

Path coefficient analysis in the variety Noor 91 revealed that the genotypic correlation was strongly positive (0.9281) between the number of pods per plant and grain yield. It also exerted a positive direct effect (0.1603) of pods per plant on grain yield. The highest positive indirect influence via harvest index strongly promoted the genotypic association towards the positive value. The indirect negative influence of pods per plant via biological yield (-0.1164), primary branches (-0.0279), days to maturity (-0.0262) and secondary branches (-0.0055) reduced the direct effect of pods per plant on grain yield (Table 102).

In variety Punjab 91, pods per plant had strongest positive direct (4.0370) contribution to grain yield, while the genotypic correlation was moderately positive (0.5411) between this pair of characters. The highest negative indirect effect via 100-seed weight (-1.9074) followed by biological yield (-1.0346), seeds per pod (-0.9351), plant height (-0.3204) and secondary branches (-0.2419) had decreased the genotypic correlation irrespective of the highest direct path influence (Table 103).

Pods per plant in variety C141 had positive direct influence (0.5353) on grain yield. The genotypic association between these two characters was also strongly positive (0.8407).

The indirect negative influence via primary branches (-0.0121), secondary branches (-0.0067), 100-seed weight (-0.0134), biological yield (-0.1019) and days to maturity (-0.0197) had reduced the direct effect of pods per plant on grain yield. The indirect effect via the rest of all the characters like plant height (0.0004), seeds per pod (0.0194), harvest index (0.4273) and days to 50% flowering (0.0122) was found to be positive (Table 104).

5. Seeds per pod Vs grain yield per plant

The results of path coefficient analysis shown in Table 102 indicated that in variety Noor 91, seeds per pod had direct positive path (0.0401) with the grain yield. The association between the two characters was positive (0.5238) at the genotypic level. The highest positive indirect contribution via harvest index (0.6759) followed by pods per plant (0.0362), primary branches (0.0145) and 100-seed weight (0.0113) had shifted the genotypic association towards the positive direction. However, the indirect negative effect via the rest of the traits decreased the direct positive path of seeds per plant on grain yield

In variety Punjab 91, seeds per pod had the strongest positive direct effect (2.0935) on grain yield and it had also exerted a positive genotypic relationship (0.4434) with grain yield. The highest negative indirect influence via pods per plant (-1.8031), followed the biological yield (-0.5770), plant height (-0.3296), harvest index (-0.1797), primary branches (-0.1501) and days to 50% flowering (-0.0584) had reduced the genotypic correlation between these two characters. However, the rest of all the component characters had the positive indirect contribution to the grain yield (Table-103).

Path coefficient analysis in variety C141, a strong genotypic correlation was found (0.6811) between seeds per pod and the grain yield. It had also exerted a positive direct effect (0.1173) on grain yield. Only the biological yield had negative indirect contribution (-0.1321) to grain yield while the rest of all yield contributing traits had indirect positive influence which shifted the genotypic association towards the positive trend (Table-104).

6. 100-seed weight Vs grain yield per plant

100-seed weight in variety Noor 91, had direct positive influence (0.0260) towards the grain yield. It had also positive and strong genotypic association (0.5600) with grain yield. The highest indirect effect (0.3769) was computed through harvest index followed by biological yield (0.1466), pods per plant (0.0463), seeds per pod (0.0174) and primary branches (0.0076). Whereas, negative indirect effects was observed via rest of the traits (Table 102).

In case of variety Punjab 91, 100-seed weight had strong positive (2.6638) effect on grain yield, while the relationship between the two characters was negative (-0.0659) at the genotypic level. The highest negative indirect effect via pods per plant (-2.8906), harvest index (2.7556), primary branches (-0.3906), secondary branches (-0.0785), days to maturity (-0.543) and days to 50% flowering (-0.0348) had shifted the genotypic correlation towards the negative trend. Whereas, indirect positive effects was observed via rest of the characters. The highest positive indirect effect was found via biological yield (1.9148) followed by seeds per pod (0.8928) and plant height (0.6673) (Table 103)

In C141 genotype, 100-seed weight had positive genotypic correlation (0.4328) and also exerted positive direct path effect on the grain yield. However, the magnitude of direct effect (0.0929) was very low than correlation value. The indirect influence of 100-seed weight via pods per plant (-0.0774), biological yield (-0.0702) and secondary branches (-0.0003) was found to be negative, whereas, rest of the traits had positive indirect influence of 100-seed weight on grain yield (Table 104).

7. Biological yield Vs grain yield per plant

Biological yield in Noor 91 genotype, had negative genotype correlation (-0.3177) with a direct positive path (0.3834) towards grain yield. The indirect influence via 100-seed weight (0.0099) and primary branches (0.0045) was found to be positive. Whereas rest of the characters had negative indirect contribution towards the negative trend (Table 102).

Path coefficient analysis in genotype Punjab 91 revealed that biological yield had strong negative effect (3.5758) on grain yield, whereas the genotypic correlation was low but

positive (0.2033). The highest positive indirect effect (4.9375) was computed via harvest index followed by pods per plant (1.1680), seeds per pod (0.3378), secondary branches (0.274) days to maturity (0.0475) and days to 50% flowering (0.0109) and had shifted the genotypic correlation towards the positive trend inspite of highest negative direct effect and indirect effect (-1.4265) via 100-seed weight (Table 103)

In case of variety C141, biological yield had positive direct effect (0.4340) on grain yield, while a negative relationship was obtained between the two characters at the genotypic (-0.2722) level. The negative genotypic association was shifted by indirect negative effect via harvest index (-0.5012) followed by pods per plant (-0.1257), plant height, (-0.0439), seeds per pod (-0.0357), days to maturity (-0.0204) and 100 seed weight (-0.0150). Whereas, rest of the component characters had positive indirect effects (Table 104).

8. Harvest index Vs grain yield per plant

In variety Noor 91, path coefficient analysis revealed that harvest index had positive and strong genotypic association (0.9550) and also exerted a highly positive direct effect (1.0457) on grain yield. By partitioning the genotypic correlation positive indirect path was computed via plant height (0.0021), pods per plant (0.1423) seeds per pod (-0.259) and 100-seed weight (0.0094), whereas the rest of the characters had negative indirect effect (Table 102).

Path coefficient analysis in variety Punjab 91 revealed that biological yield had strong negative direct effect (-5.3967) on grain yield. However, a positive genotypic association was found (0.2033) between these two characters. The highest indirect positive effect was computed by biological yield (3.2716) followed by 100-seed weight (1.3602), plant height (1.3602), primary branches (0.1996) and seeds per pod (0.0697) which had shifted the genotypic association towards the positive direction. The indirect effect of rest of the traits was negative (Table 103).

In the case of C141, harvest index had high positive direct effect on grain yield and it had also strong positive genotypic association with grain yield. However, the magnitude of direct effect (0.6432) was lower than the genotypic correlation (0.8077). The positive direct effect was reduced by the indirect negative effects via biological yield (-0.3382), secondary

branches (-0.0118) and primary branches (-0.0109). While rest of the characters exerted positive influence towards the grain yield (Table 104).

8. Days to 50% flowering Vs grain yield per plant

Days to flowering in Noor 91 had negative genotypic correlation (-0.5397) with yield but it had positive direct effect (0.0048) on seed yield. Indirect effect of days to flowering through harvest index (-0.4661), number of pods (-0.0586), biological yield (-0.0218), 100-seed weight (-0.0193) and seeds per pod (-0.0150) on grain yield was negative and contributed towards the negative genotypic association. While the rest of all the characters had positive indirect effect upon grain yield (Table 102).

In contrary to Noor 91 genotype, days to flowering in Punjab 91 had positive genotypic correlation (0.6809) with yield but it had negative direct effect (-0.0838) on seed yield. The results further indicated that indirect effect via harvest index (-2.1787), secondary branches (-0.2825), pods per plant (-0.1959) and days to maturity (-0.0064) was also negative (Table 103). The indirect effect of days to flowering through seeds per pod (1.4578), 100-seed weight (1.1062), biological yield (0.4662), plant height (0.3628) and primary branches (0.0352) was positive on grain yield and shifted the genotypic correlation towards the positive direction.

In the case of variety C141, days to flowering had negative direct effect (-0.0673) on grain yield and the genotypic correlation between these two characters was also negative (-0.6287). The indirect influence of days to flowering via plant height (0.0187) was positive but rest of all the characters had negative indirect influence on grain yield (Table 104).

10. Days to maturity Vs grain yield per plant.

In variety Noor 91, days to maturity had negative genotypic association (-0.7161) on grain yield, while the direct path was positive (0.0430) on grain yield. The highest negative indirect effect was computed by harvest index (-0.5627) followed by biological yield (-0.1055), pods per plant (-0.0977), seeds per pod (-0.0116) and 100-seed weight (-0.0181)

had shifted the genotypic correlation towards the negative trend (Table 102). The rest of the traits had positive indirect contribution to the grain yield.

In the case of Punjab 91, days to maturity had positive genotypic correlation (0.7105) with grain yield and also exerted a positive direct effect (0.1030) on grain yield. Virtually the positive genotypic association occurred due to the highest positive indirect effect (2.4198), computed via pods per plant followed by harvest index (0.8013), seeds per pod (0.4367), primary branches (0.3169), secondary branches (0.0732) and days to flowering (0.0052). The negative indirect influence of days to maturity via biological yield (-1.6481), 100-seed weight (-1.4045) and plant height (-0.3932) had reduced the direct positve effect of days to maturity on grain yield (Table 103).

Days to maturity in C141 genotype, had negative direct effect (-0.0385) on yield while it had positive genotypic correlation (0.1700) with grain yield. Days to maturity had negatively contributed indirectly through various traits including plant height (-0.0258), seeds per pod (-0.0595), 100-seed weight (-0.0450), harvest index (-0.1527) and days to flowering (-0.0112). The other traits like secondary branches (0.0053), pods per plant (0.2747) and biological yield (0.2305) had positive indirect effect on grain yield (Table 104).

Table 102: Noor 91 M_2 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variables	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harvest Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(.0167)	-.0110	.0127	.0113	-.0015	-.0158	-.2389	1.2920	.0090	.0330	-.0620
Pri. Branches	.0051	(-.0363)	.0024	.1232	-.0160	-.0055	-.047	.4600	-.0002	-.0097	.4760
Sec. Branches	-.0112	.0046	(-.0189)	.0468	.0094	.0236	.1902	.2803	-.0023	-.0302	.4922
Pods/ Plant	.0012	-.0279	-.0055	(.1603)	.0091	.0075	-.0064	.9272	-.0018	-.0262	.9281
Seeds/ Pod	-.0006	.0145	-.0044	.0362	(.0401)	.0113	-.235	.6759	-.0018	-.0125	.5238
100-Seed Weight	-.0102	.0076	-.0171	.0463	.0174	(.0260)	.1466	.3769	-.0036	-.03	.5600
Biol. Yield	-.0104	.0045	-.0094	-.0487	-.0245	.0099	(.3834)	-.6105	-.0003	-.0118	-.3177
Harvest Index	.0021	-.0160	-.0051	.1423	.0259	0094	-.2239	(1.0457)	-.0022	-.0231	.9550
Flowering Days	.0066	.0018	.0088	-.0586	-.0150	-.0193	-.0218	-.4661	(.0048)	.0189	-.5397
Maturity Days	.0128	.0082	.0133	-.977	-.0116	-.0181	-.1055	-.5627	.0021	(.043)	-.7161

Table 103: Punjab 91 M_2 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variables	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harvest Index	Flow. Day	Matu. Day	Grain (rg)
Plant Height	(-1.5219)	-.1282	.2498	.8498	.4533	-1.1681	3.2952	4.6831	.0200	.0266	.1692
Primary Branches	.3147	(.6199)	-.9500	2.8845	-.5069	-1.6783	.5590	-1.7374	-.0048	.0527	.4086
Secondary Branches	-.7166	-.1110	(.5306)	-1.8407	1.1464	-.3940	-1.389	2.5197	.0446	.0142	-.2067
Pods/ Plant	-.3204	.4429	-.2419	(4.073)	-.9351	-1.9074	-1.0346	.4345	.0041	.0617	.5411
Seeds/ Pod	-.3269	-.1501	.2906	-1.8031	(2.0935)	1.1360	-.5770	-.1797	-.0584	.0215	.4434
100-Seed Weight	.6673	-.3906	-.0785	-2.8906	.8928	(2.6638)	1.9148	-2.7556	-.0348	-.0543	-.0659
Biol. Yield	-1.4025	-.0969	.2074	1.168	.3378	-1.4265	(3.5758)	4.9375	.0109	.0475	.2075
Harvest Index	1.3206	.1996	-.2476	-.3250	.0697	1.3602	3.2716	(-5.3967)	-.0338	-.0153	.2033
Flowering Days	.3628	.0352	-.2825	-.1959	1.4578	1.1062	.4662	-2.1787	(-.0838)	-.0064	.6809
Maturity Days	-.3932	.3169	.0732	2.4198	.4367	-1.4045	-1.6481	.8013	.0052	(.103)	.7105

Table 104: C141 M_2 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield. The last column shows genotype correlation of independent variables with grain yield)

Variables	Plant Height	Pri. Branch	Sec. Brabch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harvest Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(-.0472)	.0107	.0129	-.005	-.0214	-.027	.4033	-.4129	.0267	-.021	-.0809
Primary Branches	-.0106	(.0478)	.0103	-.1357	.025	.0287	.0579	-.1467	-0186	.0008	-.1038
Secondary Branches	-.00296	.0241	(.0205)	-.1756	.0057	-.0013	.2761	-.3699	.0217	-.0100	-.2383
Pods/ Plant	.0004	-.0121	-.0067	(.5353)	.0194	-.0134	-.1019	.4273	.0122	-.0197	.8407
Seeds/ Pod	.0086	.0102	.001	.884	(.1173)	.0994	-.1321	.4073	.0615	.0195	.6811
100-Seed Weight	.0137	.0148	-0003	-.0774	.1255	(.0929)	-.0702	.2627	.0525	.0186	.4328
Biol. Yield	-.0439	.0064	.0131	-.1257	-.0357	-.015	(.434)	-.5012	.0163	-.0204	-.2722
Harvest Index	.0303	-.0109	-.0118	.3557	.0743	.0379	-.3382	(.6432)	.0180	.0091	.8077
Flowering Days	.0187	-.0132	-.0066	-.0968	-.1071	-.0724	-.1052	-.1723	(-.0673)	-.0064	-.6287
Maturity Days	-.0258	-.0009	.0053	.2747	-.0595	-.045	.2305	-.1527	-.0112	(-.0385)	.1770

III: M_3 GENERATION

1: Plant height Vs grain yield per plant

The results of path coefficient analysis in Noor 91 variety, revealed that direct contribution of plant height to grain yield was negative (-0.0288). The indirect effect of plant height via most of the traits like primary branches (-0.0581), pods per plant (-0.0143), seeds per pod (-0.283), 100-seed weight (-0.0146). harvest index (-.1878) and days to maturity (-0.0001) on grain yield was also negative. While through secondary branches (0.0004). biological yield (0.1341) and days to 50% flowering (0.1374) it had positive indirect effect on yield. A negative genotypic correlation of plant height with yield was mostly due to its indirect negative indirect effects through various traits (Table 105).

In the case of genotype Punjab 91, genotypic correlation between plant height and grain yield was positive direct effect (0.3740) and it had also exerted a positive direct effect (0.3075) on grain yield. However, the magnitude of direct effect was lower than the genotypic correlation. This decrease in indirect path occurred through the indirect path of various characters like primary branches (-0.0073), secondary branches (-0.0481), 100-seed weight (-0.5670), biological yield (-0.0516), harvest index (-0.0025) and days to 50% flowering (-0.0615). The remaining characters like pods per plant (0.6009), seeds per pod (0.1011) and days to maturity (0.1025) had contributed to grain yield through positive indirect path (Table 106).

Path coefficient analysis in variety C141 revealed that plant height had negative genotypic correlation (-0.4731) with yield, while it had positive direct effect (0.0049) on yield. Indirect effects of plant height via primary branches (0.0281), secondary branches (0.0311), pods per plant (0.5756), harvest index (0.2457) and days to 50% flowering (0.0064) was also positive. While indirect negative contribution of plant height was observed via seeds per pod (-0.9973), 100-seed weight (-0.0660, biological yield (-0.2880) and days to maturity (-0.0137) (Table 107).

2: Primary branches Vs grain yield per plant

Path coefficient analysis in Noor 91 genotype revealed that primary branches had negative and lower genotypic correlation (-0.0290) with grain yield. Partitioning of total genotypic correlation revealed that primary branches had negative direct effect (-0.1253) on

grain yield. It had also negative indirect effect of grain yield via plant height (-0.0134), secondary branches (-0.0015), pods per plant (-0.0269), 100-seed weight (-0.0118), harvest index (-0.2651) and days to maturity (-0.0001). While remaining contributing variables like, seeds per pod (0.0653), biological yield (0.2870) and days to 50% flowering (0.0628) had positive indirect effect on grain yield (Table 105).

In the case of variety Punjab 91, primary branches had positive genotypic correlation (0.6921) with grain yield and it had also exerted a direct positive effect (0.0766) on grain yield. The highest positive indirect effect (1.8520) was computed via pods per plant, contributed towards the positive genotypic correlation. Most of the remaining traits had indirect negative effect like plant height (-0.0295), secondary branches (-0.1824), seeds per pod (-0.1931), 100-seed weight (-0.7087), biological yield (-0.1079), harvest index (-0.1058) and days to 50% flowering (-0.0692) on grain yield and also reduced the direct path of primary branches (Table 106).

Genotype C141, had exhibited a strong positive genotypic association (0.7113) between primary branches and seed yield. It had also a positive direct effect (0.0649) on grain yield. The indirect positive influence of primary branches via plant height (0.0021), secondary branches (0.132), pods per plant (0.7430) and seeds per pod (0.0238) contributed towards the positive genotypic correlation. While the remaining characters through the negative indirect path via 100-seed weight (-0.0065), biological yield (-0.0894), harvest index (-0.0368), days to 50% flowering (-0.0004) and days to maturity (-0.0025) had reduced the direct path (Table 107).

3: Secondary branches Vs Grain yield per plant

It is evident from the results (Table 105) that in variety Noor 91, secondary branches had strong negative genotypic association (-0.7014) with grain yield and it had also exerted a negative direct path (-0.0020) on grain yield. The highest negative indirect path via harvest index (-1.3053) followed by primary branches (-0.0892), pods per plant (-0.0274) and days to 50% flowering (-0.0178) contributed towards the negative genotypic correlation. Secondary branches had positively contributed indirectly through various traits including plant height (0.0060), seed per pod (0.1922), 100-seed weight (0.0368), biological yield

(0.5053) and days to maturity (0.0001) were nullified by negative indirect effects and the negative direct effect (-0.0020) of secondary branches was decreased (Table 105).

In the case of Punjab 91, both the genotypic correlation and direct path due to secondary branches on grain yield was positive. However, the magnitude of direct path (0.1813) was lower than the genotypic correlation (0.2259). Various traits had positively but indirectly contributed to the grain yield like seeds per pod (0.6068), 100-seed weight (1.9531), biological yield (0.1797) and harvest index (0.1598). However, these positive indirect paths were counter balanced by the highest negative indirect path via pods per plant (-2.5661) (Table 106).

Path coefficient analysis in C141 genotype revealed that secondary branches had negative genotypic correlation (-0.4274) with grain yield. While it had positive direct effect (0.0375) on grain yield. The highest negative indirect effect was computed by the seeds per pod (-0.9141) followed by biological yield (-0.2773), 100-seed weight (-0.0631) and days to maturity (-0.0199) and shifted the genotypic correlation towards the negative trend. While the rest of the characters like plant height (0.0041), primary branches (0.0228), pods per plant (0.5459), harvest index (0.2347) and days to flowering (0.0020) had positive indirect contribution to the grain yield (Table 107).

4: Number of pods per plant Vs grain yield per plant

Path coefficient analysis (Table 105) in variety Noor 91 revealed that pods per plant had positive genotypic correlation (0.7902) with grain yield. It had negative direct effect (-0.1138) on grain yield. It had negative direct effect (0.1138) on grain yield. The partitioning of genotypic correlation revealed that characters like harvest index (1.2040) and seeds per pod had positive indirect path. While, rest of the component characters had negative indirect path.

In the case of variety Punjab 91 that pods per plant had highest positive direct effects on grain yield. It had also a positive genotypic correlation with grain yield. However, the magnitude of genotypic correlation (0.5546) was lower than the direct path (1.6758). The indirect influence of pods per plant via plant height (0.1103), primary branches (0.0847) and days to maturity (0.1620) had positive indirect effect on grain yield. The indirect effect via secondary branches (-0.2776), seeds per pod (-0.1443), 100-seed weight (-0.8389),

biological yield (-0.0949), harvest index (-0.0696) and days to 50% flowering (-0.0528) were negative and had reduced the genotypic correlation (Table 106).

In the variety c141, pods per plant had positive direct effect (0.7260) on grain yield. It had also the positive genotypic correlation (0.1457) with yield. the indirect effect via plant height (0.0039), primary branches (0.0664), secondary branches (0.0282), harvest index (0.1316) and days to 50% flowering (0.0014) were found also positive. however, only the two characters seeds per pod (-0.5645) and biological yield (-0.2341) had negative indirect effect (table 107).

5: Seeds per pod Vs grain yield per plant

Path coefficient analysis in variety Noor 91, the seeds per pod had positive genotypic correlation (0.3798) with grain yield. Yet it had slightly negative direct effect on the grain yield. The negative indirect effect of seeds per pod via plant height (-0.0055), biological yield (-0.4950) and days to maturity (-0.0001) were recorded. Whereas, the positive indirect effect of seeds per pod on grain yield was contributed via primary branches (0.0556), secondary branches (0.0027), pods per plant (0.0145), 100-seed weight (0.0030), harvest index (0.9446) and days to 50% flowering (0.0072) shifting the genotypic correlation towards the negative side (Table 105).

In variety Punjab 91, the seeds per pod had positive genotypic correlation (0.5474) with grain yield and it had also exerted a positive direct effect on grain yield (0.3630). However, the magnitude of direct path was lower than the genotypic correlation. The indirect effect via primary branches (-0.0408), pods per plant (0.6661) and days to 50% flowering (-0.0267) was negative which minimized the direct effect of seeds per pod. The indirect effect via plant height (0.0856), secondary branches (0.3030), 100-seed weight (0.4622), biological yield. (0.0070), harvest index (0.0205) and days to maturity (0.0460) was positive (Table 106).

In the case of variety C141, the path coefficient analysis revealed that seeds per pod had positive direct effect (1.1147) on grain yield yet it has negative indirect effect via plant height (-0.0044), secondary branches (-0.0308), pods per plant (-0.3677), harvest index (-0.2754) and days to 50% flowering (-0.0080), while the positive effect of this character on

grain yield was through primary branches (0.0014), 100-seed weight (0.0436), biological yield (0.2812) and days to maturity (0.0512) (Table 107).

6: 100-seed weight Vs grain yield per plant

Path coefficient analysis in variety Noor 91 revealed that 100-seed weight had positive genotypic correlation (0.5728) with grain yield yet it had slight negative direct effect (-0.0543) on grain yield. 100-seed weight also exerted the negative indirect effect via plant height (-0.0078, primary branches (-0.0271), pods per plant (-0.0583), biological yield (-0.2489) and days to 50% flowering (-0.0148). While positive indirect influence on yield was observed via secondary branches (0.0014), seeds per pod (0.0081), harvest index (0.9744) and days to maturity (0.0002) (Table 105).

100-seed weight in variety Punjab 91, had positive direct effect (0.8204) on grain yield, while the correlation between these two characters was negative (-0.4450). The highest indirect effects via pods per plant (-1.7136) followed by plant height (-0.2125), days to maturity (-0.1938) and primary branches (-0.0662), shifted the genotypic correlation towards the negative direction whereas the remaining characters contributed towards the grain yield via secondary branches (0.4316), seeds per pod (0.2045), biological yield (0.1293), harvest index (0.0914) and days to 50% flowering (0.0638) indirectly and positively (Table 106).

In variety C141, the 100-seed weight had positive genotypic correlation (0.3816) with grain yield and it had also exerted a positive direct effect (0.2159) on grain yield. However, the magnitude of direct path was lower than the genotypic correlation. The negative indirect effect via plant height (-0.0015), primary branches (-0.0019), secondary branches (-0.0110), pods per pod (-0.0001), harvest index (-0.0712), days to 50% flowering (-0.0066) and days to maturity (-0.0027). The remaining traits had positively contributed towards the yield via seeds per pod (0.2251) and biological yield (0.0355) (Table 107).

7: Biological yield vs grain yield per plant

In variety Noor 91, the path coefficient analysis revealed that biological yield had negative genotypic correlation (-1.1005) with grain yield. It had positive indirect effect (0.4425) on grain yield. The negative association of biological yield with grain yield was mainly due to high indirect negative effect via harvest index (-1.7508). The positive indirect effect via pods per plant (0.0658), seeds per pod (0.1645), 100-seed weight (0.0306), days to

50% flowering (0.0391) and days to maturity (0.0001) contributed towards the grain yield (Table 105).

In the variety Punjab 91, the genotypic correlation between biological yield and seed yield was positive (0.9385), whereas biological yield had negative direct path (-0.0699) on grain yield. The results further indicated that indirect effect via secondary branches (-0.4661), seeds per pod (-0.0038), 100-seed weight (-1.5176) and harvest index (-0.0138) was also negative. The positive association of biological yield with grain yield was mainly due to highest indirect effect via pods per plant (2.2750) (Table 106)

In variety C141, biological yield exhibited a negative genotypic correlation (-0.5134) with grain yield and also had a negative direct path (-0.2878) on grain yield. Biological yield had negatively contributed indirectly through various traits including seeds per pod (-1.0891) 100-seed weight (-0.0266) and days to maturity (-0.0153) on grain yield. Biological yield had positively affected seed yield through plant height (0.0049), primary branches (0.0201), secondary branches (0.361), pods per plant (0.5906), harvest index (0.2451) and days to 50% flowering (0.0085) (Table 107).

8: Harvest index Vs grain yield per plant

In variety Punjab 91, harvest index exerted a positive direct effect (0.0172) on seed yield yet it had negative genotypic correlation (-1.9105) with grain yield. The strong negative genotypic association was obtained through the highest indirect negative path via pods per plant (-6.7876) followed by days to maturity (-0.8537), primary branches (-0.4717), days to 50% flowering (-0.3088) and plant height (-0.0440), while the strong positive indirect path through 100-seed weight (4.3637), secondary branches (1.6850), seeds per pod (0.4329) and biological yield (0.0563) had counter balanced the negative indirect path and shifted the directed path towards positive direction (Table 106).

The results given in table 107 revealed that in genotype C141, harvest index had positive genotypic correlation (0.8166) with grain yield. Yet it had slightly negative direct effect on the grain yield (-0.2680). The strong positive genotypic association between harvest index and seed yield was observed via seed per pod (1.1456)

9: Days to 50% flowering Vs grain yield per plant

Path coefficient analysis (Table 105) in variety Noor 91 revealed that direct contribution of days to 50% flowering to grain yield was negative. The indirect effect of days to flowering via secondary branches (-0.0003), pods per plant (-0.0014), 100-seed weight (-0.0071 and biological yield (-0.1531) was also negative. While through plant height (0.0350), primary branches (0.0695), seeds per pod (0.0093), harvest index (0.3149) and days to maturity (.0001) had positive indirect effect on yield. The genotypic correlation between these two characters was low and positive.

In genotype Punjab 91, days to 50% flowering had positive genotypic correlation (-0.2837) with yield. Yet is had negative direct effect (-0.2086) on grain yield. Bifurcation of genotypic correlation revealed a negative indirect effect only through 100-seed weight (-0.2511), while the rest of all the component characters positively contributed to grain yield (Table 106).

In variety C141, a positive correlation between days to 50% flowering and grain yield (0.3597) was recorded at the genotypic level. Bifurcation of genotypic correlation revealed a negative direct effect of days to flowering on grain yield. Indirect effect via plant height (-0.0014), secondary branches (-0.0033), pods per plant (-0.0043), harvest index (-0.1187) and days to maturity (-0.0044) showed also negative indirect effect on grain yield (Table 107)

10: Days to maturity Vs grain yield per plant

In genotype Noor 91, path coefficient analysis (Table 105) revealed that days to maturity had negative direct effect (-0.0003) on grain yield, yet it had positive indirect effect via secondary branches (0.0004), 100-seed weight (0.0339), harvest index (0.5756) and days to 50% flowering (0.0526), while the negative effect of this character on grain yield was through plant height (-0.0078), primary branches (-0.0375), pods per plant (-0.0290), seeds per pod (-0.0674) and biological yield (-0.1636). These negative effects were mostly smaller in magnitude but in combination they effect the determination of yield by direct forces.

Path coefficient analysis in variety Punjab 91, showed that correlation between days to maturity and grain yield was strongly positive (.7794) at the genotypic level and it also exerted a positive direct effect (0.2285) on seed yield. The positive genotypic correlation was exerted mainly through pods per plant (1.1878). The negative indirect effect via 100-seed

weight (-0.6958), biological yield (-0.0982), harvest index (-0.0642), secondary branches (-0.0140) and days to 50% flowering (-0.0093 had reduced the direct effect of days to maturity (Table 106).

In variety C141, genotypic correlation between days to maturity and seed yield was negative and the direct path (-0.0180) on grain yield was also negative. It was further observed that negative indirect effect via the seeds per pod (-0.9392), biological yield (-0.2243) and days to flowering (-0.0056) shifted the genotypic correlation more towards the negative direction. Positive indirect influence was, however, observed via plant height (0.0037), primary branches (0.0091), secondary branches (0.0143), pods per plant (0.5247), 100-seed weight (0.0328) and harvest index (0.2016). This indirect positive impression was less important (Table 107).

Table 105: Noor 91 M_3 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variables	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harves Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(-.0288)	-.0581	.0004	-.0143	-.0283	-.0146	.1341	-.1878	.1374	-.0001	-.0600
Primary Branches	-.0134	(-.1253)	-.0015	-.0269	.0653	-.0118	.287	-.2651	.0628	-.0001	-.0290
Secondary Branches	.006	-.0892	(-.002)	-.0274	.1922	.0368	.5053	-1.3053	-.0178	.0001	-.7014
Pods/ Plant	-.0036	-.0296	-.0005	(-.1138)	.0188	-.0278	-.2558	1.204	-.0014	-.0001	.7902
Seeds/ Pod	-.0055	.0556	.0027	.0145	(-.1471)	.003	-.495	.9446	.0072	-.0001	.3978
100-Seed Weight	-.0078	-.0271	.0014	-.0583	.0081	(-.0543)	-.2489	.9744	-.0148	.0002	.5728
Biol. Yield	-.0087	-.0813	-.0023	.0658	.1645	.0306	(.4425)	-1.7508	.0391	.0001	-1.1005
Harvest Index	.0032	.0199	.0016	-.0822	-.0833	-.0318	-.4647	(1.667)	-.0214	-.0001	1.0084
Flowering Days	.0350	.0695	-.0003	-.0014	.0093	-.0017	-.1531	.3149	(-.1131)	.0001	.1539
Maturity Days	-.0078	-.0375	.0004	-.029	-.0674	.0339	-.1636	.5753	.0526	(.0003)	.3566

Table 106: Punjab 91 M_3 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variables	Plant Height	Pri. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harves Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(.3075)	-.0073	-.0481	.6009	.1011	-.567	-.0516	-.0025	-.0615	.1025	.3740
Primary Branches	-.0295	(.0766)	-.1824	1.852	-.1931	-.7087	-.1079	.1058	-.0692	.1600	.6921
Secondary Branches	-.0816	-.0771	(.1813)	-2.5661	.6068	1.9531	.1797	.1598	-.1124	-.0176	.2259
Pods/ Plant	.1103	.0847	-.2776	(1.6758)	-.1443	-.8389	-.0949	-.0696	-.0528	.1620	.5546
Seeds/ Pod	.0856	-.0408	.303	-.6661	(.363)	.4622	.0007	.0205	-.0267	.0460	.5474
100-Seed Weight	-.2125	-.0662	.4316	-1.7136	.2045	(.8204)	.1293	.0914	.0638	-.1938	-.4450
Biol. Yield	.2268	.1182	-.4661	2.275	-.0038	-1.5176	(-.0699)	-.0138	.0687	.3211	.9385
Harvest Index	-.044	-.4717	1.685	-6.7876	.4329	4.3637	.0563	(.0172)	-.3088	-.8537	-1.9105
Flowering Days	.0907	.0254	.0977	.4244	.0465	-.2511	.0230	.0255	(-.2086)	.0102	.2837
Maturity Days	.01379	.0537	-.014	1.1878	.0730	-.6958	-.0982	.0642	.0093	(.2285)	.7994

Table 107: C 141 M_3 Generation; Direct (parenthesis) and indirect effect matrix (non-dependent variable is grain yield). The last column shows genotype correlation of independent variables with grain yield.

Variables	Plant Height	Prl. Branch	Sec. Branch	Pods/ Plant	Seeds/ Pod	100-S Weight	Biol. Yield	Harvest Index	Flow. Days	Matu. Days	Grain (rg)
Plant Height	(.0049)	.0281	.0311	.5756	-.9973	-.066	-.288	.2457	.0664	-.0137	-.4731
Primary Branches	.0021	(.0649)	.0132	.743	.0238	-.0065	-.0894	-.0368	-.0004	-.0025	.7113
Secondary Branches	.0041	.0228	(.0375)	.5459	-.9141	-.0631	-.2773	.2347	.002	-.0199	-.4274
Pods/ Plant	.0039	.0664	.0282	(.726)	-.5645	0.0000	-.2341	.1316	.0014	-.013	.1457
Seeds/ Pod	-.0044	.0014	-.0308	-.3677	(1.1147)	.0436	.2812	-.2754	-.008	.0152	.7697
100-Seed Weight	-.0015	-.0019	-.011	-.0001	.2251	(.2159)	.0355	-.0712	-.0066	-.0027	.3816
Biol. Yield	.0049	.0201	.0361	.5906	-1.0891	-.0266	(-.2878)	.2451	.0085	-.0513	-.5134
Harvest Index	-.0045	.0089	-.0328	-.3564	1.1456	.0574	.2632	(-.268)	-.0103	.0136	.8166
Flowering Days	-.0014	.0012	-.0033	-.443	.3873	.0611	.1052	-.1187	(-.0231)	-.0044	.3597
Maturity Days	.0037	.0091	.0413	.5247	-.9392	.0328	-.2443	.2016	-.0056	(-.018)	-.3938

DISCUSSION

DISCUSSION

This project was conducted to study the modulation of radiosensitivity with post mutagenic application of gibberellic acid for various characters in three chickpea genotypes having different seed coat colours. Dry seeds of genotype Noor 91, Punjab 91 and C141 were irradiated at various intensities of 10, 20, 30, 40, 50, 60, 70, 90 and 110 Kr treatments. These gamma irradiated seeds along with control and post mutagenic treatment of gibberellic acid were used for cytogenetical, biochemical and seedling studies. The experiment was further extended to manipulate the variability induced in three successive generations of chickpea with two mutagenic treatments i.e., gamma irradiated separately and with gibberellic acid; and also to determine the correlation and path coefficient analysis among the yield and other yield contributing characters. Results obtained for different parameters are discussed below.

I: CYTOGENETICAL STUDIES

i: MITOTIC INDEX

The mitotic cycle is a complex phenomena consisting of many cyclic events which are mutually dependent. The integrity of these events is very essential for their precise functioning and ultimately the growth and survival of the cell or organism. An experiment was conducted to evaluate the cytotoxic effect of gamma irradiation and the recovery of the physiological phenomena by decreasing the potentially damaging effects of irradiation by the application of gibberellic acid.

It is evident from table 2 that mitotic index decreased with an increase of gamma irradiation treatments in the three genotypes except at 20, 30 and 40 Kr doses in Punjab 91 genotype. Higher doses of gamma irradiation were found intensively mitodepressive, while lower doses were less mitoinhibitory. This reduction in mitotic index was dose dependent. In the previous research similar observations for mitotic index were recorded by Savage and Worth (1970) in *Hordeum vulgare*, Khanna (1988) in *Cicer arietinum*, Khanum (1994) in *Pisum sativum* and Khan (1995) in *Gossypium hirsutum*. In this study a differential response of varieties towards the gamma irradiation was observed (Table 1). Similar differential response

of varieties towards gamma irradiation has been reported by Khanum (1994) in *Pisum sativum* and Khan (1995) in *Gossypium hirsutum*.

In a number of studies it has been visualized that gamma irradiation interferes with a number of biochemical processes especially those concerned with nucleic acid and protein synthesis. Genetic effects of gamma irradiation are of very diverse nature. These are known to cause breakage, depolymerization, formation of cross links of DNA with other molecules, production of site specific hydroxyl radicals (Oleinick et al., 1994), depletion of RNA and protein (Izvorska and Bak"Rdzhieva, 1975; Khanna, 1988), decrease in H1 histone and changes in chromatin structure and transcription (Bagi and Hidvegi, 1983). The reduction in mitotic index by the gamma irradiation has been attributed mainly to the inhibition of DNA synthesis (Haber et al., 1961; Haber and Foard, 1964) or delay in mitotic cycle (Rowley et al., 1992). Khanna (1988) determined that with gamma irradiation mitotic index decreased with the decrease of ribonuclease activity and reduction in DNA content of chickpea seedlings. Callebaut et al. (1980) determined that the delay in the mitotic cycle was due to the reduction in the GA_3 content and endomitotic DNA synthesis. A slight increase in mitotic index at 20, 30 and 40 Kr of gamma irradiation in variety Punjab 91 may be due to different genetic constitution. Low doses of gamma irradiation are reported to increase the auxin, gibberellin and cytokinin contents (Stajkov et al.,1985; Rabie et al., 1996). Khanna and Maherchandani (1981) noted higher peroxidase activity with gamma irradiation, which might increase the mitotic activity in chickpea seedlings.

The treatment of gamma irradiation probably arrests the cells at a stage of cell cycle preceding mitosis resulting in a decrease in the proportion of dividing cells observed in mitosis. The results of the present mitotic index study in the three genotypes showed that gamma irradiation may have altered the movement of cells through the nuclear cycle. Gamma irradiation of 50 Kr and above resulted in a decrease of prophase frequency and an increase in metaphase and anaphase frequency, suggesting the presence of two transition points; i) before entering the nuclear cycle and ii) movement of chromosomes at metaphase and anaphase stage of the mitotic cycle. The first transition point may be due to the reduction in DNA synthesis, while the second transition point could be due to the effect of gamma irradiation on microtubule propagation and movement of chromosomes.

From the results of table 2 it is obvious that seed treatment with GA_3 increased the mitotic index in the three genotypes. This protective effect of GA_3 against the gamma irradiation may be due to its action at several biochemical and cellular levels. Tomi et al. (1983) determined that GA_3 treatment resulted in binding of enzyme to chromatin more easily for the enhancement of template activity. Increase in rate of transcription and protein synthesis was noted by Martin et al. (1984). An increase in ribonuclease activity and microtubule synthesis and orientation was observed by Martin and Northcote, (1983) and Duckett and Lloyd (1994), respectively. Application of GA_3 increased the size of meristematic region and proportion of cells undergoing division (Loy, 1977). An increase in the synthesis of endomitotic DNA (Callebaut et al., 1980), and peroxidase activity (Khanna, 1992) was recorded with post mutagenic treatment of GA_3. It can, therefore, be concluded that the exogenous application of gibberellic acid may have increased the mitotic index by i) minimizing the potential toxic effect of peroxy radicals; ii) increasing the synthesis of DNA and proteins; iii) promoting the frequency of cells entering the mitotic cycle and iv) propagating the microtubule assembly and thereby the movement of chromosomes, which might contributed an increase in the mitotic index.

ii: CHROMOSOMAL ABERRATIONS

In all the genotypes of chickpea viz. Noor 91, Punjab 91 and C141 similar chromosomal abnormalities were observed which included fragments, bridges and laggards at anaphase. These anomalies tended to increase with an increase of gamma irradiation treatment. Bridge formation was the most common abnormality, followed by laggards and fragments. Laggards and fragments decreased while bridges increased with an increase in irradiation dosages. Similar trends with gamma irradiation have also been observed in *Pisum sativum* (Kalloo, 1972; Mujeeb and Greig, 1973; Tan et al., 1990; 1991), in bread and durum wheat (Khanna, 1990), in *Secale cereale* (Savaskan and Toker, 1991), in *Vicia faba* (Vandana, 1993). Rukmanski and Rodridges (1990) reported a parallel response between the dose rate of gamma irradiation and the chromosomal anomalies in root tip cells of french bean. Khanum (1994) observed a similar increase in mitotic anomalies with an increase of gamma irradiation in *Pisum sativum* and Khan (1995) in *Gossypium hirsutum.*

In this study a differential response of varieties towards the gamma irradiation was observed (Table 3). Similar differential response of varieties towards gamma irradiation has been reported by Ahmad and Godward (1981) in *Cicer arietinum*; Khanum (1994) in *Pisum sativum* and Khan (1995) in *Gossypium hirsutum*. It is known that gamma irradiation interferes with chromosome condensation, breakages, movement and organization (Khanna, 1988; Oleinick et al., 1994). Variants associated with such changes were increased. In the present studies mitotic abnormalities induced by gamma irradiation were of two types; i) caused by partial or complete failure of the spindle mechanism resulting in lagging of chromosomes and ii) induced chromosomal aberrations which results in stickiness at metaphase, bridges and fragmentation of chromosomes at anaphase. Differences in both quality and kind of chromosomal aberrations provided excellent criteria for the study of differential sensitivity.

In the present investigation, application of gibberellic acid reduced the mitotic anomalies in all the three genotypes. Fragments and laggards were not observed at 40 Kr with GA_3 in Punjab 91 and C141. The decrease in mitotic anomalies were more pronounced with GA_3 at higher doses of gamma irradiation in all the genotypes (Table 4). In previous studies, similar radio protective effects of GA_3 were reported by Uppal and Maherchandani, (1988); Arora et al., (1989), Khanna (1990) in wheat and Khanna (1992) in triticale. Arora et al. (1989) suggested that GA_3 probably reduces the potential damage from becoming actual detectable cytological damage and promotes the repairing process. It has been investigated that gamma irradiation produces hydroperoxides of pyrimidines, hydroxyl radicals and superoxide anion radicals in DNA or metabolic pool of the cells (Thomas et al., 1976; Feldberg and Carew, 1981; Sokolov et al., 1981). Accumulation of these radicals in the living system is very injurious in minute quantities and place a stress on cellular activity. Application of GA_3 enhance the peroxidase activity which probably decrease these free radicals and consequently the radiation potential damage (Khanna, 1992).

iii: CYTOLOGY AND NUCLEOLUS VOLUME

Considerable changes in cell volume and vacuolation with gamma irradiation separately and with GA_3 have been demonstrated in the present investigation. At lower dose of 10 Kr with both treatments i.e., gamma irradiation and post mutagenic treatment of GA_3, there was not any appreciable change in cell volume, however, vacuolation was increased. This increase in vacuolation may be due to the greater metabolic and mitotic activity (Sax, 1963). Higher dose

60 Kr of gamma irradiation demonstrated considerable changes in cell volume and vacuolation. Post mutagenic treatment of GA_3 reverses this affect resulting an increase in cell volume and vacuolation. At 110 Kr of gamma irradiation cell volume was increased and vacuoles appeared to be collapsed forming large vacuoles, suggesting the drastic effects of irradiation. There may still be some cell enlargement due to wall elongation, which is more resistant to radiation. This effect is comparable with known radiation effects (Haber and Foard, 1964; Allen and Haigh, 1973). Application of GA_3 considerably modulated the effects of gamma irradiation leading to increase in wall extensibility and vesiculation. The results therefore provide additional evidence of a change in cell wall extensibility with GA_3 (Jupe and Scott, 1992).

Size of nucleolus is taken for cytomorphological markers related to transcription and post transcription processes in eukaryotic cells, in particular to rDNA amplification. The rDNA strand protrudes from the chromosome axis in the form of loop and form the nucleolar organizer (NOR), in which most of rRNA is synthesized. NOR binds with nuclear proteins to form the nucleolus. These processes make part to the genetic activity of the nuclei. Nucleolus contains DNA, chromatin and ribonucleo-protein particles as well as other structural components (Ashraf and Sharif, 1990; Ashraf et al., 1992). Nucleolar volume appears to be regulated by many factors. The demonstration of increase in nucleolar volume with an increase of gamma irradiation is in agreement with the observation of pea roots (Khanum, 1994). The size of the nucleolus is very sensitive to the change in ribosomal synthesis (Knowland and Miller, 1970). The nucleolus is also believed to serve as a cytological indicator of structural and molecular changes that takes place normally in response to environmental stresses. This increase in nucleolar volume may be due to the response of the nucleolar organizing region to the treatments. This increase in nucleolar volume at 10 Kr may be due to the stimulation of metabolic activity and consequently the ribosomal precursor (granular region). However, at higher doses of 60 and 110 Kr, size of nucleoli may be increased due to the deformity of the nucleolar components. On the other hand application of GA_3 modulated the effects of gamma irradiation quite convincingly and nucleolar size was decreased. This may account for the influence of GA_3 at various biochemical and structural levels to reduce the potential damaging effects of gamma irradiation.

iv: NUCLEOLUS STRUCTURE

The present study provides additional information on the ultrastructural changes of the nucleolar morphology induced through gamma irradiation separately and with GA_3. The foregoing results indicate that in interphase nuclei of untreated material, the nucleolus consist of three structural components. These constitute dark fibrillar, pale fibrillar and granular regions (Bernhard, 1969; Chouinard, 1971; Lord and Lafontaine, 1973; Lafontaine and Lord, 1974; Chouinard, 1975; Ashraf and Sharif, 1990; Ashraf et al., 1992). Exposure of seeds at 10 Kr of gamma irradiation separately and with GA_3, there are no significant modifications in the structure of nucleolus except that nucleoli faintly stained. The following morphological observations have been made after 60 Kr of gamma irradiation. At this treatment nucleolus appear amorphous except some dark patches surrounding the periphery of the nucleolus. A nucleolus associated body is also seen toward the periphery of the nuclear membrane. Similar nucleolus associated body with gamma irradiation has also been observed in pea (Khanum, 1994). Drastic effects of gamma irradiation on the structural components are recorded at 110 Kr treatment. Nucleolus appears to be deformed having dark patches scattered throughout the nucleoloplasm. Nucleolus associated body is also seen attatching the main nucleolus. Granular components appears to be scattered in the nucleoplasm. These deformities of the nucleolar components could arise as an effect of gamma irradiation on the NOR. Application of GA_3 has changed these drastic effects to some extent and nucleolus appears to be reformed. A nucleolus associated body is still attached to the main nucleolus.

II: BIOCHEMICAL STUDIES

i: FRESH WEIGHT PER SHOOT (mg)

The results shown in Fig. 14-16 revealed a stimulatory response of fresh weight at lower dose of 10 Kr of gamma irradiation in all the three genotypes as compared to their respective checks throughout the developmental period. This increase in fresh weight might be due to higher contents of auxin, gibberellins and cytokinin at lower dose of gamma irradiation (Stajkov et al., 1985; Rabie et al., 1996). These growth regulators enhance the mitotic activity in the meristematic region by increasing the number of dividing cells (Sax, 1963). In the present investigation fresh weight decreased linearly with an increase in irradiation doses. The effects of gamma irradiation in a biological system are manifold; inhibit the synthesis of DNA

(Oleinick et al., 1994), induces DNA double stranded breaks and DNA protein crosslinks (Xue et al., 1994), decrease in H1 histone and changes in chromatin structure (Bagi and Hidvegi, 1983). Besides these direct effects of gamma irradiation on the genetic material, these are also known to enhance the production of hydroperoxide radicals (Harihorn and Cerutti, 1972; Feldberg and Carew, 1981; Sokolov et al., 1981; Voisine et al., 1991). These placed a stress on cellular activity and metabolism was grossly impaired at higher gamma irradiation doses. Maximum decrease in fresh weight was noticed at the highest gamma irradiation dose of 110 Kr in all the three genotypes. The genotypes also varied in the repairing process. In this case Noor 91 appeared to be more radio sensitive, because the increase in fresh weight at higher doses of 90 and 110 Kr was smaller when we compared to Punjab 91 and C141 genotype. The daily increase in fresh weight at higher doses was smaller as compared to lower doses of gamma irradiation. This small increase in fresh weight at higher doses may account for the excessive damage and impaired cellular activity.

Our observations (Fig. 14-16) showed that post mutagenic application of gibberellic acid modulated the effect of gamma irradiation and fresh weight increased. This increase in fresh weight at various levels of irradiation may account for the action of GA_3 at various biochemical and cellular levels. The effects of GA_3 in a biological system are of diverse nature, especially in relation to plant growth and development. It has been observed that GA_3 can promote growth of plants by affecting either cell division (Loy, 1977) and in reducing the duration of cell cycle (Liu and Loy, 1976). GA_3 is known to be involved in the synthesis of RNA and protein in the treated plants (Bewley and Black, 1978; Jacobsen, 1977; Jacobsen et al., 1979; Martin and Northcote, 1983). Callebaut et al. (1980) studied that application of GA_3 restores the endomitotic DNA synthesis and cell elongation in epicotyls of irradiated seeds of pea.

ii: CATALASE ACTIVITY (mM)

It is evident from the results (Fig. 17-19) that catalase activity was similar in all the three genotypes. In the present study it was observed that lower doses of 10 and 20 Kr of gamma irradiation did not have much effect on catalase activity. However, stimulation in activity at doses above 20 Kr was recorded on the 4^{th} and 5^{th} day and then a sharp decrease was observed gradually with increasing doses on the following day of the developmental period.

Specific changes in catalase activity were reported by Stajkov et al. (1985) at lower does of gamma irradiation in pea. Following gamma irradiation production of various peroxy radicals have been reported (Mead, 1976; Thomas et al., 1976; Teole et al., 1974; Harihorn and Cerutti, 1972; Voisine et al., 1991). The radiation protection affects of catalase is due to the removal of H_2O_2 (Donny et al., 1976). At higher doses of gamma irradiation a decrease in catalase activity may be due to the chain reaction of organic hydroperoxides after 5^{th} day of the developmental period. Accumulation of these hydroperoxides place a stress on cellular activities and consequently the rate of catalase activity decreased.

From the results of Fig. 17-19, it is obvious that the post mutagenic application of gibberellic acid changed the radiosensitivity and a stimulation in catalase activity was recorded. Catalase activity increased gradually with an increase of gamma irradiation and also throughout the development period. This increase in catalase activity with application of GA_3 may be accounted for the removal and inhibition of chain reaction of hydroperoxides.

iii: PEROXIDASE ACTIVITY (o.d.)

It is seen from the results (Fig. 20-22) that response of three genotypes towards the peroxidase activity was found almost similar in all the three genotypes. Peroxidase activity increased with an increase of gamma irradiation during the early developmental days at irradiation doses of 10, 20, 30, 40, 50 and 60 Kr from 3^{rd} to 5^{th} day and then decreased gradually from 6^{th} to 8^{th} development day. Stajkov et al. (1985) also reported specific peroxidase activity at lower doses of gamma irradiation. However, at higher doses the increase was upto 6^{th} developmental day. In previous studies it has been investigated that gamma irradiation causes damage to the tissues by producing H_2O_2 and organic peroxy radicals (Mead, 1976; Thomas et al., 1976; Teole et al., 1974; Harihorn and Cerutti, 1972; Motoji and Sergio, 1989; Sokolov et al., 1981; Izvorska and Bak "Rdzhieva, 1975; Voisine et al., 1991; Oleinick et al., 1994). The increase in peroxidase activity may be due to the production of organic peroxy radicals (Jain et al., 1990). These results are similar to Khanna and Maherchandani, (1981) in chickpea seedlings, where they also observed stimulation in peroxidase activity as a stimulus of the peroxy radicals. Peroxidase activity decreased on the later developmental days at higher doses of irradiation. This decrease in peroxidase activity may be due to the extensive radiation damage resulting in impaired cellular activity.

Post mutagenic application of gibberellic acid enhanced the peroxidase activity at various irradiation doses. The results obtained in this study are in line with those of Khanna, (1992) that peroxidase activity increased after the post irradiation treatment with gibberellic acid. Treatment with GA_3 restored the cellular activity by minimizing the potential toxic effects of gamma irradiation. Similar findings have also been reported in other studies (Callebaut et al., 1980; Pak et al., 1982; Khanna, 1992).

iv: INDOLE-3-ACETIC ACID (IAA) OXIDASE ACTIVITY (μg)

It is evident from the results (Fig.23-25) that IAA oxidase activity was stimulated at lower doses of 10 and 20 Kr of gamma irradiation throughout the developmental period in all the three genotypes. This increase in IAA oxidase activity at lower doses may be due to the stimulation of auxin contents in seedlings (Stajkov et al., 1985; Rabie et al., 1996). It is also seen from the present results (Fig. 23-25) that IAA oxidase activity decreased with increasing irradiation dosages and also the activity decreased across the developmental period. This decrease in IAA oxidase activity may be due to the production of higher amounts of peroxy radicals including phenols or the reduction in auxin content (Rabie et al., 1996). The inhibitory effects of various peroxy radicals as a damaging action of gamma irradiation is the activation of either peroxidase or IAA oxidase activity. Whether peroxy radicals or IAA are oxidized would depended upon the nature of peroxy radicals and concentration of peroxy radicals/IAA. Post mutagenic application of GA_3 increased the activity of IAA oxidase at all the levels of gamma irradiation and also across the various days of the developmental period. Application of GA_3 may stimulate the production of IAA and elimination of peroxy radicals, which ensure the stimulation of IAA oxidase activity.

v: PROTEIN CONTENTS (mg)

Results presented in Fig. 26-28 revealed that protein contents decreased progressively with an increase in gamma irradiation dosages. However, at 10 Kr treatment stimulation in protein contents was observed. In the previous research, similar results have been reported by Stajkov et al. (1985). The response of protein contents was found to be similar in all the three genotypes. Stimulation at lower doses may be due to the increase in auxin contents as a response to gamma irradiation (Rabie et al., 1996). Our results of higher doses of gamma

irradiation are also in line with previous studies where gamma irradiation decreased the protein content (Izvorska and Bak"Rdzhieva 1975; Damayanti and Sharma, 1990).

It is obvious from the results (Fig.26-28) that the application of GA_3 increased the synthesis of proteins (Bewley and Black, 1978; Jacobsen, 1977; Jacobsen et al., 1979; Martin et al., 1984). GA_3 is also known to stimulate the formation of polysomes (Martin and Northcote, 1983), advancing the production of rRNA and mRNA and consequently accelerating the rate of total protein synthesis during germination (Martin and Northcote, 1982).

vi: RIBONUCLEIC ACID (RNA) CONTENTS (μg)

It is evident from the results shown in Fig. 29-31 that all the three genotypes responded similarly to both mutagenic treatments i.e. gamma irradiation separately and with GA_3. RNA contents decreased regularly with an increase in gamma irradiation doses. Similar results on the depletion of RNA with increasing doses of gamma irradiation have also been reported in wheat (Haber and Foard, 1964), chickpea seedlings (Khanna, 1988), pea (Damayanti and Sharma, 1990). However, at lower doses of irradiation, stimulation in RNA contents was observed in all the three genotypes. This could be due to an effect of the peroxy radicals (Khanna and Maherchandani, 1981; Jain et al., 1990) or the increase in growth regulators (Rabie et al., 1996). Post mutagenic treatment of GA_3 reduced the effects of gamma irradiation and increases the RNA contents. This increase in RNA contents may be due to the enhancement of template activity of chromatin (Tomi et al., 1983), and the rate of transcription (Jacobsen, 1977; Jacobsen et al., 1979; Martin and Northcote, 1982).

vii: DEOXYRIBONUCLEIC ACID (DNA) CONTENTS (μg)

It is seen from the results presented in Fig. 32-34 that DNA contents decreased gradually with an increase in gamma irradiation dosages except at 10 Kr where stimulation over control was observed in all the three genotypes. Genetic effects of gamma irradiation on the inhibition of DNA synthesis was noted in wheat (Haber et al., 1961; Haber and Foard, 1964; Rowley et al., 1992; Bagi and Hidvegi, 1983; Kuzin et al., 1981; Khanna, 1988). Besides this gamma irradiation [illegible] is known to cause breakage and depolymerization of DNA, degradation and dissociation of histone H1 (Bagi and Hidvegi, 1983), double stranded breaks and DNA protein crosslinks (Oleinick et al., 1994; Xue et al., 1994). Radiolytic products,

adenine-7-oxide (Yamamoto, 1980), thymine hydroperoxide (Thomas et al., 1976), pyrimidine and thymine hydroperoxide (Teole et al., 1974), hydroperoxides (Harihorn and Cerutti, 1972), stable macroradicals in DNA (Sokolov et al., 1981) are also produced which may be applied to biological system as a radiation induced damage. Stimulation in DNA contents at lower doses may be due to an increase in auxin, gibberellin and cytokinin contents (Stajkov et al., 1985; Rabie et al., 1996), or the increase in peroxidase activity as a stimulus of the peroxy radicals (Khanna, 1992).

From this study it becomes apparent that there was an appreciable increase in DNA contents with the post mutagenic application of GA_3. Its application restored the endomitotic DNA synthesis (Callebuat et al., 1980), reduced chromosomal damage (Narsinghani and Kumar, 1976; Uppal and Maherchandani, 1988; Arora et al., 1989) by preventing the potential damage and promoting the repairing process.

III: SEEDLING STUDIES

Mutation breeding has been used in recent years as a valuable supplement to the method of plant breeding in the development of better crop cultivars. The gamma irradiation treatments have been used more frequently. Determination of a suitable radiation dose for a particular cultivar is of primary importance in mutation breeding. In mutation breeding treatments with low physiological damage and high genetic effects are desirable. Physiological effects of gamma irradiation are changed with the treatment of gibberellic acid without much interference in the genetic changes. An experiment was, therefore, conducted to determine the proper dose for obtaining maximum genetic variation and also to evaluate the modulation of radiosensitivity with gibberellic acid.

i: GERMINATION PERCENTAGE

Our observations showed (Table 9) a minor difference of germination percentage in the three varieties under study. Punjab 91 exhibited the highest overall seed germination of 98.25 as compared to 97.62 and 95.25% of C141 and Noor 91, respectively. Germination was found not to be affected upto 30 Kr in Punjab 91 as compared to 20 Kr and 10 Kr doses in C141 and Noor 91, respectively. Noor 91 exhibited a drastic decrease in germination % at 40 Kr

treatment and again an increase in germination percentage at 50 and 60 Kr doses of gamma irradiation. However, this type of behaviour was not seen in the other two varieties. A buffering action was observed at 40, 50 and 60 Kr treatments in Punjab 91 and C141. At higher doses decrease in germination percentage was gradual and minimum germination percentage was observed at 110 Kr dose of gamma irradiation. Hassan and Javed (1991) and Haq et al., (1992) while treating dry seeds of different chickpea genotypes and Veeresh et al., (1995) winged been genotypes with gamma irradiation showed that there was more reduction in germination at higher doses as compared to lower doses. Reduction in germination percentage with gamma irradiation have been reported in *Corchorus* (Rahman and Mia, 1970), chickpea *Cicer arietinum* (Rajput, 1977; Khanna, 1981; Bhatnagar, 1984; Mahto et al., 1989; Ahmad and Godward, 1990; Hassan and Javed, 1991), *Pennisetum americanum* (Aslam and Siddiqui, 1979), lentil *Lens culinaris* (Sinha and Chaudhry, 1987; Sarkar and Sharma, 1989;), Soybean (Mehetre and Mahajan, 1996), common bean (Colaco et al., 1995), pea (Khan et al., 1990), french bean (Svetleva and Petkova, 1992), faba bean (Rabie et al., 1996 and Kumar et al., 1993) and in green gram (Mallick et al., 1997). The results obtained in this study are also in line with Mahto et al., (1989) and Ahmad and Godward (1990) where, they found that the germination percentage in chickpea was not affected with irradiation at lower doses.

Germination percentage increased with the post mutagenic treatment of gibberellic acid at various levels of irradiation. Treatment of gibberellic acid modulated the behaviour of the three varieties of chickpea more or less in an equal manner. Germination was not affected in Punjab 91, C141 and Noor 91, upto 70 Kr, 60 Kr and 50 Kr dosages of gamma irradiation , respectively (Table 9). Maximum recovery of 12.50% was observed in Noor 91 at 110 Kr treatment. These results are in line with those of Arora et al. (1989), where studying the post-mutagenic effects of GA_3 in wheat resulted in higher germination percentage. Similarly higher germination percentage by modulating effects of growth regulators IAA and Kinetin is reported in chickpea by Ali and Ansari (1989). Vasilev and Mekhandzhiev (1991) determined the radio protective effect of N.allyl and Phenyl-N.2-pyridyl thiourea in *Pisum sativum* and *Glycine max*. On the basis of germination percentage they determined that the appplication of chemicals increased the resistance against radiation. On account of these studies it was thought that treatment with radio protective compounds in the course of mutation breeding would increase the frequency of induced mutant survival.

Germination was highly significantly ($p<0.01$) affected by gamma irradiation treatment (Table 8) while genotype-treatment interaction was non-significant ($p>0.05$). However, the difference between genotypes was significant ($p<0.05$). The differential response of varieties towards the gamma irradiation have also been reported in various studies, Aslam and Siddiqui, (1979) in *Pennisetum americanum*, Sinha and Chaudhry, (1987), Tripathi and Dubey, (1992 b), in *lens culinaris*, Ahmad and Godward, (1990) and Hassan and Javed (1991), Haq et al., (1992) in chickpea, Mehetre and Mahajan, (1996) in soybean and Mallick et al., (1997) in green gram. Reduction in germination with gamma irradiation might be due to an increase in the production of active radicals responsible for seed lethality while the protective effect of GA_3 could be due to the removal of these potential dangerous radicals.

ii: SHOOT LENGTH (cm)

A marked effect of gamma irradiation on shoot length in three genotypes has been observed in our study. Punjab 91 exhibited the highest overall shoot length of 13.88 cm as compared to 10.30 and 9.73 cm of Noor 91 and C141, respectively (Table 11). At lower doses of gamma irradiation a stimulating effect on shoot growth was observed in all the genotypes. In Punjab 91 the stimulating effect was observed at 10, 20 and 30 Kr, while in Noor 91 and C141 it was observed at 10 Kr dose. Stimulating effects of low doses of ionizing irradiation have also been reported in chickpea (Khanna and Maherchandani, 1981; Rao, 1988; Haq et al., 1992), in soybean (Vasti and Keerio , 1974) and in lentil (Tripathi and Dubey, 1992 b). Stajkov et al. (1985) and Rabie et al. (1996) attributing stimulating effect of low doses of gamma irradiation on auxin, gibberellin and cytokinin balance in irradiated seedlings. A similar observation on auxin balance in chickpea seedlings was observed by Sax, (1963). This stimulation in growth could be due to the increased cell expansion and greater mitotic activity in chickpea seedlings raised after low gamma irradiation treatment.

Shoot length decreased gradually with an increase in doses of gamma irradiation (Table 11). Gamma irradiation causes damage to the tissues by producing H_2O_2 and organic peroxy radicals (Mead, 1976; Thomas et al., 1976; Teole et al., 1974; Harihorn and Cerutti, 1972 and Voisine et al., 1991) and peroxidases are the internal mechanism for removal of these radicals. The increase in enzyme activity at lower doses could be a response of the tissues to the increase in peroxides (Khanna and Maherchandani, 1981; Croci et al., 1987; Croci et al., 1991; Shen et

al., 1991). At higher doses the entire cellular metabolism is grossly impaired resulting in lower enzyme activity. Reduction in shoot length with increasing doses of gamma irradiation have also been reported in soybean (Hassan et al., 1985) lentil *Lens culinaris* (Sinha and Chaudhry, 1987; Shaikh and Begum, 1990), chickpea *Cicer arietinum* (Hassan and Javed, 1991; Haq et al., 1992), sorghum (Asghar and Khan, 1988), bean (Colaco et al., 1995), winged bean (Veeresh et al., 1995), faba bean (Rabie et al., 1996).

In the present research application of gibberellic acid decreased the intensity of radiation damage resulting in an increase in the seedling height at various levels of irradiation. Maximum stimulating effect of GA_3 with an increase of 5.65 cm in height was observed at 20 Kr dose in Noor 91. Stimulation in the seedling growth with the post mutagenic treatment GA_3 have also been reported in Triticale (Khanna, 1992), wheat (Uppal and Maherchandani, 1988; Arora et al., 1989). Ali and Ansari (1989) reported that the post mutagenic treatment of IAA and kinetin in chickpea seedlings reduced the inhibition of shoot growth caused by irradiation.

The effect of GA_3 on growth, development and metabolism is due to the action of GA_3 at several biochemical levels. It has been established that GA_3 can promote growth of plants by affecting either cell division (Loy, 1977) and also reduces the duration of cell cycle (Liu and Loy 1976). GA_3 has also been known to be involved in the synthesis of RNA and protein (Bewley and Black, 1978; Jacobsen, 1977; Jacobsen et al., 1979; Martin and Northcote, 1983). Callebaut et al. (1980) noted that application of gibberellic acid elongates the cells in epicotyls of irradiated seeds of pea. Due to multiple physiological functions, therefore, GA_3 treatment restores the growth of plant by minimizing the potential toxic effects of gamma irradiation at different metabolic processes.

Analysis of variance for the effect of different doses of gamma irradiation and with GA_3 on shoot length (Table. 10) revealed that varieties, treatments and genotypes x treatment varied highly significantly ($p<0.01$) for shoot length. This indicated that the genotypes did not perform uniformly at different doses of mutagenic treatments. The results obtained in this study are closely related to those of Hassan and Javed, (1991) and Haq et al., (1992) in chickpea and Eser et al., (1991) and Tripathi and Dubey (1992 b) in lentil.

iii: ROOT LENGTH (cm)

Punjab 91 exhibited the highest overall root length of 11.72 cm compared to 10.82 and 9.20 cm of C141 and Noor 91, respectively. In this parameter contrary to shoot length stimulation was not observed at lower doses in the three genotypes (Table 13). Root length decreased gradually with an increase in gamma irradiation dosages. Maximum reduction was observed in C 141 (10.10 cm) followed by Noor 91 (9.40 cm) and Punjab 91 (8.93 cm). The reduction in root length with increasing doses of gamma irradiation have also been reported in lentil (Sinha and Chaudhry, 1987), in rye (Savaskan and Toker, 1991), in chickpea (Hassan and Javed, 1991). Most pronounced reduction in root length was observed at higher doses as compared to lower doses similar to those reported by Veeresh et al., (1995) in winged bean.

Seeds when treated with GA_3 root length increased differentially in the three genotypes at various irradiation doses. Stimulation in root length with post mutagenic treatment of growth regulators has been reported by Ali and Ansari, (1989) in chickpea. Analysis of variance for the effect of different doses of gamma irradiation and with GA_3 on root length is presented in table 12. ANOVA shows that the genotypic, mutagenic and genotypes x mutagen interaction was highly significant ($p<0.01$) which indicates that the varieties did not perform uniformly at the different mutagenic treatments. Genotypic variation in sensitivity have been detected in lentil (Sinha and Chaudhry, 1987) in chickpea (Hassan and Javed, 1991 and Haq et al., 1992).

iv: NUMBER OF ROOTS

Punjab 91 exhibited the highest number of roots 15.77 per plant followed by 11.88 and 10.57 per plant in Noor 91 and C141, respectively. From the results (Table 15) it is revealed that the number of roots decreased with an increase of gamma irradiation in all the three genotypes. Maximum reduction in number of roots was observed at 110 Kr treatment of gamma irradiation. The reduction in number of roots with increasing doses of gamma irradiation has been reported in chickpea by Hassan and Javed, (1991).

Application of gibberellic acid after gamma irradiation of seeds had increased the number of roots in the three genotypes. Stimulation in the number of root over the respective control was recorded at 10 Kr in Noor 91 and Punjab 91 genotype. Analysis of variance for the effect of different doses of gamma irradiation and with GA_3 on number of roots (Table 14) revealed that the genotypic, mutagenic and genotype x mutagen interaction was highly

significant ($p<0.01$) indicating that differences exist among the varieties regarding sensitivity to mutagenic treatments.

From the above mentioned results, it appeared that out of four parameters studied, shoot and root length can be used with equal reliability for estimating the appropriate doses of gamma irradiation on a large scale in a breeding programme. The higher doses of gamma irradiation showed an overall reduction in all the parameters studied. This may be partly due the fact that the cells, which had relatively more chromosomal damage and grossly impaired cellular activity due to injurious active compounds could not compete with the normal cells and thus prevented from making any further contribution. Mutagenic treatments with low physiological effects and strong genetic effects are, therefore, desirable. From the results, it appeared appropriate to use a dose range of 40-60 Kr of gamma irradiation, which were effective to reduce about 20-40% shoot and /or root length. These doses have also been determined suitable by Haq et al. (1992) for inducing variability in chickpea. Post mutagenic application of gibberellic acid changed the physiological effects of gamma irradiation by restoring the growth, so as to enhance the mutants survival for breeding program.

IV: MUTATION STUDIES IN M_1 GENERATION

i: PLANT HEIGHT (cm)

It is revealed from the results (Table 16) that plant height in chickpea was highly significantly effected ($p<0.01$) by varieties, treatments as well as by the genotypes- treatment interaction. It indicates that varieties did not perform uniformly across the various doses of irradiation. It is also obvious from table 17 that plant height was significantly reduced with both mutagenic treatments as compared to their respective controls. Decrease in plant height with gamma irradiation have also been reported in chickpea (Khanna, 1981; Rao, 1988; Mahto et al., 1989), lentil (Eser et al., 1991; Tripathi and Dubey, 1992 b), faba bean (Kumar et al., 1993), mungbean (Sarkar et al., 1996), wheat (Hassan et al., 1988), pea (Khan et al., 1990) and french bean (Svetleva and Petkova, 1992).

It is concluded from the results (Table 17) that the three genotypes responded differentially to both mutagenic treatments. In Noor 91, both mutagenic treatments increased the plant height. However, application of GA_3 significantly increased the plant height at 40 and 50 Kr, while decreased at 60 Kr. In genotypes Punjab 91 and C141 plant height decreased with both mutagenic treatments. Plant height decreased significantly with GA_3 at 50 and 60 Kr in C141. It suggests that post mutagenic application of GA_3 modulated the effects of gamma irradiation. Differential response of varieties have also been reported in various crops; broad bean (Kasim et al., 1977), chickpea (Khanna, 1981), lentil (Tripathi and Dubey, 1992 b; Eser et al., 1991), *Pennisetum* (Aslam et al., 1985) and in *Cajanus cajan* (Kumar and Sinha, 1989).

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

Highly significantly ($p<0.01$) variance components of treatments and genotype-treatment interaction (Table 18) indicate that relative performance of genotypes for number of primary branches per plant is highly inconsistent across the different treatments. It is similar to the findings of Khanna (1981) in chickpea and Mehetre et al. (1990) in mung bean. A marked change in radio sensitivity with GA_3 is revealed in this study and number of primary branches per plant increased significantly at 40 Kr, while decreased at 60 Kr. The present results implies that radio sensitivity is changed with the treatment of GA_3. Stimulation in the number of primary branches was recorded at 50 Kr of gamma irradiation and at 40 Kr with GA_3 treatment as compared to their respective

controls. In the previous research, similar results have been reported by Khanna (1981) in chickpea. In this experiment maximum inhibitory effects of irradiation were noticed at highest dose. Our results are also in line to those of Sinha and Chaudhry (1987); Tripathi and Dubey (1992 b) in lentil, Kumar and Sinha (1989) in *Cajanus cajan*, Hassan et al. (1988) in wheat where tillers per plant were decreased with gamma irradiation.

An obvious change in the effects of gamma irradiation with gibberellic acid has been visualized in present findings. Number of primary branches per plant decreased at 40 Kr and increased at 50 and 60 Kr in Noor 91, while reverse was true for Punjab 91. Application of GA_3 significantly increased the number of primary branches at 40 Kr and decreased at 50 and 60 Kr in Noor 91, while decreased at 40 Kr in Punjab 91. In C141 both mutagenic treatments significantly decreased the number of primary branches at 60 Kr, while increased at 40 Kr with the post mutagenic treatment of GA_3. A differential response of the number of primary branches per plant has also been recorded in mung bean by Mehetre et al. (1990) and in chickpea (Khanna, 1981).

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

The presence of highly significant (Table 20) differences due to genotypes, treatments and genotype-treatment interaction for number of secondary branches per plant can be attributed to high variation in response of genotypes to the different doses. The present results reveal that the number of secondary branches per plant were significantly ($p<0.01$) decreased at various levels of irradiation. Application of GA_3 modulated the effects of irradiation and significant increase at 40 Kr and decrease at 50 and 60 Kr was noticed. The interaction effect revealed that the response of the genotypes (Table 21) at various doses in the two treatments was also varied. Post mutagenic application of GA_3 either increased or decreased the number of secondary branches per plant in the three genotypes. It implies that the application of GA_3 modulates the genetic effects of gamma irradiation to create extra genetic variability for this character.

iv: NUMBER OF PODS PER PLANT

It is obvious from the highly significant variance components of genotypes, treatments and also their interaction (Table 22) that performance of genotypes is highly inconsistent at the various irradiation dosages. Table 23 shows that number of pods per plant decreased significantly ($p<0.01$) at various levels of irradiation in both mutagenic treatments as compared to controls. An

appreciable change with the post mutagenic application of GA3 for this character was noted. The number of pods per plant significantly increased ($p<0.01$) at 40 and 50 Kr while, a negative effect was recorded at 60 Kr treatment. These results are in agreement with those of Rajput (1977) and Khanna (1981), Mahto et al. (1989) in chickpea; Sinha and Chaudhry (1987), Eser et al. (1991), Tripathi and Dubey (1992 b) in lentil; Hassan (1986), Mehetre et al. (1990), Hassan et al. (1990) in mungbean; Kumar and Sinha (1989) in *Cajanus cajan*; Hassan et al. (1988) number of spikelets in wheat; Khan et al. (1990) in pea and Kumar et al. (1993) in faba bean ; Svetleva and Dimeva (1991) in *Phaseolus vulgaris* where number of pods per plant were decreased with irradiation as compared to control. However, Hassan (1986); Hassan et al. (1990) and Sarkar et al. (1996) obtained higher number of pods per plant in mung bean at lower doses. Yousaf et al. (1991) investigated the non-significant inhibitory effect with gamma irradiation on pods per plant in lentil. This confliction in the results might be due to the different breeding material and environmental conditions.

The interaction effect revealed a varying response of varieties towards the both mutagenic treatments. Number of pods per plant decreased in Noor 91, Punjab 91 and C141 as compared to control with gamma irradiation except at 60 Kr in Noor 91. Similar observation have been noticed by Hassan (1986), Eser et al. (1991) and Tripathi and Dubey (1992 b). Application of GA_3 had changed the irradiation response in the three genotypes. Number of pods per plant decreased at 40 and 50 Kr doses in Noor 91 and Punjab 91, however, in C141 a stimulation in pods per plant even over the control was recorded at 40 and 50 Kr treatment.

v: NUMBER OF SEEDS PER POD

The highly significant differences due to genotypes, mutagen treatment and the interaction of varieties with treatments (Table 24) indicate highly inconsistent response of genotypes for this character across the various doses. It is evident from the results of table 25 that number of seeds per pod decreased at various levels of irradiation as compared to control. Decrease in seeds per pod at higher doses was also noted in chickpea (Rajput, 1977), in mungbean (Khan, 1984; Hassan, 1986; Sarkar et al. 1996), lentil (Yousaf et al., 1991; Tripathi and Dubey, 1992 b). Post mutagenic application of GA_3 reduced the inhibitory effects of irradiation and increased the seeds per pod at 40 and 50 Kr.

Highly significantly genotypic-treatment interaction indicated a differential response of

different chickpea varieties to various mutagenic treatments. In Noor 91 and Punjab 91, less number of seeds per pod was obtained with gamma irradiation. However, in C141 a stimulation over control was recorded at 50 and 60 Kr. Varietal differences for seeds per pod towards the gamma irradiation was also reported by Tripathi and Dubey (1992 b) in two lentil varieties. Application of GA_3 increased the seeds per pod at all levels of irradiation in Noor 91 and at 40 and 50 Kr in Punjab 91 and C141 genotype. This increase in the number of seeds per pod may be accounted for the radio protective effects of gibberellic acid which may increase the fertility and survival of pollen grains and ovules..

vi: 100-SEED WEIGHT (g)

From the results table 26 it is obvious that performance of genotypes is highly inconsistent at various irradiation levels. 100-seed weight decreased significantly ($p<0.01$) with an increase in gamma irradiation. However, heavier seeds were produced with the application of GA_3 (Table 27). In the previous research, similar observations for this character was recorded by Rajput, (1977); Khanna, (1981) and Rao, (1988) in chickpea, Khan, (1984) in mungbean, Aslam et al. (1985) in *Pennisetum* and Din et al. (1988) in sunflower. 100-seed weight reduced variably at various irradiation dosages in all the three varieties. However, the application of GA_3 produced heavier seeds. This increase in 100-seed weight may be due to the radio protective effects of GA_3 and enhancement of template activity.

vii: BIOLOGICAL YIELD PER PLANT (g)

Highly significant variance components of varieties, mutagenic treatments and also by their interaction indicate that relative performance of genotypes was markedly inconsistent across the various doses (Table 28). Both the mutagenic treatments decreased the biological yield significantly as compared to their respective controls. The irregular response of biological yield with gamma irradiation may be due to the kind and extent of biological damage, while the consistent decrease in biological yield may be accounted for the protective and repairing activity of GA_3. Table 29 showed that in the three genotypes biological yield decreased inconsistently at various gamma irradiation dosages. Post mutagenic application of GA_3 significantly changed the

biological effects of gamma irradiation either in positive or negative direction. This suggests that the treatment of GA_3 could be useful for inducing extra variability.

viii: GRAIN YIELD PER PLANT (g)

It is concluded from the highly significantly variability (Table 30) of genotypes, mutagenic treatment and the interaction between varieties and treatments that performance of genotypes was highly inconsistent across the different treatments. It is evident from the results (Table 31) that grain yield reduced significantly at various levels of irradiation. However, with the application of GA_3 grain yield increased significantly at 40 and 50 Kr and decreased at 60 Kr. Rajput (1977); Khanna, (1981); Rao, (1988) and Mahto et al. (1989) have also observed the same trend in ckickpea. Similar results for this character were obtained in various crops; in lentil (Sinha and Chaudhry, 1987; Eser et al., 1991; Tripathi and Dubey, 1992 b), in *Pennisetum* (Aslam et al., 1985), in sunflower (Din et al., 1988), in *Cajanus cajan* (Kumar and Sinha, 1989), in mungbean (Hassan et al., 1990) and in *Phaseolus vulgaris* (Svetleva and Dimeva, 1991).

The genotype-treatment interaction (Table 31) indicate a wide range in the performance of genotypes for this character. Grain yield decreased significantly or non-significantly with gamma irradiation in the three genotypes as compared to their respective controls. However, the response of genotypes varied greatly at different doses. Genotypic differences due to various gamma irradiation were also observed by Rajput (1977) and Mahto et al. (1989) in chickpea and Aslam et al. (1985) in *Pennisetum.* Application of GA_3 had changed the effects of gamma irradiation significantly in the three genotypes except at 60 Kr in Punjab 91 indicating the possibility of increasing the variability for grain yield in chickpea.

ix: HARVEST INDEX (%)

Statistically highly significant differences ($p<0.01$) for genotypes, treatments and also for interaction (Table 32) indicate that response of this character is highly variable at various doses. Gamma irradiation decreased the harvest index significantly with both treatments as compared to their respective controls. However, application of GA_3 significantly increased the harvest index percent at 40 and 50 Kr by modulating the effects of gamma irradiation. Contrary to this, Yousaf et al. (1991) have recorded little variation in harvest index percentage under different gamma irradiation in lentil. The change in the results might be due to different genotypes and places of experimentation.

Highly significant interaction between genotype and treatment indicate varied response of harvest index towards the various doses of gamma irradiation. Gamma irradiation decreased the harvest index differently at all the treatments in the three genotypes as compared to control. However, with the application of GA_3 stimulation in harvest index over control was recorded in C141 genotype. The results of the present study further reveal that the application of GA_3 has changed the effects of gamma irradiation, which might increase the variability for this character in chickpea.

x: FLOWERING DAYS

It is obvious from the highly significant variance components (Table 34) of genotypes, treatment and genotype-treatment interaction that performance of genotypes is highly inconsistent across the various treatments. Table 35 shows that gamma irradiation significantly and progressively increased the number of days to 50% flowering at various levels of irradiation as compared to control. However, post mutagenic treatment with GA_3 decreased the time to 50% flowering. Late flowering in M_1 generation as compared to control have also been reported in pea (Khan et al., 1990; Amjad et al., 1993), french bean (Svetleva and Petkova, 1992). However, a non-significant delay in flowering with gamma irradiation was reported by Yousaf et al. (1991) in lentil. But Kasim et al. (1977) did not agree with these results and reported earliness in flowering in broad bean.

Highly significant interaction of varieties and doses indicate that the response of varieties to various levels of irradiation is quite variable. The results show a relative delay in 50% flowering over control in the three varieties. Application of GA_3 reduced the number of days to 50% flowering at different intensities of gamma irradiation. This decrease in time could be due to the repairing process of GA_3, which might bring the population to a physiological state for early flowering.

xi: MATURITY DAYS

It is concluded from the highly significant variation for genotypes, treatments and genotype-treatment interaction that crop maturity of genotypes was highly inconsistent across the different doses (Table 36). The effect of gamma rays (Table 37) revealed a consistent delay in crop maturity

with an increase of radiation intensity as compared to control. Post mutagenic application of GA3 decreased the time to crop maturity. The results obtained in this study are in line with those of Hassan et al. (1988) in wheat, Tripathi and Dubey (1992 b) in lentil and Kumar et al. (1993) in pea.

Highly significant interaction between varieties and doses (Table 37) revealed a variable response of genotypes at various doses. A linear increase in time to crop maturity was recorded with gamma irradiation in the three genotypes as compared to control. However, with GA_3 treatment less number of days were taken to maturity at various levels of irradiation in the three genotypes.

V: MUTATION STUDIES IN M_2 GENERATION

i: PLANT HEIGHT (cm)

It is revealed from the present study that plant height in M_2 population of chickpea was significantly and highly significantly effected due to treatments and genotypes ($p<0.01$) as well as by genotype and treatment interaction (Table 38). It indicates that a differential genetic variability obtained in the genotypes at different doses. Variability in plant height with gamma irradiation have been reported by Charumathi et al. (1992) in black gram. The results regarding the main effect of gamma irradiation revealed that plant height decreased non-significant ($p>0.01$) at 40 and 50 Kr treatment, while at 60 Kr dose a significant reduction ($p<0.01$) was observed with the two mutagenic treatments. Present results are compatible with Rao, (1988) in chickpea, Shakoor et al. (1978 a and b) in mungbean, Vadher et al. (1988) in sorghum and Ramani and Jadon (1991) in groundnut who reported a decrease in plant height with gamma irradiation in M_2 population. But findings of Rao et al. (1988) in pigeon pea and Khan et al. (1989) in sorghum did not agree with these results. They observed that plant height increased with the application of gamma irradiation.

The interaction between varieties and irradiation intensities was highly significant indicating that a differential pattern of mutants was obtained in three genotypes with the mutagenic treatments. Plant height increased non-significantly ($p>0.01$) in Noor 91 at all gamma irradiation dosages, while in Punjab 91 and C141 decreased as compared with untreated check. The application of gibberellic acid modulates the effects of gamma irradiation variably in the three

genotypes. Similar results were obtained by Shakoor et al. (1978 a & b) in mungbean, Kasim et al. (1977) in broad bean in M_2 generation of mutants with gamma irradiation.

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

Statistically highly significant variation of genotypes, treatment and their interaction (Table 40) indicate a marked variation in genetic spectrum obtained at various doses in the three genotypes. The main effect of radiation frequencies, showed that primary branches per plant were stimulated at all levels of gamma irradiation with the two mutagenic treatments. Maximum number of primary branches was obtained at 50 Kr in the two mutagenic treatments. Charumathi et al. (1992) reported similar findings with gamma rays in blackgram.

The interaction between genotypes and irradiation doses revealed that there exists differential response of genotypes at variable doses of the two mutagenic treatments regarding number of primary branches per plant. Number of primary branches per plant increased with gamma irradiation except at 60 Kr with the two mutagenic treatments in C141 genotype. However, the response of the genotypes varied at different doses. It implies that mutational spectrum is changed at different doses in the two treatments.

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

A highly significant variation in number of secondary branches per plant was noted in various chickpea genotypes, treatments ($p<0.01$) as well as for interaction between treatment and genotypes (Table 42) This indicates a different genetic spectrum obtained at various doses in M2 generation. From table 43 it is revealed that secondary branches per plant decreased significantly except at 40 Kr treatment with gibberellic acid where a non-significant ($p>0.01$) increase was observed when compared with untreated check. The interaction between genotype and treatment was highly significant for this character. It reveals a marked variation in mutants is obtained at different doses in three genotypes. Charumathi et al. (1992) also observed a marked variability in the number of branches in black gram with gamma irradiation.

iv: NUMBER OF PODS PER PLANT

The variation in number of pods per plant in chickpea was highly significantly effected

($p<0.01$) by genotypes, treatments and their interaction (Table 44) indicating the existence of a sufficient variability among the population in the three genotypes at various doses. In the previous research similar results have been reported by Shakoor et al. (1978 a & b) in mungbean, Khan et al. (1989) in sorghum, Alexieva and Nikolov (1991) in soybean, Bhatnagar (1991) in chickpea, Sarma et al. (1991) in green gram and Charumathi et al. (1992) in black gram. The main irradiation effect (Table 45) on number of pods per plant in M_2 population of chickpea showed that number of pods increased with both mutagenic treatments.

Highly significant interaction between varieties and doses revealed that cultivars were found independent in their response to different irradiation intensities for this character. In Noor 91 and C141 number of pods per plant at various irradiation dosages increased while, in Punjab 91 increased at 50 Kr only. With the application of gibberellic acid a wide variation in number of pods per plant was observed at different doses. Present investigations are in line to those of Shakoor et al. (1978 a) in mungbean, Sinha and Bharati (1990) in Urdbean and Bhatnagar (1991) in chickpea where they observed that the number of pods per plant were increased at various irradiation dosages irrespective of the cultivars.

v: NUMBER OF SEEDS PER POD

Number of seeds per pod in M_2 generation of chickpea was highly significantly effected ($p<0.01$) due to various genotypes, treatments and by their interaction (Table 46) indicating a marked variation in mutants obtained at different doses. Similar variability in seeds/pod have also been recorded by Sarma et al. (1991), Charumathi et al. (1992) and Gupta and Sharma (1996). It is obvious from the results (Table 47) that number of seeds per pod were not effected by gamma irradiation, while with gibberellic acid the number of seeds per pod increased significantly ($p<0.01$) as compared with control. The results of Khan (1984) were partially in line with these studies as that with the treatment of GA_3.

The interaction between varieties and doses showed that the genotype Noor 91 and C141 number of seeds by pod increased non-significantly with gamma irradiation while, significantly ($p<0.01$) with gibberellic acid as compared to control. In Punjab 91, number of seeds per pod decreased significantly ($p<0.01$) except at 40 Kr dose of gamma irradiation. Statistically non-significant ($p>0.01$) increase as compared to control was recorded with GA_3. Our results are partially compatible with Shakoor et al. (1978 a) in mungbean where the number of seeds per pod

were decreased with gamma irradiation in M_2 population as in the genotype Punjab 91. Similarly Kumar and Sinha (1989) recorded a non-significant difference for number of seeds per pod in M_2 generation of *Cajanus cajan* and *Moghania*. These results are in agreement to present study in genotypes C141 and Noor 91 where the number of seeds obtained were statistically similar to their untreated control.

vi: 100-SEED WEIGHT (g)

Highly significant variation in 100-seed weight by divergent genotypes, radiation doses and their interaction (Table 48) revealed a marked variation in genetic spectrum obtained at different doses. In the previous research, similar findings have also been reported by Sarma et al (1991) in green gram, Charumathi et al (1992) in black gram and Gupta and Sharma (1996) in horse gram. It is evident from the results (Table 49) that the size of seed responded differentially to the two mutagenic treatments. Gamma irradiation decreased the 100-seed weight at 40 and 60 Kr as compared to control. Application of gibberellic acid probably modulated the effects of gamma irradiation and 100-seed weight increased significantly ($p<0.01$) at 40 Kr dose as compared to control. Khan (1984) reported contrary findings to these results in mungbean. This could be due to the different breeding material and experimental conditions.

The interaction between varieties and doses revealed that various genotypes responded differentially to different mutagenic treatments. Gamma irradiation in Noor 91 decreased while, gibberellic acid increased 100-seed weight as compared to control. In Punjab 91 the weight of 100-seed weight increased non-significantly ($p>0.01$) at 40 and 50 Kr, while a significant decrease ($p<0.01$) was observed at 60 Kr treatment as compared to control. The weight of 100-seeds decreased except at 50 Kr with gibberellic acid. In C141 100-seed weight decreased with gamma irradiation. However, the application of gibberellic acid increased the weight of 100-seeds significantly ($p<0.01$) at all levels of irradiation when compared to untreated check. This increase in variability with the application of GA_3 might widen the germ pool and enhance the selection for better genotypes.

vii: BIOLOGICAL YIELD PER PLANT (g)

The variability in biological yield in M_2 generation of chickpea (Table 50) was highly significantly effected ($p<0.01$) due to genotypes and treatments as well as by their interaction. This indicates that a sufficient variability is induced at various doses in the three genotypes. The biological yield decreased with the two mutagenic treatments at all the levels. The decrease in biological yield with gamma irradiation was significant ($p<0.01$) as compared to control. Application of gibberellic acid modulated the effects of gamma irradiation and at 40 Kr and 60 Kr dose a significant increase and decrease ($p<0.01$) in biological yield was observed.

The interaction between varieties and the mutagenic treatments indicate a sufficient variability in the three genotypes. In Noor 91 the biological yield decreased non-significantly with gamma irradiation, while increased significantly with the application of gibberellic acid except at 40 Kr as compared to control. Punjab 91 exhibited a marked decrease with gamma irradiation, however, the decrease in biological yield with gibberellic acid at all levels of irradiation was non-significant ($p>0.01$) as compared to control. Behaviour of C141 towards the two mutagenic treatments was identical and biological yield decreased progressively with an increase in irradiation dosages. However, it was significantly decreased ($p<0.01$) as compared to control.

viii: GRAIN YIELD PER PLANT (g)

Highly significant variance components of genotypes, treatments as well as their interaction (Table 52) revealed that a marked variability is created at various doses in the three genotypes. In the previous research, similar observations have been reported by Sarma et al. (1991) and Charumathi et al. (1992). Table 53 reveals that grain yield increased with gamma rays significantly ($p<0.01$) as compared to control. Application of gibberellic acid changed the effect of irradiation and a marked increase in grain yield was observed. The present results are similar to those of John (1995). However, Rao (1988) had different results. He reported that gamma irradiation had adverse affect on grain yield in chickpea. This confliction in the results may be due to the dissimilar material and environmental conditions of the experiment.

The interaction between varieties and doses revealed that the varieties Noor 91 and C141 showed a positive response to the mutagenic treatments. However, a marked increase in grain yield

with the application of gibberellic acid was observed. In Punjab 91, gamma irradiation decreased the grain yield, while with gibberellic acid the response of grain yield at various doses was inconsistent. The results advocated in present study are similar to those of Larik et al. (1980) in wheat, Charumathi et al. (1992) in black gram and Kalia and Gupta (1989) in lentil who reported that the genotypes varied for grain yield in M_2 population. Results of Shakoor et al. (1978 a) are similar to genotype Punjab 91, while the results of Rao et al. (1988) are partially in line with the genotypes Noor 91 and C141.

ix: HARVEST INDEX (%)

The variation in harvest index was highly significantly ($p<0.01$) effected by genotypes, doses as well as by their interaction (Table 54) revealed that a marked variation is induced at various doses in the three genotypes. From the results (Table 55) it is revealed that harvest index responded differentially to the various doses. Harvest index increased significantly ($p<0.01$) with gamma irradiation while, the application of gibberellic acid modulated the effect of gamma irradiation and harvest index decreased significantly ($p<0.01$) at 40 Kr and then a significant increase was observed at 50 and 60 Kr. It reflects the induction of extra variability with the treatment of GA_3.

Highly significant interaction between varieties-doses revealed that a marked genetic variation is induced at different doses in the three genotypes. In Noor 91 and C141 harvest index increased significantly ($p<0.01$) at all levels of gamma irradiation as compared to control. A marked increase in harvest index was recorded with the application of gibberellic acid. In Punjab 91 the harvest index increased at various doses of gamma irradiation. Treatment of gibberellic acid altered the effects of irradiation and a marked variation in harvest index was observed at various levels of irradiation. Kalia and Gupta (1989) reported sufficient variation in harvest index induced due to gamma irradiation in M_2 population of lentil.

x: FLOWERING DAYS

Highly significant differences in time to flowering for genotypes, treatments and their interaction (Table 56) revealed that there exists a sufficient variability for this character in the three genotypes. Variability for this character has also been reported by Charumathi et al. (1992).

Gamma irradiation delayed the 50% flowering, while with the application of gibberellic acid an earliness in 50% flowering was observed.

Highly significant interaction of varieties to the mutagenic treatments (Table 57) indicated that a marked variation is present with regards to 50% flowering in the three genotypes. With gamma irradiation a non-significant change in 50% flowering was recorded in Punjab 91, whereas, in Noor 91 an earliness was observed as compared to control. C141 exhibited a significant delay in 50% flowering ($p<0.01$) at 40 and 50 Kr treatments. Application of gibberellic acid markedly decreased the days to 50% flowering in the three genotypes. The results obtained in present study are comparable to Khan et al. (1989), where the delay in flowering was observed in M_2 generation of sorghum. Similarly Kasim et al. (1977) in broad bean, Jayamanne and Jayasuriya (1991) in green grain, Charumathi et al. (1992) in black gram and Kalia and Gupta (1989) in lentils obtained a high degree of variation in days to 50% flowering in M_2 generation. Our results are in contrary with Vadher et al. (1988) who reported earliness in flowering of sorghum.

xi: MATURITY DAYS

The variation in days to maturity was highly significantly effected ($p<0.01$) due to various genotypes, doses and their interaction (Table 58). This indicates that an appreciable variability for crop maturity is present in the mutants obtained in M_2 population. Charumathi et al. (1992) also observed high variation for this character with gamma irradiation in black gram. Gamma irradiation non-significant ($p<0.01$) decreased the crop maturity as compared to control. Treatment of gibberellic acid changed the crop maturity and it was significantly decreased ($p<0.01$) at 40 and 50 Kr doses as compared to control.

Highly significant interaction of genotype-dose (Table 59) revealed an enormous variability for crop maturity in the mutants recovered at various doses in M_2 population. An earliness in maturity was recorded in Noor 91 and Punjab 91 with both mutagenic treatments. Days to maturity in C141 increased at 40 Kr, while decreased at other doses of gamma irradiation. Application of gibberellic acid modulated the effects of gamma irradiation and an earliness in maturity was recorded.

VI: MUTATION STUDIES IN M_3 GENERATION

i: PLANT HEIGHT (cm)

The differences in plant height in M_3 population of chickpea were highly significant ($p<0.01$) for genotypes, treatment as well as by their interaction (Table 60) indicating the presence of highly significant genetic variability in mutants obtained at different doses in the three genotypes. In the previous research, similar observations have been reported by Charumathi et al. (1992) in black gram. It is revealed from table 61 that plant height of mutants significantly ($p<0.01$) decreased with both mutagenic treatments as compared to untreated check. Decrease in plant height at higher doses of gamma irradiation has also been reported by Kumari (1995) in *V. hirsuta*. However, the plant height obtained at various levels of irradiation was statistically identical to each other in the two mutagenic treatments.

It is obvious (Table 61) from the highly significant interaction of variety-treatment that a sufficient variability is present for this character among mutants. In Noor 91, statistically non-significant variability in plant height was observed with both mutagenic treatments. Height of plants in Punjab 91 and C141 decreased significantly at various levels of irradiation in the two treatments as compared to control. However, a marked variability was recorded with GA_3 at different doses.

ii: NUMBER OF PRIMARY BRANCHES PER PLANT

The variation in number of primary branches per plant was highly significantly ($p<0.01$) effected by various treatments and the interaction between genotypes and treatments. However, the variation created due to chickpea genotypes was non-significant (Table 62). This indicates that an appreciable genetic variability is present in mutants at various doses. It is revealed (Table 64) that number of primary branches decreased non-significantly at various levels of irradiation except at 60 Kr with gibberellic acid. Similar results were also reported by Kumari (1995) in *V. hirsuta*. However, the application of gibberellic acid modulated the effects of gamma irradiation and sufficient variability was induced for this character.

The effect of mutagen via varieties (Table 64) revealed that a marked genetic variability is induced at various intensities of irradiation in the three genotypes for this character. Noor 91 exhibited a non-significant decrease or increase at different doses with the two mutagenic

treatments as compared to control. In Punjab 91, significant decrease in number of primary branches was recorded at 50 Kr with the application of gibberellic acid. However, in C141 significant decrease in number of primary branches was recorded at 40 Kr with both mutagenic treatments as compared to control. Application of GA_3 significantly increased the number of primary branches at 60 Kr by modulating the effects of gamma irradiation.

iii: NUMBER OF SECONDARY BRANCHES PER PLANT

The number of secondary branches per plant in M_3 generation was highly significantly effected ($p<0.01$) due to treatments. While the genotypes and interaction between varieties and doses were effected significantly ($p<0.05$) (Table 64). This indicates that a marked genetic variability is existed in mutants in the three genotypes. These results are in line to the findings of Charumathi et al. (1992). From table 65 it is obvious that irradiation had a direct effect on number of secondary branches per plant and decreased at various levels of irradiation. Application of gibberellic acid modulated the effects of irradiation and a sufficient variability is induced at various treatments.

The interaction between varieties and doses was significant ($p<0.05$) indicating that among genotypes an extra variability is induced at different does. In Noor 91 and Punjab 91 non-significant increase in secondary branches per plant was observed at 50 Kr with gamma irradiation. Post mutagenic treatment of GA_3 markedly induce genetic variability at different doses. In C141, number of secondary branches decreased significantly ($p<0.01$) at various dosages of irradiation in two mutagenic treatments except at 50 Kr dose of gamma irradiation as compared to control.

iv: NUMBER OF PODS PER PLANT

The analysis of variance showed that number of pods per plant in M_3 generation of chickpea were highly significantly effected by ($p<0.01$) varieties, treatments as well as by their interaction (Table 66). This indicates that various mutagenic treatments had induced an appreciable genetic variability in the three genotypes. Sufficient variability in pods per plant have been reported by Sarma et al. (1991) and Charumathi et al. (1992). It is obvious from table 67 that the application of gibberellic acid had changed the effects of gamma irradiation at various doses. Maximum number of pods was obtained at 50 Kr with gamma irradiation while, the minimum number of pods was

noticed at the same treatment of gibberellic acid.

The interaction of varieties via mutagen (Table 67) revealed that in all the varieties a sufficient variability is induced at various mutagenic treatments. These results are similar to the findings of Gupta and Sharma (1996). Number of pods increased significantly ($p<0.01$) in Noor 91 at various levels of irradiation in two mutagenic treatments except at 60 Kr with gamma irradiation. In Punjab 91, pods per plant decreased across the various doses except at 60 Kr of gamma irradiation. In C141, pods per plant decreased at all levels of irradiation as compared to untreated check. However, marked genetic variations were observed with GA_3 in the three genotypes.

v: NUMBER OF SEEDS PER POD

Highly significantly variance components due to varieties, treatments as well as by their interaction (Table 68) revealed that an extra genetic variability is induced at various doses in the three genotypes. In the previous research, similar results were reported by Sarma et al. (1991), Charumathi et al. (1992) and Gupta and Sharma (1996). From table 69 it is revealed that the application of gibberellic acid modulated the effects of irradiation and more genetic variability is induced. Gamma irradiation increased the number of seeds per pod, however, more seeds per pod were obtained with gibberellic acid.

The interaction effect between varieties and treatments revealed that gamma irradiation in Noor 91 and C141 stimulated the number of seeds per pod. Modulation of radio sensitivity with gibberellic acid for this character was observed and more number of seeds per pod were recorded at various doses. In Punjab 91 number of seeds per pod decreased significantly ($P<0.01$) with gamma irradiation at 40 and 60 Kr, while with gibberellic acid no appreciable change was observed numerically as compared to control.

iv: 100-SEED WEIGHT (g)

The variation in 100-seed weight was not affected by varieties ($p>0.05$). Whereas, irradiation doses and varieties-doses interaction had showed highly significant ($p<0.01$) effect on the variation in 100-seed weight (Table 79). This indicates that the mutants obtained in M3 generation have sufficient variability. Variability in 100-seed weight have also been reported by Sarma et al. (1991), Charumathi et al. (1992) and Gupta and Sharma (1996). It is obvious from the table 71 that the application of gibberellic acid changed the genetic spectrum and increased the

weight of 100-seeds at various doses.

The effect of interaction between varieties and doses revealed that in Noor 91 and Punjab 91 mutagenic treatments increased the 100-seed weight except at 60 Kr dose of Noor 91 where a decrease was observed as compared to control. In C141 the effect of irradiation on 100-seed weight was inconsistent at both mutagenic treatments. Post mutagenic application of GA_3 induce variability in 100-seed weight in the three genotypes.

vii: BIOLOGICAL YIELD PER PLANT (g)

The variability in biological yield was statistically non-significant due to genotypes ($p>0.05$) while, it was highly significant ($p>0.01$) due to treatments and for the interaction of genotypes and treatments (Table 72): This shows that a marked variability is induced at different doses in the three genotypes. The mean data regarding the biological yield effected by various doses of irradiation revealed that the biological yield decreased significantly ($p<0.01$) in two mutagenic treatments as compared to control. However, the difference in biological yield was non-significant among the different doses of irradiation (Table 73). Gupta and Sharma (1996) had reported different results for this character where biological yield increased with gamma irradiation in horse gram. The confliction in the results may be due to the dissimilar material and environmental conditions of the experiment.

The interaction between chickpea genotypes and treatments (Table 73) revealed that a divergent response was observed at various doses in the three genotypes. It is similar to the findings of Gupta and Sharma (1996). The biological yield decreased significantly ($p<0.01$) in the three genotypes except at 40 and 50 Kr in Noor 91 as compared to control. Application of GA_3 modulated the effects of gamma irradiation non-significantly at various treatments of irradiation.

viii: GRAIN YIELD PER PLANT (g)

Highly significantly variance components (Table 79) due to genotypes ($p<0.01$), treatments as well as their interaction revealed that a sufficient variability was induced at different doses in the three genotypes. It is similar to the findings of Sarma et al. (1991) and Charumathi et al. (1992). As regards the main effect of radiation doses (Table 75), the results revealed that grain yield increased significantly ($p<0.01$) at various irradiation doses except at 50 Kr dose of gamma irradiation, where the increase was non-significant ($p>0.01$) as compared to control. However, more yield grain was recorded with gibberellic acid at various doses. In the previous research, similar results by Khan

(1984), Khan et al. (1989), John (1995), Kumari (1995) and Gupta and Sharma (1996) were reported.

Highly significant interaction between varieties and gamma rays revealed that a marked variability is obtained at various doses in the three genotypes. Similar results were obtained by Gupta and Sharma (1996). The application of gibberellic acid modulated the effects of gamma rays and more grain yield was recorded in the three genotypes. In Noor 91 and C141 grain yield increased at various irradiation doses as compared to control. However, more grain yield was produced by gibberellic acid. In Punjab 91, grain yield decreased at various doses except at 50 Kr dose of gamma irradiation and at 40 Kr dose of gibberellic acid, however, the change in mean from control was non-significant ($p>0.01$).

ix: HARVEST INDEX (%)

The variability in harvest index was found to be highly significant ($p<0.01$) due to genotypes and treatments. However, it was non-significant ($p>0.05$) for the interaction between genotypes and treatment (Table 76). This indicates that a similar variability is induced at different doses in the three genotypes. The main effect of gamma irradiation (Table 77) revealed that percentage of harvest index increased significantly ($p<0.01$) at various levels of irradiation in the two mutagenic treatments as compared to control. However, more percentage of harvest index was obtained with the application of gibberellic acid.

The data regarding the interaction between genotypes and treatments revealed that harvest index percent increased significantly ($p<0.01$) at all the irradiation intensities in Noor 91 and C141 as compared to control. However, in Punjab 91 significance as compared to control was found at 50 Kr of gamma irradiation and at 40 Kr of gibberellic acid .

x: FLOWERING DAYS

Days to 50% flowering were highly significantly effected ($p<0.01$) by varieties, doses and their interaction (Table 78) indicating that a marked variation is induced at different doses in the three genotypes. The results are in line to the findings of Charumathi et al. (1992). It is obvious from the results (Table 79) that the application of gibberellic acid created extra genetic variability at different doses of gamma irradiation for this character. The flowering was found late at various levels of gamma irradiation except at 40 Kr. However, less days to 50% flowering was taken with the application of gibberellic acid.

The genotypes into doses interaction (Table 79) revealed that the varieties had responded independently to varying levels of mutagenic treatments for time to flowering. Modulation of radio sensitivity with gibberellic acid was observed in the three genotypes. In Noor 91 significant difference on days to 50% flowering was observed with GA_3 except at 50 Kr dose. In Punjab 91 the days to 50% flowering decreased at 40 Kr, while a significant increase ($p<0.01$) was observed at 50 and 60 Kr doses of gamma irradiation as compared to control. While with gibberellic acid the decrease or increase to 50% flowering was non-significant ($p>0.01$) as compared to control. In C141 gamma irradiation increased the days to 50% flowering regularly with an increase of irradiation dosages, while with gibberellic acid the change was inconsistent.

xi: MATURITY DAYS

The statistical differences in days to maturity were highly significant ($p<0.01$) for genotypes, treatments and the interaction of doses and genotypes (Table 80). This indicates that the mutants varied in their genetic spectrum at various doses in the three genotypes. Variability in days to maturity with gamma irradiation was also observed by Charumathi et al. (1992). From table 81 it is evident that maturity delayed with gamma irradiation. However, the application of gibberellic acid had changed this pattern and less number of days were taken to crop maturity at various levels of irradiation.

Highly significant interaction between varieties and treatments (Table 81) revealed that a sufficient variability is present for this character in mutants of the three genotypes. In Noor 91 maturity delayed significantly with gamma irradiation, while with gibberellic acid less number of days were taken to crop maturity. In Punjab 91 and C141 earliness in maturity was observed at all levels of irradiation with both mutagenic treatments.

VII: CHLOROPHYLL AND MORPHOLOGICAL MUTATION SPECTRUM IN M_2 GENERATION

1. CHLOROPHYLL MUTATIONS

I: MUTATION FREQUENCY

Several types of chlorophyll deficiencies occurred in mutagenically treated plant material. These mutants included *albina, xantha, chlorina* and *viridis*. The data on chlorophyll mutation frequency on M_2 population basis as well as family basis are presented in table 82. There was an increase in the frequency of chlorophyll mutations with an increase in the dose of gamma irradiation in the three genotypes. However, this trend was not observed with the post mutagenic application of GA_3. Dose dependent increase in the mutation frequency has been reported in mung bean (Yadav and Singh, 1988) and in one chickpea genotypes (Haq et al., 1994). Erratic effects with GA_3 may be due to its radio protective effects with respect to the kind of the damage. The frequency of occurrence of mutations, however, indicated that gamma irradiation alone were more effective in inducing chlorophyll mutations. Comparing the mutability of different varieties, it was observed that Noor 91 produced the maximum number of chlorophyll mutations with both mutagenic treatments, followed by Punjab 91 and C141. The varieties also varied to achieve the saturation effect. Genotypic control of mutation frequencies has been reported in chickpea (Haq et al., 1994).

II: SINGLE AND MULTIPLE MUTATIONS

It is evident from the results presented in table 84, that most of the progenies segregated for one chlorophyll mutation type in genotypes Noor 91 and Punjab 91, while in C141 segregation for the second mutant type was not recorded with both mutagenic treatments. Haq et al. (1994) has reported reduction in recovery of multiple mutations with an increase in gamma irradiation in chickpea.

III: SPECTRUM OF MUTATIONS

The chlorophyll deficiencies in the seedlings were classified into four groups viz; *albina, xantha, chlorina* and *viridis* (Table 86). Some of these deficiencies like *albina* and *xantha* are lethal, while some of the seedlings with partial deficiencies like *chlorina* and *viridis* continue to survive. No chlorophyll mutation type was associated with a specific genotype as reported in chickpea by Nerkar and Mote, (1978). Application of GA_3 modulated the chlorophyll deficiency spectrum irrespective of the varieties. On the overall basis of three genotypes, the highest frequency of mutations was that of *xantha* types, followed by *chlorina, alba* and *viridis* types. The results are in agreement with earlier reports in other studies, Kalia et al. (1981) in chickpea, Dixit and Dubey, (1986) and Singh et al. (1989) in lentil also reported the predominance of *xantha* among chlorophyll mutant types. The results are contrary to the findings of Athwal et al. (1970) and Haq et al. (1994) that *albina* constituted the largest single category of mutants in gamma rays treated M_2 generation of chickpea genotypes.

The frequency of chlorophyll mutations indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1. Differences were also observed in frequencies of chlorophyll mutations in different genotypes. Such differences in radiation response indicate that several physical and biological factors are involved in the mutational process and that makes it extremely difficult to predict the occurrence of mutations in different varieties of crop plants. Neverthless, it can be concluded that radiation doses used in the present studies were by and large, most appropriate for inducing mutations in chickpea.

2. MORPHOLOGICAL MUTATIONS

I: MUTATION FREQUENCY

It is seen from the results given in table 83, a dose dependent increase in morphological mutations with both mutagenic treatments i.e., gamma irradiation alone and with GA_3. More morphological mutants were obtained with the post mutagenic application of GA_3 except in Punjab 91 where more morphological mutations were obtained with gamma irradiation. The stimulation in mutation frequency with GA_3 may be due to the elimination of gross chromosomal changes, while creating point mutations, which were being expressed.

Results of a similar nature have been reported in lentil (Dixit and Dubey, 1986; Tripathi and Dubey, 1992 a) where the simultaneous application of gamma rays, NMU and EMS increased the frequency of segregating families.

From table 86, it could be concluded that the proportion of chlorophyll mutations was greater as compared to morphological mutations (709:645). Dixit and Dubey (1986) also reported similar results in lentil. The correlation studies (Table 85) between various chlorophyll mutants and the morphological mutants have also shown a negative relationship between them. These results are contrary to Haq et al. (1994), where a significant positive correlation was observed between chlorophyll and morphological mutants in chickpea.

II: SINGLE AND MULTIPLE MUTATIONS

Most of the families were segregated for one type in the three genotypes, although the genotypes varied in their response to the two mutagenic treatments (Table 84). Noor 91 segregated for two and three types with both mutagenic treatments. A relationship was observed between frequency of families segregating for three types. Punjab 91 segregated for three types with gamma irradiation while, two types with GA_3. In C141 three types were only observed with GA_3. These segregating families increased with an increase in gamma irradiation in Punjab 91 and C141 as reported by Nerker and Mote (1978). The present results are contrary to the findings of Haq et al. (1994) where no such relationship was recorded in three Kabuli chickpea genotypes.

III: SPECTRUM OF MUTATIONS

The mutagenic treatments induced mutations affecting plant height, growth habit, branching and stem structure, stem and foliage colour, leaf size, flowering and maturity, seed and pod size (Table 86). There were differences in mutation spectrum between the genotypes and also between the mutagens. The results suggested that induced mutability is governed by the genetic architecture of the material used. Various morphological mutations have also been reported in lentil (Dixit and Dubey, 1983; Dixit and Dubey, 1986, Tyagi and Gupta, 1991; Tripathi and Dubey, 1992 a), chickpea (Haq et al., 1994) bengal gram (Nerker and Mote, 1978)

and in *Vicia faba* (Filippetti et al., 1982). The vital mutations recovered in the present study can provide a valuable germplasm, which could be released as new mutant varieties. The desirable induced mutations can be used in recombination breeding. Some mutations, such as tall, increased branching, large pod and seed size, can be used directly in developing a variety. Other mutations like early maturing, long pod, and various leaf mutants, show desirable characters from a breeder's point of view, and thus increase the wealth of germplasm for breeding program. The various beneficial mutants recovered in the present study suggest that mutation breeding can reasonably be expected to play a pivotal role in the evolution of new types suited to the new needs and niches.

VIII: HERITABILITY AND GENETIC ADVANCE

Response to selection for quantitative traits is directly proportional to the function of its heritability, genetic advance and its genotypic variance. Heritability enable the plant breeder to recognize the genetic differences among traits and the genotypic variance indicates the potential for the improvement of a particular trait. Mutation breeding provide us means for enhancing the genetic variation and extra genetic variability may also be created by modulating the effects of that very mutagen.

I: M_1 GENERATION

The results depicted in table 87 displayed a wide range of genotypic (GCV) and phenotypic (PCV) coefficient of variability among the different characters in M_1 generation. The values of PCV were higher than the GCV, which indicates that these may be easily influenced by the environment. It is also evident from the results that application of GA_3 proved as a useful tool in modulating the radiosensitivity resulted in new variability in the three genotypes. Heritability in broadsense indicates the effectiveness with which the selection of genotypes can be based on phenotypic performance. However, it has been suggested that heritability and genetic coefficient of variation provide no indication for the amount of genetic progress that can be achieved through selection (Sivasubramanian and Menon, 1973). However, when the estimates of heritability are used in conjuction with genetic advance it indicates the feasibility of improvement in different traits. High heritability values were obtained for all the characters. It was ranged from 60.16% for primary branches in Noor 91 to

98.66% for 100-seed weight in C141 genotype with gamma irradiation. However, with the application of GA_3 minimum heritability of 25.74% for maturity days in C141 to highest 99.65% for plant height were recorded in C141. These results are in line with those of Majid et al., (1982), Malik et al. (1987 b), Ramana and Singh, (1987), Ilhamuddin et al. (1989), Miah and Bhadra, (1989) and Singh, (1988 b). While results of Malik et al. (1988 b) and Aslam et al. (1992) contradicted to these findings. Lower genetic advance values with gamma irradiation were obtained for plant height, 100-seed weight, flowering and maturity days in the three genotypes. Aslam et al. (1992) also reported low genetic advance for maturity in soybean. It is revealed from the results (Table 87) that considerable level of improvement can be achieved in traits like primary and secondary branches, biological yield, grain yield and harvest index in the three varieties. The high broad sense heritability with high genetic advance was observed for secondary branches, pods per plant, grain yield and harvest index in the three genotypes. This could be due to additive gene effects. However, seeds per pod with the combined treatments in M_1 gave negative estimates of genotypic variance and are usually regarded as estimates of zero. This means that all genetic variance exhibited in connection with seeds per pod in M_1 generation is dominance or environmental variance.

II: M_2 GENERATION

The estimation of coefficient of variability revealed that the magnitude of phenotypic coefficient of variability was greater than respective genotypic coefficient of variability for all the characters studied. Similar findings to these results where higher PCV than GCV have been reported by Kumari, (1996) and Dev and Gupta, (1997) in faba and kidney bean, respectively. Application of GA_3 modulated the induced genetic variation due to gamma irradiation and an appreciable amount of variability was recorded for secondary branches, pods per plant, grain yield and harvest index in the three genotypes (Table 88). High levels of PCV and GCV due to irradiation were recorded by Bhatnagar, (1991) in chickpea for branching per plant and pods per plant. Ignacimuthu and Babu (1992; 1993) also reported sufficient amount of variability for various yield characters. The results of other workers Charumathi et al. (1992), Baruah and Talukdar (1994), Ramani and Jadon (1991) Kamala (1990), Mehetre et al. (1994). Gupta and Sharma (1996) are in agreement with present observations. The results also revealed the negative values of GCV for seeds per pod, 100-seed weight, biological yield, grain yield and

harvest index in Noor 91 and of seeds per pod biological yield in Punjab 91 with gamma irradiation. However, negative GCV estimates were obtained for seeds per pod, grain yield and harvest index in C141 genotype. The results are reflected from the fact that environmental variance for these characters is high.

The estimation of broad sense heritability revealed that higher h^2 (99.72%) was calculated for grain yield in C141 followed by secondary branches (99.25%) in C141 and pods per plant (98.82%) in Punjab 91 with gamma irradiation. Ignacimuthu and Babu (1992; 1993) obtained high heritability values for these characters in mungbean. Baruah and Talukdar (1994) also observed high heritability for biological yield in green gram. High level of heritability was recorded by Kamala (1990) for seed yield in sesame. Kumari (1996) and Dev and Gupta (1997) reported similar findings in faba bean and kidney bean, respectively. In present studies high heritability was associated with high genetic advance for secondary branches in Noor 91, primary and secondary branches grain yield, biological yield and harvest index in Punjab 91 while, for primary and secondary branches in C141 with gamma irradiation. This indicates that additive gene effects are important for determining these characters. Additive gene effects have also been reported by Kamala (1990); Baruah and Talukdar (1994); Mehetre et al. (1994); Kumari (1996) in various crops.

It is evident from the results (Table 88) that for plant height, primary branches and days to maturity in Noor 91, pods per plant and days to flowering and maturity in Punjab 91 while, 100-seed weight, biological yield and days to flowering and maturity C141 with gamma irradiation high heritability was coupled with low genetic advance thereby, indicating the influence of non-additive gene action (dominanance or epistasis). Malik et al. (1987 b) has reported the association of high heritability with low genetic advance for plant height in mungbean.

It is evident from the results (Table 88) that the application of GA_3 changed the genetic spectrum in the three genotypes. Prominent effects on heritability were recorded for plant height, pods per plant, seeds per pod, 100-seed weight, biological yield grain yield and harvest index in Noor 91 while, 100-seed weight, biological yield and days to flowering in Punjab 91. Similar effects were also recorded for plant height, pods per plant, 100-seed weight, grain yield and harvest index in C141 genotype. This provides an extra variability for the improvement and selection of desired traits in chickpea cultivars.

III: M_3 GENERATION

The results revealed (Table 89) that with the advancement of generation different characters stabalize themselves and genetic spectrum was changed. Phenotypic coefficient of variability ranged from 0.37% for maturity days in Noor 91 to 13.24% for number of secondary branches in C141 with gamma irradiation. Similarly, GCV ranged from 0.002% for maturity days in Noor 91 to 8.23% for number of secondary branches in C141 with gamma irradiation. Higher PCV than GCV were obtained for all the characters indicating the influence of environment on these characters. Similar results were reported by Kamala (1990); Charumathi et al. (1992); Mehetre et al. (1994); Gupta and Sharma (1996); Dev and Gupta (1997) in different crops.

It is also evident from the results (Table 89) that application of GA_3 modulated the genetic variability induced through gamma irradiation for various characters in the three genotypes. This extra genetic variability may provide a scope for selection of better genotypes in chickpea.

A lot of variation in broad sense heritability was found with both mutagenic treatments in the three genotypes ranging from 2.39% for days to maturity in Noor 91 to 98.84% for 100-seed weight in Noor 91 with gamma irradiation. Among the estimated heritability, high h^2 was associated with high genetic advance for primary branches, pods per plant and seeds per pod with gamma irradiation and for number of primary and secondary branches and harvest index with GA_3 in Noor 91. Similarly, higher h^2 was related with greater genetic advance for number of primary branches and harvest index with gamma irradiation and for number of pods per plant grain yield and harvest index with GA_3 in Punjab 91. However, high h^2 was associated with higher genetic advance for number of secondary branches, 100-seed weight and harvest index with gamma irradiation and for number of primary branches and grain yield with GA_3 in C141. It could be due to additive gene action for these characters (Khan and Chowdhury, 1975). Similar results have also been reported in various crops, branches per plant and grain yield per plant in soybean (Mehetre et al., 1994); grain yield per plant in faba bean (Kumari, 1996); branches per plant and seed yield per plant in pigeon pea (Rao et al., 1988); in sesame (Kamala, 1990); biological yield in green gram (Baruah and Talukdar, 1994). High heritability coupled with moderate to low estimates of genetic advance for plant height, seeds per pod and

100-seed weight was probably due to non-additive gene (dominance and epistasis) effects (Singh et al, 1977).

The present study revealed that the selection based on biological yield, seed yield and harvest index could be exploited for the improvement of yield in chickpea cultivars and significant gain could be achieved through selection in early generations. On the basis of these studies it is suggested that GA_3 could be used quite reliably as a post mutagenic agent to modulate the radio sensitivity and to widen the genetic spectrum in chickpea.

IX: CORRELATION COEFFICIENT STUDIES

Induced mutation breeding offers an effective alternate to conventional breeding for creating genetically variable genotypes with better yield potential and other desirable traits. Although breeders strive to improve all these characters which have economic importance, increase in yield has been their main objective. The availability of genetic variability is essential for genetic improvement of crop plants. The identification of plants with suitable combination of characters from a population with genetic variability is dependent upon the knowledge of breeder on that population.

Grain yield being polygenic trait is greatly influenced by its component characters. Therefore, direct plant selection on the basis of yield is often misleading. Studies on the genetic variation and character association are of great importance for improvement of chickpea crop. The estimates of coefficient of correlation analysis is of prime importance for better understanding of the relationship between yield components. In present studies eleven characters like plant height, number of primary branches per plant, number of secondary branches per plant, number of pods per plant, seeds per pod, 100-seed weight, biological yield, grain yield, harvest index, days to 50% flowering and days to maturity were subjected to correlation analysis. The results thus achieved are discussed in the following paragraphs.

I: M_1 GENERATION

The results regarding genotypic and phenotypic correlation coefficients for M_1 population are given in tables 90-92 revealed that the genotypic correlations were higher than the phenotypic for most of the characters. The findings of Rani and Rao (1981), Singh et al. (1985), Sinha et al. (1986), Malik et al. (1987 a), Malik et al. (1988 a), Ghafoor et al. (1990), Wadud and Yaqoob (1988), Hussain et al. (1991), Bakhsh et al. (1991) are in agreement with present results. The higher magnitude of genotypic correlation that of phenotypic, indicated the masking effect of environment (Asawa et al. 1981).

In the case of M_1 generation in the three varieties, the correlation of yield was positive with all characters except days to flowering and maturity where association was negative. In Noor 91, the genotypic correlation was highly significant for pods per plant, seeds per pod and harvest index while a significant correlation was observed for biological yield. Majid et al. (1982) also recorded highest correlation value between seed yield and pods per plant. Malik et al. (1987 a) and Malik et al. (1988 a) observed that seed yield was positively and significantly correlated with pods per plant and biological yield. Positive and significant association of seed yield have been reported for pods per plant in chickpea (Wadud and Yaqoob, 1988; Yaqoob et al., 1990), pods per plant and seeds per pod in chickpea (Khan et al., 1983). The correlation between yield and other traits like plant height, primary branches, secondary branches and 100-seed weight was positive but non-significant. Ghafoor et al. (1990) also recorded positive correlation with these characters.

Genotypic correlation in the case of variety Punjab 91, was highly significant for pods per plant, seeds per pod and harvest index. Malik et al. (1987 a) also reported positive and significant association between yield and pods per plant. While, a significant association between yield and other characters like plant height, primary branches, secondary branches, 100-seed weight and biological yield was observed. Positive and significant correlation between seed yield and all these traits except biological yield has been observed by Khan et al. (1983) in chickpea, while in *Vigna radiata* (Malik et al., 1987 b). However, in variety C141, highly significant correlation was observed for seed yield with pods per plant and harvest index

while a significant association was recorded for primary branches. Whereas, the remaining characters like plant height, secondary branches, seeds per pod, 100-seed weight and biological yield were positively and non-significantly associated with grain yield.

The positive association between yield and these parameter in the three varieties revealed that any increase in the height, number of primary branches, number of secondary branches, number of pods per plant, seeds per pod, 100-seed weight, biological yield and increase in harvest index, would have direct and proportionate impact on the grain yield. These strong relationships between yield and other parameters would enhance the grain yield. The present results are in line with those of Singh et al. (1985), Ali (1985), Sinha et al. (1986), Malik et al. (1987 b), Singh (1988 b), Malik et al. (1988 a), Ghafoor et al. (1990) and Bakhsh et al. (1991). They also recorded a strong positive association of these characters with grain yield in various crops. Malik et al. (1988 b) also reported a positive and significant relationship in mutant population of mungbean for grain yield with number of pods and seeds per pod. The reports of Wadud and Yaqoob (1988) and Yaqoob et al. (1990) for days to maturity were similar to the present investigation, where they obtained a negative relationship between grain yield and days to maturity in chickpea. Baruah and Talukdar (1994) observed in green gram that biological yield was positively correlated with seed yield which is in line with the present findings, while contrary to our results for harvest index a negative relationship with seed yield was reported. Hussain et al. (1991) also reported a negative association between grain yield and harvest index in chickpea. Wadud and Yaqoob (1988) reported a negative and non-significant correlation between grain yield and plant height in chickpea. This deviation may be due to differences in genotypes used and different ecological conditions.

II: M_2 GENERATION

Some modifications in association of various characters (Table 93-95) in M_2 population of all the varieties with seed yield were observed as compared to their respective association in M_1 generation. In the case of variety Noor 91, grain yield was highly positively and significantly associated with pods per plant and harvest index similar to M_1 generation. Positive association

of pods per plant with grain yield has been reported by Mehetre et al. (1994), while contrary to our results a negative relationship of harvest index was also reported. However, a negative relationship was established for plant height and biological yield with seed yield in contrast to positive association in M_1 generation. Number of primary and secondary branches, seeds per pod and 100-seed weight had positive association with seed yield. Positive relationship for these characters with grain yield was also established by Mehetre et al. (1994) in soybean.

Grain yield in variety Punjab 91 was negatively correlated with secondary branches and 100-seed weight in M_2 population whereas in M_1 generation this relationship was positive. Harvest index was positively and highly significantly correlated with grain yield in M_1 population while in M_2 population this association was negative and highly significant. Baruah and Talukdar (1994) reported strong negative association between harvest index and grain yield in green gram, which is similar to our findings. Relationship between days to maturity and seed yield was modified from negative to positive direction in M_1 and M_2 population, respectively. Plant height, number of primary branches, pods per plant, seeds per plant and biological yield were positively correlated with seed yield.

In the case of variety C141, the relationship between seed yield and plant height, primary branches, secondary branches and biological yield was changed from positive to negative direction in M_1 and M_2 population, respectively. While a reverse case also established with days to maturity in the respective generations. The seed yield was positively and significantly correlated with pods per plant. Seeds per pod and 100-seed weight were also positively correlated with seed yield. Similar positive association of grain yield with pods per plant and seeds per pod has also been reported by Malik et al. (1988 b) in mutants of mungbean. Wadud and Yaqoob (1988) also reported a positive and significant relationship between pods per plant and seed yield in chickpea.

III: M_3 GENERATION

Grain yield relationship in M_3 population of variety Noor 91 with other characters was changed as in the case of M_2 generation. A negative relationship for seed yield was obtained with plant height, primary branches, secondary branches and biological yield in M_3 generation, while the association with these characters was positive in M_1 generation. Days to 50% flowering and days to maturity were positively associated with grain yield in M_3 generation, however, these were negatively associated with seed yield in M_1 generation. Pods per plant were positively and significantly associated with grain yield in M_3 generation. However, a strong but non-significant association was obtained between seed yield and harvest index. Seeds per pod and 100-seed weight were positively correlated with seed yield (Table 90, 93 and 96).

In case of Punjab 91 variety, the association of seed yield with 100-seed weight and harvest index was changed from positive to negative, while the association for days to 50% flowering and days to maturity was changed from negative to positive direction in M_1 and M_3 generations, respectively. Plant height, number of primary and secondary branches, pods per plant and seeds per pod were positively correlated with grain yield. The seed yield was highly significantly and positively associated with biological yield (Table 97).

The correlation of yield in variety C141 with plant height, secondary branches and biological yield was changed from positive to negative direction while days to 50% flowering was changed from negative to positive direction in M_1 and M_3 population, respectively. The seed yield was positively correlated with number of primary branches, pods per plant and 100-seed weight. Seeds per pod was positively and significantly associated with grain yield (Table 92, 98).

In above discussion it has been observed that the association of different characters with seed yield was modified in a different way in the three varieties in different generation. This change

in association may be attributed due to the differences in mutant genotypes, segregation pattern and different ecological conditions. As a result in most of the cases with the advance of the generations, the linkage of the grain yield with most of the characters became loose. For instance, in the case of M_1 generation of variety Noor 91, eight out of ten characters were strongly associated with grain yield, while in M_2 generation only six characters had shown positive correlation with yield. Similarly, in M_3 generation six characters were found positively correlated with grain yield. It was also observed that the M_2 mutants did not follow M_1 generation regarding genotypic correlation pattern. The genotypic correlation pattern in M_2 generation was almost similar to M_3 generation in most of the cases. It was further observed that the characters showing positive correlation with grain yield in M_1 generation did not necessarily had positive association with grain yield in advance generations. Similarly, the characters showing negative relationship in M_1 mutants may change then correlation pattern in M_2 and M_3 generation stages. These results are in line with the findings of Awan (1995) who reported similar correlation patterns of grain yield with other characters in the irradiated populations of different generations of mungbean.

In M_1 population, plant height was positively correlated with all the characters except 100-seed weight in Noor-91, days to 50% flowering and days to maturity in Punjab 91 and also harvest index in addition to days to 50% flowering and days to maturity in C141 variety, where it was negative. These results are in close agreement with Gull (1995) and Malik et al. (1987 b), who found similar relationship of plant height with other characters. Number of primary branches were positively correlated with other characters except seeds/pod and 100-seed weight in Noor 91, while days to 50% flowering and days to maturity in Punjab 91 and C141 variety it was negative. Number of secondary branches were positively correlated with other characters except days to 50% flowering and days to maturity in the three varieties. The present findings are similar to Ali, (1985) and Bakhsh et al. (1991). Pods per plant had highly significant correlation with biological yield, grain yield and harvest index, which could be considered important yield contributing characters. The positive association of pods per plant with other yield components had been reported by Malhotra et al. (1974) and Khalid et al. (1984) except with 100-seed weight, where it was negative.

There was a strong and positive association between biological yield and plant height, primary branches, secondary branches, pods per plant and grain yield, 100-seed weight expressed either positive or negative but low correlation with pods per plant. Hussain et al. (1991) also reported non-significant but negative correlation between 100-seed weight and pods per plant.

It has also been observed that with the advance in generation, the association among various characters become loose. For example in variety Noor 91, plant height had positive correlation with nine out of ten characters, while in M_2 generation positive relationship plant height was observed for only five characters. Similarly in M_3 generation six characters were positively correlated with plant height. It was also observed that the characters showing either positive or negative correlation did not necessarily had the same trend in advance generations. These results are in close agreement with those reported by Awan (1995) in irradiated population of mungbean.

In the above discussion it has been observed that grain yield had strong positive correlation with pods per plant, seeds per pod, 100-seed weight and harvest index, so yield can be increased by increasing pods per plant, seeds per pod, 100-seed weight and harvest index. It may be concluded that yield in chickpea can be increased by improving the above positive responsive parameters.

X: PATH COEFFICIENT STUDIES

Path coefficient analysis is simply a standardized partial regression coefficient and as such measures the direct and indirect effect for one variable upon an other and permits the separation of the correlation coefficient into components of direct and indirect effect (Dewey and Lu, 1959). Path coefficient studies provide an opportunity to study the magnitude and direction of association of yield with its direct and indirect components and also among various components. To accumulate optimum combination of yield contributing characters in a single genotype, it is essential to know the implications of the inter relationship of various characters.

Using path coefficient analysis, it is easy to determine which yield component is influencing the yield substantially. Having this information, selection can then be based on that criterion thus making possible great progress through selection in limited time. Dewey and Lu (1959) demonstrated the validity of path analysis in effective plant selection that results in selection of desirable genotypes.

I: M_1 GENERATION

A critical examination of the data (Table 99) showed that in M_1 generation of variety Noor 91, harvest index had maximum direct contribution to grain yield. A glance over the path coefficient computed for various characters, would indicate that the pods per plant, seeds per pod, 100-seed weight, biological yield, harvest index and days to maturity had direct positive effect on grain yield per plant. Malik et al. (1987 b) and Ghafoor et al. (1990) have also found a positive direct effect of harvest index and biological yield on grain yield. However, the remaining characters plant height, primary branches, secondary branches and days to 50% flowering had negative direct effect on grain yield per plant. Malik et al. (1987 b) also reported negative direct effect of days to flowering on grain yield.

In case of variety Punjab 91 in M_1 generation, harvest index had maximum direct contribution to grain yield followed by biological yield, however, the rest of all the characters had negative direct effect on grain yield (Table 100). Highly positive direct estimate for biological yield and harvest index were reported by Malik et al. (1987 b) and Ghafoor et al. (1990) in mungbean and mashbean, respectively.

The contribution of different characters to grain yield in variety C141 of M_1 generation, revealed that 100-seed weight had maximum direct positive effect on grain yield per plant followed by days to 50% flowering (Table 101), while biological yield had strong negative direct effect on grain yield per plant. Harvest index and seeds per pod also had negative direct contribution to grain yield.

II: M_2 GENERATION

In M_2 generation of variety Noor 91, path analysis showed that harvest index and biological yield had direct positive effect on grain yield and harvest index had the maximum contribution to grain yield per plant. Primary branches and secondary branches had negative direct effect on grain yield in M_2 generation, while all other characters had negative direct effect on grain yield per plant (Table 102).

In case of variety Punjab 91, large variations in path analysis were observed in M_2 generation (Table 103). In contrary to M_1 generation, strong negative direct effect of harvest index and biological yield on grain yield was observed in M_2 generation. Plant height, and flowering days also had negative direct effect on grain yield. Pods per plant, seeds per pod and 100-seed weight had strong direct effects, however, these had positively contributed to the grain yield per plant.

In the case of variety C141 (Table 104) harvest index had maximum direct positive effect on grain yield per plant followed by pods per plant and biological yield. Plant height, flowering and maturity days had negatively contributed to grain yield. Rest of the characters had positive but low direct contribution to seed yield.

III: M_3 GENERATION

In case of variety Noor 91 harvest index had maximum direct positive contribution to grain yield and its genotypic correlation to seed yield was also maximum and positive (Table 105). Direct effect of biological yield was positive but had negative genotypic correlation with grain yield. Rest of all the characters had negatively contributed to grain yield.

In M_3 generation of variety Punjab 91 pods per plant had the maximum direct contribution to grain yield followed by 100-seed weight, seeds per pod, plant height, maturity days, secondary branches, primary branches and harvest index. Biological yield and flowering days had negatively contributed to grain yield (Table 106).

Maximum direct contribution to grain yield in M_3 generation of C141 was exhibited by seeds per pod followed by pods per plant, 100-seed weight, primary branches, secondary branches and plant height (Table 107). However, the other characters had negatively contributed to grain yield.

In case of path coefficient analysis, it was observed that the mutants in the three genotypes showed different pattern regarding the direct and indirect effect of various traits on the grain yield. The variability in the path response from generation to generation might be due to the differential response of the genotypes towards the two types of treatments i.e. gamma irradiation alone and with gibberellic acid, which might be lead to a different genotypic spectrum in the three genotypes.

In improving the yield potential of chickpea varieties under the present investigation, harvest index and biological yield would be used a reliable criterion for selection in Noor 91, while pods per plant and 100-seed weight would be used in variety Punjab 91 and C141, as these showed highest direct effect on grain yield. These informations would ultimately lead to the determination of suitable ideotype in chickpea.

SUMMARY

The investigation on the modulation of radio sensitivity with gibberellic acid for cytogenetical, biochemical and genetic spectrum were carried out on three chickpea (*Cicer arietinum* L.) genotypes having different seed coat colours, namely Noor 91 (white), Punjab 91 (brown) and C141 (black). Dry seeds were exposed to gamma irradiation at 10, 20, 30, 40, 50, 60, 70, 90 and 110 Kr dosages. A part of the seeds were treated with 0.5mM solution of gibberellic acid for 16 hours prior to various studies.

For cytogenetical studies seeds were sown in petri plates having moistened whatmann filter paper. Mitotic index and chromosomal abnormalities were studied in root tip cells at the dose range of 10 to 110 Kr including control as check with acetocarmine stain, while the nucleolus size and structure were examined at 10, 60 and 110 Kr dosages along with the control after a standard method of fixation and staining. The results revealed wide variation in mean values for mitotic indices and chromosomal anomalies in three genotypes. Significant differences were recorded due to genotypes, treatments and their interaction. Mitotic indices decreased, while chromosomal aberrations increased with an increase of gamma irradiation. However, chromosomal anomalies were not recorded upto 30 Kr in the three genotypes. Post mutagenic application of gibberellic acid increased the number of cells entering the mitotic cycle, while the chromosomal aberrations decreased and maximum response was noticed at higher doses. Chromosomal aberrations included fragments, bridges and laggards at anaphase stage of the cell cycle. Frequency of cells having enlarged nucleolus volume increased progressively with an increase in gamma irradiation. However, the treatment of GA_3 reverted this situation towards the normal values.

For biochemical studies, treated seeds along with their respective checks were sown in plastic cups with four replications having ten seeds in each replication. Biochemical parameters included quantitative estimation of peroxidase, catalase and IAA oxidase enzyme activities, soluble proteins, ribonucleic acid (RNA) deoxyribonucleic acid (DNA) contents and fresh weight of the shoots. These studies were carried out from 3rd to 8th developmental days after sowing. From the results it is revealed that fresh weight, protein, RNA and DNA contents decreased with an increase in gamma irradiation except at lower doses of 10 or 20

Kr where a stimulation as compared to control was observed. Application of GA_3 changed the effects of gamma irradiation showing an increase in fresh weight, protein, RNA and DNA contents. Catalase activity was stimulated on 4th and 5th day at higher doses of gamma irradiation and then a gradual decrease was recorded on the following days of the developmental period. Post mutgenic application of GA_3 increased its activity throughout the developmental period. Peroxidase activity increased with an increase of gamma irradiation during the early developmental days and then a decrease was noted on the following days. This activity was further increased with application of GA_3. Stimulation in IAA oxidase activity at lower doses was noted upto 30 Kr of gamma irradiation and it was enhanced with the application of GA_3. However, at higher doses it was decreased progressively with the application of GA_3.

For seedlings studies, ten seeds in each replication were sown in plastic cups having sand with four replications in a randomized complete block design. Data on germination percentage, shoot length, root length and number of roots per plant was recorded for various treatments ranging from 10 to 110 Kr including the respective checks. The mean values for germination percentage, shoot length, root length and number of roots were highly significantly effected due to genotypes, treatments and their interaction. However, this interaction was non-significant for germination percentage. These parameters decreased with an inrease in irradiation dosages and drastic effects were observed at the maximum dose in the three varieties. However, the application of GA_3 restored the growth and their mean values increased. Gamma irradiation decreased the shoot and root length from 20-40%. Seeds exposed at these doses were selected for field studies.

To evaluate the genetic variability created by mutagens i.e., gamma radiation separately and with GA_3, field trials were conducted at the Barani Agriculture Research Institute (BARI), Chakwal under the natural conditions. The irradiated seeds of three genotypes along with their respective controls were sown in randomized complete block design with split plot arrangement having three replications to raise the M_1 generation. The genotypes were assigned to the main plots, while the irradiation doses including control were kept in sub plots. Twenty plants from each treatment were marked for recording the data on plant

height, number of primary branches and secondary branches, number of pods, seeds per pod, 100-seed weight, biological yield, grain yield, harvest index, days to flowering and days to maturity. Seeds of 250 plants from each treatment were harvested separately while, from the controls seeds were bulked. The seeds obtained from M_1 mutants including control were sown for M_2 generation in the succeeding year. The data of chlorophyll deficiencies were recorded at the seedling stage while agronomic and yield characters were recorded as M_1 generation but on 40 plants. M_3 generation was raised following the previous methodology of crop sowing and management.

The data recorded on all the generations were statistically analyzed using appropriate procedure. Results revealed a wide range of variability induced in all the characters in different generations. Results in M_1 generation showed that main genotypic effects were highly significant for all the characters except number of primary branches. The effect of gamma ray frequencies was highly significant for all the characters under study. The response of different characters in both mutagenic treatments also varied. Post mutagenic application of GA_3 either increased or decreased the means of different characters at various levels of irradiation. Both mutagenic treatments decreased the mean of various characters except days to flowering and maturity were delayed. The interaction effect was highly significant for all the characters. Post mutagenic treatment of GA_3 either increased or decreased the various characters in the three varieties. Very high magnitude of genetic variability for all the characters was recorded in M_2 and M_3 generation. Grain yield and harvest index increased in M_2 and M_3 generation, while seeds per pod and 100 seed weight increased with GA_3 in M_2 and M_3 generation. Plant height secondary branches and biological yield decreased with both mutagenic treatments as compared to control.

Albina, xantha, chlorina and *viridis* chlorophyll deficiencies were recorded in the M_2 generation of three genotypes at seedling stage. Gamma irradiation increased the chlorophyll frequencies while, the GA_3 treatment changed the frequency of the chlorophyll mutants in the three genotypes. Most of the families were segregated for single chlorophyll deficiency. Various morphological mutations including stem, leaf, pod, seed, flowering and maturity were also reported. The frequency of these mutants increased with GA_3.

Association analysis revealed higher magnitude of genotypic correlation than that of phenotypic for most of the characters. A strong positive correlation was observed for grain yield with pods per plant, 100-seed weight, harvest index and biological yield in M_1 generation of three chickpea genotypes. However, the association of various characters was changed with the advancement in generation. Association of plant height and biological yield with grain yield was positive in M_1 generation, while it became negative in M_2 generation of Noor 91. Grain yield in Punjab 91 was negatively correlated with secondary branches and 100 seed weight in M_2 population whereas in M_1 generation this relationship was positive. In genotype C141 the relationship between seed yield and plant height number of primary and secondary branches and biological yield was changed from positive to negative direction in M_1 and M_2 population, respectively. Just like the change in association of seed yield with other characters in M_2 population, grain yield in M_3 generation of variety Noor 91 with plant height, number of primary and secondary branches and biological yield was negative, while it was positive in M_1 generation. In case of variety Punjab 91, the association of seed yield with 100 seed weight and harvest index was changed from positive to negative direction in M_1 and M_3 generation, respectively. The correlation of yield in variety C141 with plant height, secondary branches and biological yield was changed from positive to negative direction in M_1 and M_3 generation, respectively.

Path analysis reflected that in M_1 population of Noor 91 and Punjab 91 had direct contribution to grain yield. However, in C141 genotype, 100-seed weight had maximum direct contribution to grain yield. In M_2 and M_3 population of variety Noor 91, harvest index and biological yield had direct positive effect on grain yield, while in variety Punjab 91, these had negative direct effect on grain yield in M_2 generation. However, in M_3 generation a low but direct positive effect of harvest index on grain yield was observed. Pods per plant and 100 seed weight had strong positive direct effect in M_2 and M_3 generation of Punjab 91. In case of variety C141, harvest index and biological yield had negative direct effect in M_1 and M_3 generation, while in M_2 generation these had a direct positive effect on grain yield. Pods per plant and 100- seed weight had a moderate to strong positive direct effect on grain yield in the three generations. So harvest index and biological yield would be used as a reliable criterion in genotype Noor 91, while pods per plant and 100-seed weight in variety Punjab 91 and C141.

CONCLUSIONS

The conclusions drawn from the present studies with the present set of genotypes and treatments are presented here.

- Post mutagenic application of gibberellic acid proves as a useful tool for modulating the effects of gamma irradiation. Mitotic indices increase while chromosomal aberrations decrease quite comprehensively as a modulating effect of gibberellic acid at the genetical level.
- Change in activity of various peroxidases with gibberellic acid, is an indication to reduce the irradiation damage at the biochemical level.
- Growth of seedlings increased as a protective measure of gibberellic acid, which increases the genetic recombination and the survival of mutants.
- Gamma irradiation decrease the shoot and root length values about 20-40% at 40-60 Kr, these doses are considered as appropriate for induction of genetic variability.
- Genotype-treatment interaction is highly significant ($p<0.01$) for all characters indicating that an appreciable genetic variability is induced at different doses in the three genotypes.
- Application of gibberellic acid widens the morphological spectrum, which could be helpful for future breeding programs in chickpea.
- In case of correlation analysis it was observed that with advancement of the generations, the genotypic linkage of the grain yield with most of the characters become loose. It is, therefore, suggested that the selection in M_1 generation could be misleading.
- Selection should be made in the advanced generations when the mutants fully stabilize for yield contributing characters.

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