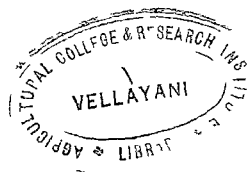


**STUDIES ON
THE BIOLOGICAL EFFECTS OF
X-RAYS ON CHILLIES (*Capsicum annum* L.)**



By

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THESIS

Submitted in partial fulfilment of the requirements for the award of the Degree of Master of Science in Agriculture (Agricultural Botany — Cytogenetics & Plant Breeding) of the University of Kerala

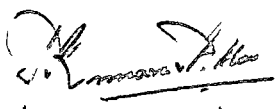
**DIVISION OF AGRICULTURAL BOTANY
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VELLAYANI, TRIVANDRUM**

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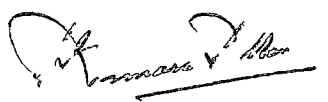
This is to certify that the thesis herewith submitted contains the results of bonafide work carried out by Shri. M. Kamaluddin Sahib, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.



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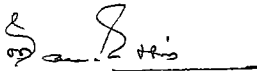
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INTRODUCTION

I N T R O D U C T I O N

The sustenance of human race on earth is absolutely dependent on agriculture. His development through the ages was closely related to the development of better types of cultivated plants, both for his food and for his several other basic needs. But it was only with the beginning of this century and with the growth of genetics and its allied field of plant breeding that these efforts were systematised and oriented as a science.

The myriads of genetic variation already existing and being multiplied by itself in nature has rendered a vast field for the plant breeders for the search and selection of better cultivable types. But natural variations were not always to the desired extent and patterns in selected materials as to enable efficient handling of their germ plasm.

It was in this context that the discovery of the mutagenic agents like radiations and certain chemicals was important for the scientists, as a tool for inducing prolific genetic variations.

Stadler in his pioneering work with X-rays on maize and barley had established the possibility of inducing very high variations in plants. Since then numerous workers all over the world

have studied effects of X-rays in the induction of mutations.

Eventhough the end result of X-irradiation in plants is the production of mutations, its biological effects on the living cell have also been assessed by the high frequency of chromosomal aberrations, depression of mitotic activity, depression of the synthesis of DNA and cell death and a variety of morphological changes. At least a few of these and other secondary effects were skilfully harnessed and utilised in practical agriculture, as well as in the study of the fundamental behaviour of the various units of the genetic material.

The present work was undertaken to investigate some of the biological effects of X-rays on the chillies plant (Capsicum annum L.). The seeds of a well established commercial variety, the K-1 variety evolved in Madras State, was subjected to X-irradiation with four dosages of 1000, 4000, 8000 and 15000 r units. The effects of X-rays in the M_1 and M_2 generations were studied in detail, the methods and results of which are presented and discussed in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The inherent ability of plants to produce mutations has been grasped by plant breeders and considerable effort was directed towards utilizing this potentiality in practical breeding in the last three decades.

The mutagenic activity of X-rays was first successfully exploited in the Nineteen twenties. Pioneers in this field were Muller and Stadler, who made extensive studies on the effect of X-rays on genes and on their mode of action, employing *Drosophila* and microorganisms. Since then many scientists, led by Nilsson-Ehle and Gustafsson in Sweden and Bauer in Germany have experimented X-irradiation in plants. The earlier studies were rather oriented to the exploration of morphological and physiological changes caused by X-rays. In later years, however, these studies were directed to the investigation of genetic effects. In the Asian countries like Japan and India work on this field had to wait till after the second world war.

Extensive studies, however, proved the limitation of X-rays as a useful means for effecting economic mutations. The far more deleterious effects caused by irradiation, as well as, the very great variability induced leading to difficulty in

accommodating them in limited experiments are but two important obstacles worthy of mentioning.

A short review on the various aspects of employing X-rays in plant breeding is given here under, although no work in chillies have come across during reference.

EFFECTS OF X-RAYS ON PLANTS:

I GERMINATION OF SEEDS AND SURVIVAL OF SEEDLINGS:

There are numerous reports on the effects of acute and chronic irradiation on many plants. In 1898, just three years after the discovery of X-rays by Roentgen, dormant seeds of Convolvulus and Lepidium were treated with X-rays by Maldiney and Thouvenin, and found that the treatment hastened germination of seeds.

Pfeiffer and Simmermacher (1915) reported that short exposure to X-rays increased germination in Vicia faba, but reduced by long exposures. But Ancel (1924) reported that irradiation has not hastened germination of hundreds of seeds she studied.

Kumar and Joshi (1939) reported the effects of X-irradiation on seeds of Brassica juncea, Nicotiana tabacum, and Pennisetum typhoides. They found that X-rays had adverse effects

on germination in all the cases.

Gustafsson and his associates reported the effects of ionizing radiations in various crop plants. Gustafsson (1941) found that different plants react differently to X-rays. Cruciferous plants were especially insensitive, in which case they tolerated upto 100 Kr. units and even more without significant reduction in germination percentage and survival. Peas (Pisum sativum) were, however, quite sensitive, their optimum doses being 10 Kr. to 20 Kr. The same is true with sunflower and safflower. He also found that storage of irradiated seed enhanced the effects.

Krishnaswamy and Rangaswamy Ayyangar (1941) reported that in Pennisetum typhoides no reduction in germination percentage was caused by X-rays, but survival was reported to be low in the treated cases.

Froiler and Gustafsson (1944) reported that larger the X-rayed seeds, the more will they endure irradiation and that their germinability and sprouting ability were better than the smaller seeds.

Higher germination percentages were reported by Jacob (1949) in irradiated seeds of Jute (Corchorus spp). He also

reported higher survival percentage in some varieties.

Datta and Datta (1953) observed that germination percentage was not inhibited by X-rays.

But Caldecott (1954, 1955, 1956, 1961) reported reduction in germination and seedling survival after X-ray and also neutron irradiation. Wohrmann (1955) found in Alonecurus pratensis that germination was very adversely affected by dosages between 18 and 26 Kr. units-the effects varying in linear proportion to the dose of X-ray used.

Increased germination percentage and survival with low doses of X-rays was reported by Ehrenberg (1955). He found that the irradiation appeared to promote earlier germination and that the seedlings displayed no definite lethal effects.

Spencer and Cabanillas (1955) found no inhibition of germination by X-rays in the Trailing indigo, Indofogera endecaphylla. It was characteristic in this experiment that some irradiation effects in X_1 were exhibited in X_2 generation as well. For the reduction of germination, the lowering trend of catalase activity in the irradiated plants has been pointed out as a probable cause.

Giles (1956) found that doses below 2 Kr. units had no effect on germination percentage in seeds of Solanum acule and

above this germination reduced proportionate to the higher doses. Lesley and Lesley (1956) reported reduced germination in irradiated tomato.

Kankis and Webster (1957) found that Sorghum spp. are very sensitive to X-rays as regard to inhibition of germination.

Beard et al (1958) found that dosages of 32 Kr. were optimal for barley and maize and 160 Kr. for mustard as regards germination factor, and beyond this the higher dosages were lethal for survival and growth.

Micke (1958) treated seeds of Melilotus albus with doses ranging from 10 Kr. to 100 Kr. and found that the germination capacity was unaffected but lethality occurred after about two weeks. Gottschalk and Scheibe (1960) working on leguminous plants reported that germination of seeds was independent of X-ray doses. Similar reports came from Kundu et al (1961) in Chorchorus spp., Shastry & Ramiah (1961) in Oryza; Jain et al (1961) in Chrysanthemum; Sjodin (1962) in Vicia faba and Katayama (1963) in Oryza sativa.

Swaminathan (1963) working on polyploid wheat also came to the conclusion that germinability of seeds was unaffected by low and moderate doses of X-rays.

Babayan et al (1964) suggested positive correlation between age of seeds and sensitivity to irradiation, as they found that fully matured seeds were more tolerant than seeds in which embryonic development was not complete.

P.N.R. Nair (1964) reported in cow peas irradiated with X-rays at doses between 1 Kr. to 15 Kr. units, that, the germination percentage was unaffected by the X-rays in the dry seeds, but in the soaked seeds there was significant reduction of germination.

Kamara and Simak (1966) working with horticultural plants suggested that while the lower doses caused chromosomal aberrations, even higher doses did not bring about any undesirable influence on the germination and growth of seedlings.

Gopalakrishna Raju (1967) in rice reported that germination percentage has been reduced slightly by all dosages excepting the very low doses. He found that doses upto 60 Kr. of X-irradiation was not lethal in the rice variety he tried. But Veluswamy (1966) found in the same CO.13 variety of rice that even 50 Kr. gamma rays were lethal to the plants.

II PLANT GROWTH:

One of the common after effects of irradiation on plants is the inhibition of growth.

As early as in 1904 Kornicke reported that in Brassica and Vicia X-ray treatment of seeds caused growth checking followed by an acceleration of growth which was transitory when the doses were not too high. The same author (1915) reported that X-rays caused growth retardation with stronger dosages, but growth stimulation with weaker doses.

Miege and Coupe (1914) conducted irradiation experiments in Raphanus and Lepidium and reported a stimulating effect which manifested itself in increased weight of leaves as well as an increase in the total weight. Schwartz (1914) also found accelerated growth in Vicia faba with weak doses of X-rays.

Revera (1920) considered increased development of aerial buds following irradiation as a stimulation. Jangling (1920) observed that with light doses root growth was unaffected while shoot growth was increased. However, on the basis of experimental evidences obtained on twelve different species of plants, Schwartz et al (1923) denied the stimulating action of X-rays.

Transitory acceleration of development has been observed by Altman et al (1923) in Phaseolus vulgaris irradiated with X-rays. According to them the stimulating dose varied with the stage of development. Ancel (1926) based on her extensive experiment concluded that the so called stimulating effect of X-rays on development

of plants did not exist.

Geller (1924) after examining the results of his experiments conducted for thirteen years stated that lower doses of X-rays caused acceleration of development while higher doses produced a depressing action. There was no optimum dose for retarding or stimulating plant growth since the effects were dependent on several factors.

Arntzen and Krebs (1925) working on peas found that light doses of X-rays caused stimulating effects when exposure was for 24 hours and in certain cases for 48 hours but beyond these there was retarded growth.

Capizzaro (1926) reported retardation of development in proportion to the dose, time of exposure and quality of rays used.

But Koltsov and Koltsov (1925) could not observe any stable and stimulating effect in treating dry and sprouting seeds of wheat, and peas with various doses of X-rays.

Goodspeed and Olson (1928) pointed out that a definite acceleration of growth and development may result by proper doses of X-irradiation.

In Pisum sativum Maisin and Masy (1928) reported significant growth retardation. With soft X-rays the same result was observed in bean seedlings by Glocker et al (1929).

Patten and Wigoder (1929) subjected the seed of beans, mustard and barley to radiation, but could not get any marked effects.

Cattle (1931) was among the first to report the high sensitivity of wheat seedlings to X-rays.

Johnson (1931) described excessive branching of stem of irradiated Helianthus and Lycopersicon. In a later work (1936) he found more tillering in wheat with doses of 1 to 5 Kr. units of X-rays.

Shull and Mitchell (1933) observed that stimulating effects could be obtained by employing appropriate conditions. Accelerated development could be obtained in seedlings of wheat, oats and sunflower when 30-120 r units of filtered X-rays was used by them.

Haskins and Moore (1935) supported the above view on working in citrus plants. Bless (1938) found no change in growth of corn and radish treated with X-rays. But lettuce showed an increase in height.

Wort (1941) reported increased growth of coleoptile in wheat when seeds were subjected to low dosages of 114 r units.

Kersten et al (1943) found that appropriate dosages did cause a stimulation in plant growth.

The inhibition of plant growth was explained to be due to chromosomal changes and this correlation was substantiated by many workers such as Lee (1947) and Gray and Scholes (1951).

Jacob (1949) reported gigantic plants in X-rayed jute plants. Gordon & Weber (1950) have indicated a low level of oxygen content in irradiated plants which pointed towards the physiological disturbances as the chief factor affecting plant growth. Effects like dwarfing of the stem often caused by irradiation can be explained this way (Sarda, 1959). Quastler and Baer (1950) have shown that growth was not affected in irradiated Phaseolus aureus.

Sparrow and Christensen (1950) using acute X-irradiation in tulip and potato observed that inhibition of plant growth was not proportional to the dose given. They observed a reduction in yield of potato in materials treated with 1200 r and above.

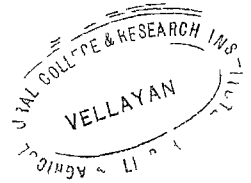
Datta and Datta (1953) recorded growth inhibition in rye at doses between 2 to 5 Kr. units of X-rays, but at higher levels of 10 to 12 Kr. units the severity was less and the seedlings were normal.

Ganckel et al (1953) reported in Tradescantia that reduction in growth, injury and death all occurred in certain cases

of X-irradiation. But Riley (1954) observed no growth retardation in X-irradiated Avena seedlings.

Bruns (1954) employed doses at 1 to 20 Kr. in Trifolium pratense and reported both dwarf and giant mutants. Gelin (1954) also reported X-ray mutants, in the case of peas and vetchers where he recorded " vitality mutants", such as dwarfs, chlorophyll defectives, plants showing inhibition of flowering etc. These " minus mutants" were found to revert back to normal types in subsequent generations. A 'gigas' form was also noted by him.

Kuzin (1956) summarising the works of Soviet scientists observed that data obtained are indicative of a stimulating effect and the absence of a stable positive action. He reported increased root elongation in rye following seed irradiation with 100 r units. In pea and radish root length was reported to be significantly greater than the controls in 900 r irradiated groups. He also reported that a radish variety irradiated with 100 r units gave earlier ripening. Schwartz and Bay (1956) established a 'seedling height-dose curve'. He found that at low levels there was the expected inverse relation, but a point was reached beyond which the original trend was reversed and any further increase in radiation dose resulted in an increase in seedling height. In an earlier paper the same author (1954) has noted that at very high doses around 500 Kr. the seedlings



were turgid and looked perfectly normal, but growth ceased after about five days. Because no dividing cells were observed it was suggested that the growth was entirely due to the elongation of cells already present in the embryo.

Giles (1956) reported that in Solanum acaule X-rays caused retardation of seedling growth leading to dwarfing. He reported two plants in the irradiated lot which flowered profusely and produced larger berries than the controls. A slight increase in height and weight was observed by Spencer and Cabanillas (1956) in Indigofera endecaphylla following X-irradiation.

Sparrow and Gunckel (1956) found in a number of species of Nicotiana that these plants grow better when subjected to chronic gamma radiation. The same authors have reported that gamma radiation produce stem swellings and also lack of internodal elongation with higher dosages.

Yagy and Morris (1957) found that in X-irradiated tomato, the size of the first two leaves showed a linear correlation with the dose.

Beard (1958) found such linear relation between plant height and dose in irradiated barley, maize, mustard and safflower.

Natarajan, Sikka and Swaminathan (1958) believed that the stimulation resulting in acceleration of plant growth may arise

from increased cell expansion, with cell division proceeding at the normal rate.

Bora and Rao (1958) in an elaborate study on induced mutations in rice reported that the X_1 plants from seeds exposed upto 75 Kr. were normal in appearance, but showed slow growth in the seedling stage. Jones and Plummer (1960) in Trifolium observed that gamma irradiation at 43 Kr. units inhibited growth for 60 days and thereafter the plants made up the growth.

Beachel (1961) working on rice using both X-rays and neutron sources found that with increased doses the plants were stunted, with broad leaves and premature tillering habit.

Scossiroli et al (1961) reported X-ray irradiation in Triticum where they observed that both in the tetraploid and hexaploid wheat root length was significantly reduced.

Shastri and Ramiah (1961) studied the effects of X-rays on several varieties of rice and supported the views of Beachel.

Plummer (1962) found initial growth retardation in gamma ray irradiated Trifolium.

Basu and Babindrakrishna (1962) irradiated five varieties of Jute with X-rays and B-rays and found that stem abnormalities persisted till maturity. Leaf abnormalities did not persist over 7th leaf stage. However, these abnormalities were not evident in the S_1 generation.

Venkatanadhachari (1963) working on irradiated rice found premature tillering, broad leaves and stunted growth, confirming the works of Beachel and also of Shastri and Ramiah.

P.N.R. Nair (1964) observed significant reduction of seedling height in soaked seeds of cow pea irradiated with X-rays. No reduction was found in the case of dry seeds subjected to irradiation. Even in the soaked series, the reduction of height was transient as the plants attained normalcy in height later on.

Surrey & Kenneth (1965) reported that X-irradiation reduced the growth markedly upto 100 Kr. units. Howard and Alma (1966) observed that Vicia faba primary roots exposed partially to 300 Kr. units caused decrease in the number and growth rate of lateral roots arising in the irradiated regions.

Gopalakrishna Raju (1967) in rice reported that the coleoptile length in the X_1 generation was adversely affected by higher doses. Similar inhibitory effects were observed by him on the growth of first and second leaves and primary roots in the X_1 generation. He also found that the effects of various dosages of irradiation had the same pattern of action on seedling growth in the X_1 and X_2 generation.

Broke (1967) observed that in Arabidopsis thaliana thermal neutrons and gamma rays exposure resulted in increased variance

of flowering dates, shifting the mean to lateness, and of growth rate with a reduction in mean plant weight. Thermal neutrons produced more leaf shape mutants while gamma rays produced more growth form and chlorophyll mutants. He found a negative correlation between flowering time and growth rate. It indicated that the mean of a character not subjected to selection after induction of mutation will be determined by its previous selection history and by any genetic correlation existing between it and a character under selection. Such correlation responses may enhance or oppose the effect of mutation on the mean character.

Maszynski (1967) also showed a definite correlation between the number of plants which attain maturity (those which survive the radiation treatment) and the degree of growth retardation of the seedlings.

III EFFECT ON FLOWERING AND FERTILITY

X-ray irradiation cause marked effect on flowering and fertility of plants, as has been observed by several workers.

Harlan and Pope (1922) found mutants having changes in fertility. Gustafsson (1937) distinguished two kinds of sterility in induced mutants. The first type of mutants had reduced viability in the vegetative stage showing poor seed setting. There were fully or partially sterile mutants due to structural changes on spike or flowers. The other type arises due to disturbed meiosis

caused by recessive factors.

Hastings and Moore (1935) reported early blooming in X-irradiated citrus.

Smith (1939) in Triticum monacoccum observed sterility due to structural changes of floral parts and poor seed setting. He observed a mutant having no anthers.

Levitsky (1940) found a strong correlation between decreased fertility and structural abnormalities.

Center and Brown (1941) stated that in Phaseolus vulgaris there was no difference in pollen sterility in the control and treated plants.

Freisleben and Lein (1943) and Gustafsson (1944) reported that fertility in barley decreased with increasing radiation.

Johnson (1948) in Kalanchoe observed earliness in flowering and better fruit set in irradiated plants.

Leopold and Thimman (1949) recorded 11% increase in the initiation of flowering in X-irradiated barley. They opined that this was due to changes in auxin levels created by irradiation. However, Mackey (1951) reported decrease in fertility in proportion to increase in radiation.

Tedin and Bagberg (1952) found no difference in fertility between treated and control materials in Lupinus.

Gunkel *et al* (1953) working in Tradescantia paludosa have recorded high percentage of sterility.

Ramakrishnan and Jacob (1955) in Sesamum have reported earliness in blooming and good fruit set due to X-irradiation. Hackbarth (1955) found no difference in fertility between the treated and control in Lupinus plants. Wehrmann (1955) studied pollen sterility in Alonecaris pratensis and obtained dose proportionality with regard to distribution on fertility classes and to mean fertility. Ehrenberg (1955) in barley reported a linear correlation between pollen sterility and dose of irradiation.

Chandhari and Das (1956) found that in X-ray treated Sesamum orientale the percentage of pollen sterility increased with the dosage only upto 40 Kr. In two varieties they found pollen sterility decreased with further increase in dose, while in another variety they found the percentage of pollen sterility increased with further increase in dose. Most workers are in agreement with the finding that pollen sterility is linearly related to dose.

Zacharias (1956) irradiated Soybean (Glycine soja) and obtained a difference of 3% pollen sterility between control and treatments, but no difference was observed between doses.

Kaulis and Webster (1957) found in X-rayed Soratum vulgaris seeds, stunted nature of plants with delayed flowering and reduced

duration of flowering period. They also reported a high range of sterility in proportion to various doses.

Beard et al (1959) in barley and Kandu et al (1961) in jute have reported the existence of a linear correlation between pollen sterility and dosages.

Beckendam (1961) reported in rice that the percentage of sterility increased with dose only upto a certain limit, indicating a saturation point. Thus the fertility in 150 Kr. lot was 58.2% and in the 320 Kr. lot 59.4%. This shows that the linear relation will hold good only upto a certain dose limit.

P.N.R. Nair (1964) have summarised from his work on cow pea that flower production was significantly different between control and treatments, 9 Kr. producing more numerous flowers than others in a range of 1 to 15 Kr. unit treatments. He also observed that percentage of fruit set was unaffected by X-rays, but number of seeds per pod was reduced (linearly) proportional to dose increase.

Bauitti and Ragazzini (1965) using gamma irradiation in Dianthus carvophyllum reported a progressive reduction in flower production in relation to treatments.

Veluswamy (1966) also using gamma rays in rice found that high sterility has been induced by irradiation. He could not observe any earliness in flowering or increase in seed set in spite of the fact

that he observed an increase in flower production.

Ivanov et al (1967) found in gamma irradiated Arabidopsis that fertility mutants were obtained in the dose range of 100 - 120 Kr.

IV CYTOLOGICAL EFFECTS

As a result of elaborate work done by various workers it has been repeatedly shown that apart from the genetically transmissible changes, irradiation cause a number of cytological abnormalities. Formation of micro nuclei, restitution nuclei, multinucleate cells, multipolar spindles, as well as, abnormal contraction of chromosomes and stickiness of chromosomes may be mentioned as some of the important cytological abnormalities. The frequencies and kinds of aberrations have shown to be effective yardsticks for the cytological assay of radiation damage. A comparison of this data with the genetical and physiological studies has given an idea as to how individual cells are killed or altered temporarily or permanently by radiation.

Lopriore (1898) was the first to record cytological effects of X-rays. He found in Valisnaria spiralis an acceleration of photoplasmic streaming which was subsequently checked by longer exposures. Seckot (1902) found similar effects in Oxalis and Mimosa. The viscosity of cytoplasm was changed due to X-irradiation, as observed by Williams (1923).

Muller (1927, 1928) and his associates could repeatedly observe the production of abundant structural changes in chromosomes of Drosophila.

Goodspeed (1928) reported failure of some chromosomes to pair after irradiation.

Patten (1929) and Nakagawa (1931) observed considerable mitotic depression after X-irradiation.

Levitsky and Araration (1932) in Crepis, Vicia and Secale and Miller and co-workers (1937, 1938) observed chromosome breakages. Stadler (1932) found chromosome breakages in maize.

Lea and Catchside (1942) found in Tradescantia that chromotid breaks and isochromatid breaks were induced at the prophase, while chromosome breaks and chromosome inter-changes were induced at the interphase. Sax (1943) have reported that the primary genetic event in irradiation is the breakage of the chromosome thread.

In Triticum vulgare it has been shown by Wagner (1950) that the neighbourhood of the centromere and the ends of the chromosomes were much more susceptible to X-ray effect and that breakages are taking place mostly in these regions.

Sparrow (1950) showed that there is a significant increase in the frequency of the micronuclei on radiation.

In barley Mackey (1951) reported that the frequency of cells with chromosome fragments gave a linear relationship to the dose with a slope down at higher doses due to a saturation effect.

Sparrow and Singleton (1952) found that micronuclei were of much more common occurrence in meiotic cells than in mitotic cells of irradiated plants.

Koller (1952) reported a number of spindle abnormalities at doses of 152 to 250 r X-irradiation. Errors in spiralization of chromosomes were also observed, which stimulated chromatid breakages.

In Allium cepa Rasch (1952) observed the following effects of X-rays; temporary inhibition of mitosis, abnormal nuclear divisions caused by all types of chromosomal aberrations, abnormal cell divisions and finally degenerations in the nuclei and cells which were severely damaged by the original treatment or had suffered subsequent disorientation. The frequency of aberration was directly related to the dose.

Caldecott et al (1954) obtained a linear relationship in interchange frequency to X-ray dosages.

D' Amato (1954) reported formation of pseudochiasmata in the somatic cells following irradiation.

Miller (1954) stated that the frequency of gross structural changes varied as a power of the dose higher than one when X-rays or gamma rays are applied in ordinary doses since with such irradiation the broken ends which unite are usually produced by independently arising breaks and the products, the structurally changed chromosomes therefore represent a concatenation of effects.

Larter and Elliot (1956) showed decrease in survival and fertility as well as increase in chromosome interchange frequency with increasing dosage of X-irradiation.

Swanson (1956), and Yagyu and Morris (1957) found non-disjunction as one of the reasons for cytological abnormalities. The latter workers reported that in X-ray treated seeds of tomato the most observable features were the anaphase bridges and fragments, their frequency increasing proportionate to higher dosages.

Davidson (1957) observed in Vicia faba interstitial union of chromatids mostly at homologous and rarely at nonhomologous points and terminal union of chromatids or point unions. Such point unions did not occur in a limited segment about the centromere.

Sparrow (1961) reported chromosome stickiness and clumping as an immediate result of irradiation with high doses.

Sparrow (1961) and Evans (1961) recorded persistent nucleoli in irradiated material.

Fatil and Bora (1961) recorded multineucleoli formation followed by X-irradiation of seeds of Arachis hypogea.

Haque (1961) confirmed that pseudochiasmata do occur in somatic cells of irradiated material.

Basu (1962) confirmed the existence of the linear relationship of formation of anaphase bridges and fragments with increase in dosage from his work in irradiated root tips of jute.

P.N.R. Nair (1964) recorded an increased frequency of abnormal anaphases and a linear relation to dosage in X-irradiated cowpea.

Budaskina and Scapova (1965) observed ring formation along with bivalents in 10 Kr. gamma irradiated Triticum dicoccum.

Revell (1965) measured real chromatid breaks, isochromatid breaks, and lesions and gaps separately in X-rayed materials. He suggested that the general opinion that chromatid break is linearly related to X-ray dose is due to an over estimation of breakage due to inclusion of the gaps and lesions.

Scott and Evans (1965) studying chromosome aberrations in X-irradiated Vicia faba found non linear response for intrachanges, supporting Revell's hypothesis that these aberrations are produced as a result of an exchange process.

Evans (1967) concluded that radiation does not immediately break chromosomes and may not produce direct breakages of chromosomes

at all. Radiation produce damaged zones or lesions on the chromosomes and restoration of a single lesion results in the restoration of the original structure. But if two lesions become associated at the time of repair there is an opportunity for misrepair and hence aberrations. The misrepair hypothesis accounts for chromatid type aberrations induced in the DNA synthesis or Post-DNA synthesis periods of interphase.

V MORPHOLOGICAL MUTANTS

Various kinds of morphologically distinct mutants have been reported by several workers in the irradiated populations, mostly in the M_2 generation.

Colour variants:

Goodspeed (1930) noted flower colour changes in the progenies of irradiated tobacco.

Bruns (1954) reported flower colour changes in Trifolium.

Hoffmann and Zeschke (1955) in Linum recorded flower colour changes.

Sjodin (1961) obtained interesting flower colour changes in the M_2 generation of Vicia faba.

P.N.R. Nair (1964) obtained two plants in X-irradiated cowpea, having stems with deep purple in the bottom and diluted colouring on

the upper parts. He also obtained plants with purple nodes, purple peduncle and purple streaked sepals.

Chimeric plants:

Monti (1965) studying formation of chimaeras among mutant pea plants observed that 63.64% of the M_1 mutated branches are derived from one initial cell.

Gichner (1966) working in Arabidopsis thaliana found that M_1 root length and frequency of chimeras were proportional to the degree of sterility and to the frequency of mutants in the M_2 .

P.N.R. Nair (loc.cit.) recorded one chimeric plant in the 3 Kr. unit treated cow peas, in which one branch was chimera. The leaves of one side were normal and on the opposite side the leaves were of the mutant type with green and yellow patches.

Leaf types:

Goodspeed (1928) reported crinkled and puckered leaves in segregating populations of irradiated tobacco.

Horlacher and Killough (1931, 1932 & 1933) obtained forked-leaf mutants which were inherited as simple recessives.

Gustafsson (1947) stated that both broader and narrower leaved mutants were common in the X_2 and X_3 generations of irradiated barley.

Athwal (1961) in Cicer reported similar narrow and broad-leaved mutants.

P.N.R. Nair (loc.cit.) obtained one plant with thicker and larger leaves in the 5 Kr. treated cow peas.

Growth habit:

Changes in the pattern of growth of plants have been found to be caused by irradiation.

Gelin (1954) obtained multi branched robust mutants from the X₂ of irradiated Vicia sativa.

Batler (1954) reported two distinct types of tomato plants, named "propeller" and "rosette".

Wohrmann (1956) noted that X-irradiation caused trailing nature in non-trailing types of Alopecurus pratensis.

Gunckel (1957, 1961), and Gunckel and Sparrow (1961), have extensively studied the effect of ionizing radiation on plant growth, morphology and physiology, as well as their biochemical aspects.

Athwal (1961) reported bushy mutants in the X₂ of irradiated Cicer, characterized by very small internodes and closely packed leaves and leaflets.

VI CHLOROPHYLL VARIANTS IN M_2 GENERATION

One of the effects of X-irradiation is the production of disturbances in the organisation of chlorophyll.

Horlacher and Killough (1932) in cotton observed that chloroplasts are sensitive to X-radiation.

Johnson (1936) observed similar effects using X-rays. Gustafsson (1940) recorded various types of chlorophyll variants in the segregating generation of X-rayed barley such as "Albina", "xantha", "chlorina" etc. But Mantzing (1942) found no such disorganisation of chlorophyll in his studies. Freislben and Lein (1943) obtained chlorophyll mutants similar to those got by Gustafsson. Levin (1944) could not find any change in chlorophyll in the M_1 generation. But Troiler (1946) obtained chlorophyll mutants in irradiated barley.

Gustafsson (1947) stated that chlorophyll disorganisation is one of the many effects of irradiation. But Lawrence (1955) reported that chlorophyll disorganisation does not occur in plants in the M_1 generation.

Godner and Shlik (1956) using X-rays observed significant changes in the chlorophyll content in the M_2 generation plants.

Gailey and Telbert (1958) reported that very high doses of gamma radiation to the extent of 50 Kr. units did cause disorganisation of chlorophyll.

Sjodin (1961) observed that chlorophyll mutations were comparatively rare in the segregating generations of leguminous plants. In leguminous plants he found the 'viridis' type most commonly, which furnished various shades of green. In one case he obtained a segregation ratio of 19 normal:1 albina in the X_1 generation.

Blixt (1961) reported a type of chlorophyll mutant in the X_2 and X_3 of pea plants which has been named as 'chlorotica variomaculata', characterised by patches of green and yellow spots.

P. N.B. Nair (1964) in cow peas observed various grades of spotting in the X_2 generation seedlings.

Veluswamy (1966) using gamma rays (10 to 60 Kr.) in rice found no chlorophyll changes. He concluded that the absence of any significant effect in his experiment might probably be due to the reason that the dry seeds he treated were not quite sensitive to gamma irradiation and rice chlorophyll emerge only with germination of seeds. But he observed in the 40 Kr. treatment that chlorophyll did not deteriorate even after the harvest was delayed for 15 days, while such disorganisation is normal at harvest time.

Popovic and Zecevic (1966) studying mutation changes in winter barley due to gamma irradiation observed a change in ear colour in some plants. They also recorded several chlorophyll mutants.

Kasyanenko and Timofeyev-Rosevsky (1967) in Arabidopsis thaliana L. found in Co^{60} treated cases all the 130 chlorophyll mutants as single recessive, six considered to be particularly interesting in that, one had increased pigmentation but less assimilation rate, another reduced pigmentation but showed greater vitality etc.

Kasyanenko and Guiller (1967) also working in Arabidopsis thaliana recorded varying quantities of pigmentation, carotenes and chlorophyll in gamma irradiated mutants.

Bedei (1967) found that in X-rayed Arabidopsis, the treatment induced the formation of more chlorophyll temporarily in the somatic condition.

MATERIALS AND METHODS

M A T E R I A L S A N D M E T H O D S

The present study was undertaken in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani, during the year 1968-1969.

A. MATERIALS:

The material selected for study was the K-1 variety of chillies (Capsicum annuum L.). It is a well known commercial variety, evolved by selection from a line of B 72 A - 14, at the Regional Research Station, Kevilpatti, Madras State. The original home of the variety is Assam. It bears long, conical, pendent fruits. Flowers are white, and normal immature fruit colour is green. Other important features of the strain are given in Appendix-I. For the present study, the pure seed was obtained from breeders' stock from the Research Station.

B. METHODS:

Seeds of apparently uniform size were carefully selected out for irradiation. The X-ray irradiation was given at the Division of Genetics, Indian Agricultural Research Institute, New Delhi, by using a Philips (Holland) Model Superficial Therapy Medical X-ray Unit. No filter was used. The unit was operated at 50 KV - 2 mA of tube current.

The seeds were treated in dry condition. The entire lot of seeds used for the experiment were divided into five samples, and four samples were treated at four different doses of X-rays. The doses employed were 1000 r, 4000 r, 8000 r and 15000 r units. The fifth sample of seeds was retained for raising the control plants.

Seeds were sown in petridishes lined with moist filter paper for conducting germination tests.

Root tips were taken from germinated seeds for study of mitotic behaviour. When the roots attained about 2 cm length, they were cut and fixed in 1:3 acetic alcohol.

2 Field technique:

250 seeds from each of the treatments were sown in separate uniformly manured pots for raising seedlings. Seeds were sown with sufficient spacing for normal growth of the seedlings. Observations were made for morphological differences. Seedling height was measured first on the 25th day. The survival of seedlings was counted on the 45th day.

The 45 days old seedlings were transplanted in the main field with a spacing of 80 cm x 80 cm. 20 plants from each of the treatments and control were randomly selected for detailed studies.

The observations made on the M_1 generation are listed below:

- i. Germination of seeds, as measured by germination percentage of the treated seeds.
- ii. Survival of seedlings as measured in percentage of the seedlings survival on the 45th day.
- iii. Seedling height on the 25th day and 60th day.
- iv. Duration of flowering from date of sowing.
- v. Pollen sterility in percentage.
- vi. Morphological abnormalities.
- vii. Cytological effects.
 - (a) in mitosis, using root tips.
 - (b) in meiosis, studied in P.M.Cs.

In addition, the following study on the segregating M_2 generation was also undertaken:

Frequency of chlorophyll mutations.

- (a) M_1 basis
- (b) M_2 basis
- (c) mutation spectrum.

In all the above studies, excepting items (vi) and (vii) in the M_1 generation, the comparative effects of the various doses of X-rays employed on the standard experimental material was subjected to detailed studies.

3 Methods of observations:

M₁ generation:

(i) Germination percentage:

From the four treatments and the control 100 seeds each were sown in petridishes lined with moist filter paper. Germination counts were taken from the second day onwards and continued to the 12th day. The final data were taken for the analysis.

(ii) Percentage of Survival:

Survival counts were taken in the pot-sown seedlings. Percentage were worked out for the different treatments based on the number of seeds sown and the number of seedlings survived till the time of transplanting on the 45th day.

(iii) Plant height and growth:

Plant heights were measured on the 25th day and 60th day. It gives an idea about the general growth and vigour of the plants. Heights from the base of the plant at soil level to the tip were measured. The data were analysed.

(iv) Duration of flowering:

The date of the blossoming of the first flower in each of the M₁ plants was recorded. The number of days taken from the date of sowing to the day of flowering were calculated and compared.

(v) Pollen sterility:

Pollen sterility was studied in all the 20 plants in each of the treatments and control. Flowers born in the early part of the flowering periods were selected. Anthers were squashed and the pollen grains stained in glycerine - aceto carmine. 30 fields in each of the slides, and thereby for each treatment 600 microscopic fields were studied and recorded. Sterility percentage were worked out.

The acetocarmine staining technique enables to count the fertile and sterile pollens separately.

(vi) Morphological abnormalities:

Several morphological abnormalities were observed in the M_1 plants compared to the control. They were recorded and presented.

(vii) Cytological studies:

(a) Mitosis:

Root tips of about 2 cm long collected and fixed in 1:3 acetic alcohol were subjected to mitotic study. The root tips were first hydrolysed at 60°C in N HCl. Acetocarmine preparations were made using the hydrolysed material.

Another technique employed was that the roots were treated with 0.05% colchicine solution between 10 A.M. and 2 P.M. and then

to cut the root tips, fix them and hydrolyse before preparation of slides.

(b) Meiosis:

The flower buds of M_1 plants were fixed in 1:3 acetic alcohol and preparations made with 1% acetocarmine.

M_2 Generation:

Chlorophyll mutations:

Samples of 100 seeds each taken from the first ten ripened fruits of the 20 plants studied in the different treatments of M_1 generation were sown separately in pots. Seedlings were observed and scored on the 6th to 15th days when they had 2 to 4 leaves.

Percentages of mutants in the total population were estimated on M_1 plant basis and M_2 plant basis. Mutation spectrum is worked out.

Statistical techniques:

Data on the quantitative estimates were analysed adopting the analysis of variance technique.

EXPERIMENTAL RESULTS

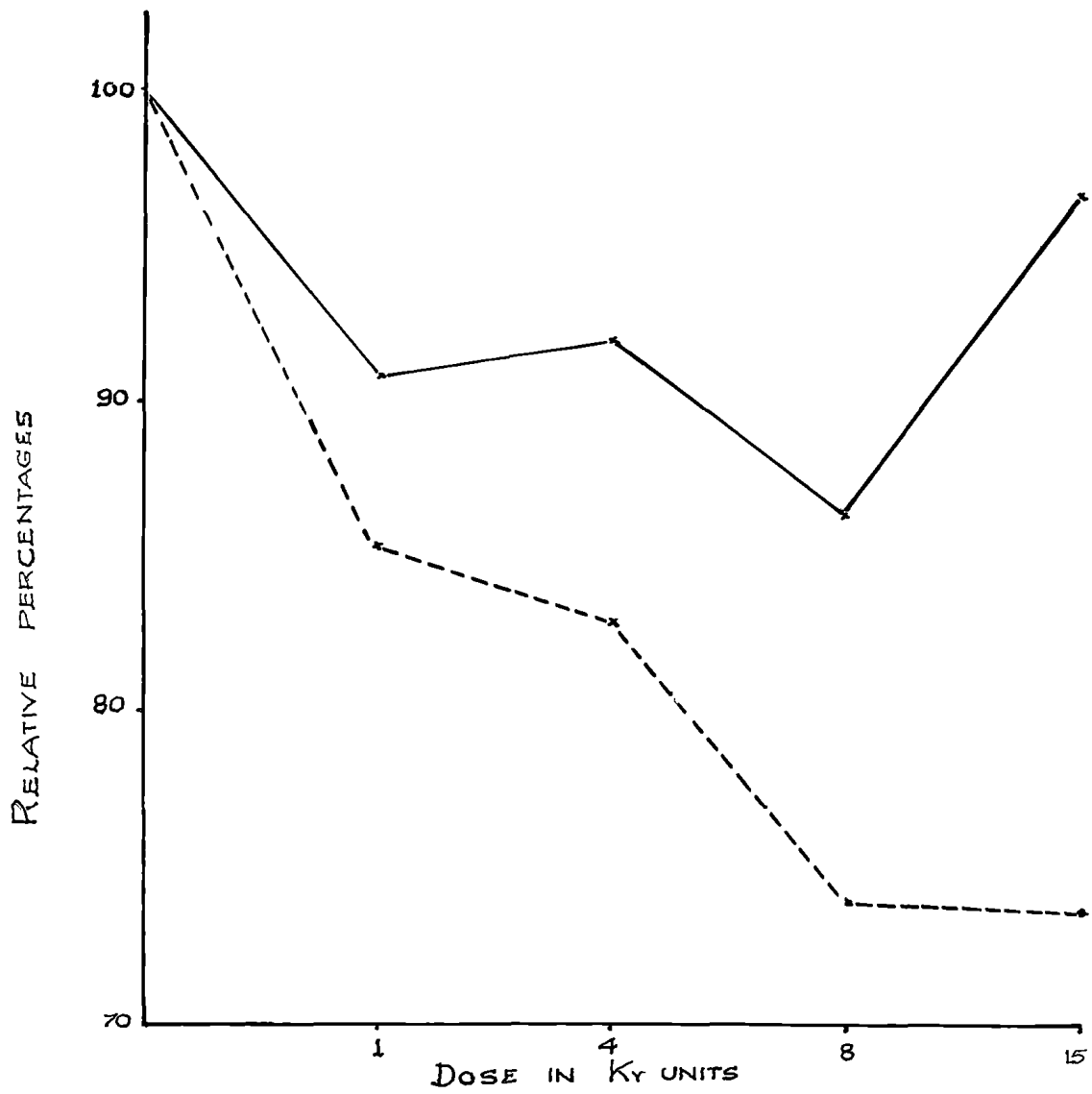


TABLE - 1
 DATA ON GERMINATION AND SURVIVAL PERCENTAGE OF
 SEEDS - M₁ GENERATION

Treatments	Germination		Survival	
	Germina- tion per- centage	Relative percen- tage	Survival percen- tage	Relative percen- tage
Control	88.0	100.0	88.0	100.0
1 Kr.	80.0	90.9	75.2	85.4
4 Kr.	81.0	92.0	73.2	83.1
8 Kr.	76.0	86.3	65.2	74.0
15 Kr.	85.0	96.6	65.0	73.8

Fig. I.

**Graph showing data on germination
of X-irradiated seeds and seedling
survival.**



— GERMINATION OF SEEDS
--- SURVIVAL OF SEEDLINGS

FIG I

reveals that with increase in dose of irradiation there was a reduction in the survival percentages from 94% in the 1 Kr. treatment to 82% in the 15 Kr. treatment.

It was observed that lethal effects occur only in the early days of the seedlings. Such seedlings had their growth arrested, presented a dehydrated appearance and wilted by the 4th to 6th leaf stage. No lethal effects were noted beyond the 25th day.

The data is represented in Figure-1.

Plant height and growth:

The seedling height measured on the 25th day and 60th day gave an idea about the effect of irradiation on growth rate of the M_1 plants.

Table-2 and Appendices-II to V presents data on this subject.

There were wide fluctuations on the individual plant heights within the treatments. Also, certain plants which suffered physical damages consequent to transplanting gave minimal heights in the second recording.

Significant variations in the effects of the various doses of X-rays could be seen in the 25th day measurements. When the mean plant height in the control plants was 6.48 cm, it rose to 7.39 cm in the 4 Kr., 8.05 cm in the 15 Kr., 8.36 cm in the 8 Kr., and 9.52 cm in the 1 Kr. treatments. The lowest dose showed the largest

TABLE - 2

DATA SHOWING PLANT HEIGHT (in cm) M₁ GENERATION

Treatments	25 days old plants				60 days old plants			
	Mean height	Relative percentage	Range		Mean height	Relative percentage	Range	
			Minimum	Maximum			Minimum	Maximum
Control	6.48	100.0	5.3	7.4	14.2	100.0	5.0	27.0
1 Kr.	9.52	147.2	8.5	10.7	17.2	121.1	9.0	33.0
4 Kr.	7.39	114.0	6.3	8.2	14.8	100.7	5.5	26.0
8 Kr	8.36	129.0	7.3	9.2	17.2	121.1	9.0	31.0
15 Kr.	8.05	124.2	7.3	8.6	16.4	115.4	6.0	26.0

effect.

The analysis of variance technique revealed that against a critical difference of 0.98, all the treatments gave significant increase in plant height compared to the control and between all the treatments excepting the 8 Kr. and 15 Kr. doses. The 1 Kr. treatment gave the highest mean immediately followed by 8 Kr., 15 Kr. and 4 Kr. treatments.

In the 60th day heights no significant effects could be observed. The mean plant heights recorded were 14.2 cm in control, 14.3 cm in the 4 Kr., 16.4 cm in the 15 Kr., 17.2 cm in the 8 Kr. and 17.2 cm in the 1 Kr. treatments. Even by the 60th day the initial ranks of treatment means remained unaltered.

In both the cases the means showed no linear relationship between dose and plant heights.

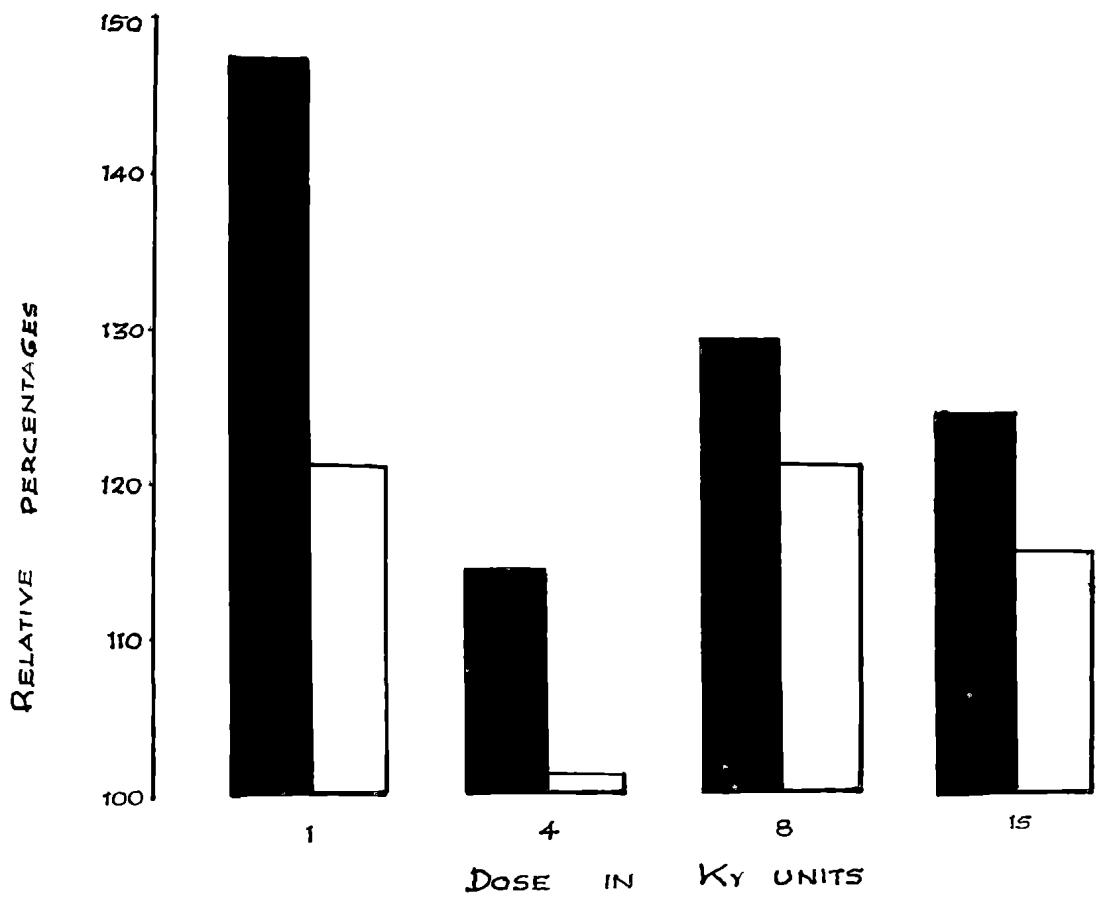
Figure-II gives a graphical representation of the two data.

Duration of flowering:

It was investigated whether X-irradiation induce variation in flowering in chillies.

Significant effects were recorded in the number of days taken from the date of sowing to the day of first blossoming in the different treatments.

Fig. II. **Graph showing plant height**
on the 25th and 60th days.



■ HEIGHT OF 25 DAYS OLD SEEDLINGS
□ HEIGHT OF 60 DAYS OLD SEEDLINGS

FIG II

Table-3 and Appendices VI and VII presents data on duration of flowering.

Wide fluctuations were observed within both the control and the treatments. The analysis of variance gave significant variance ratio for the treatments. Against a critical difference of 4.12, the ranked mean duration in flowering showed that the different doses induced significant earliness in flowering. Such significant differences were observed between the 15 Kr. treatments and the 8 Kr. and 4 Kr. treatments, 15 Kr. being most effective. 1 Kr. showed better effect than the 4 Kr. treatment and the control. Between 1 Kr. and 15 Kr. and also between the control, 4 Kr. and 8 Kr. there were no significant differences.

Similarly, no linear relationship between duration of flowering and doses could be observed from the study.

It was observed on studying the entire populations for this particular character, that, in the 4 Kr. treatment one plant blossomed on the 60th day and in the 8 Kr. on the 63rd day (as early as in the 15 Kr. treatment). These two plants were not included for the statistical analysis as they did not come within the 20 selected plants in the two treatments.

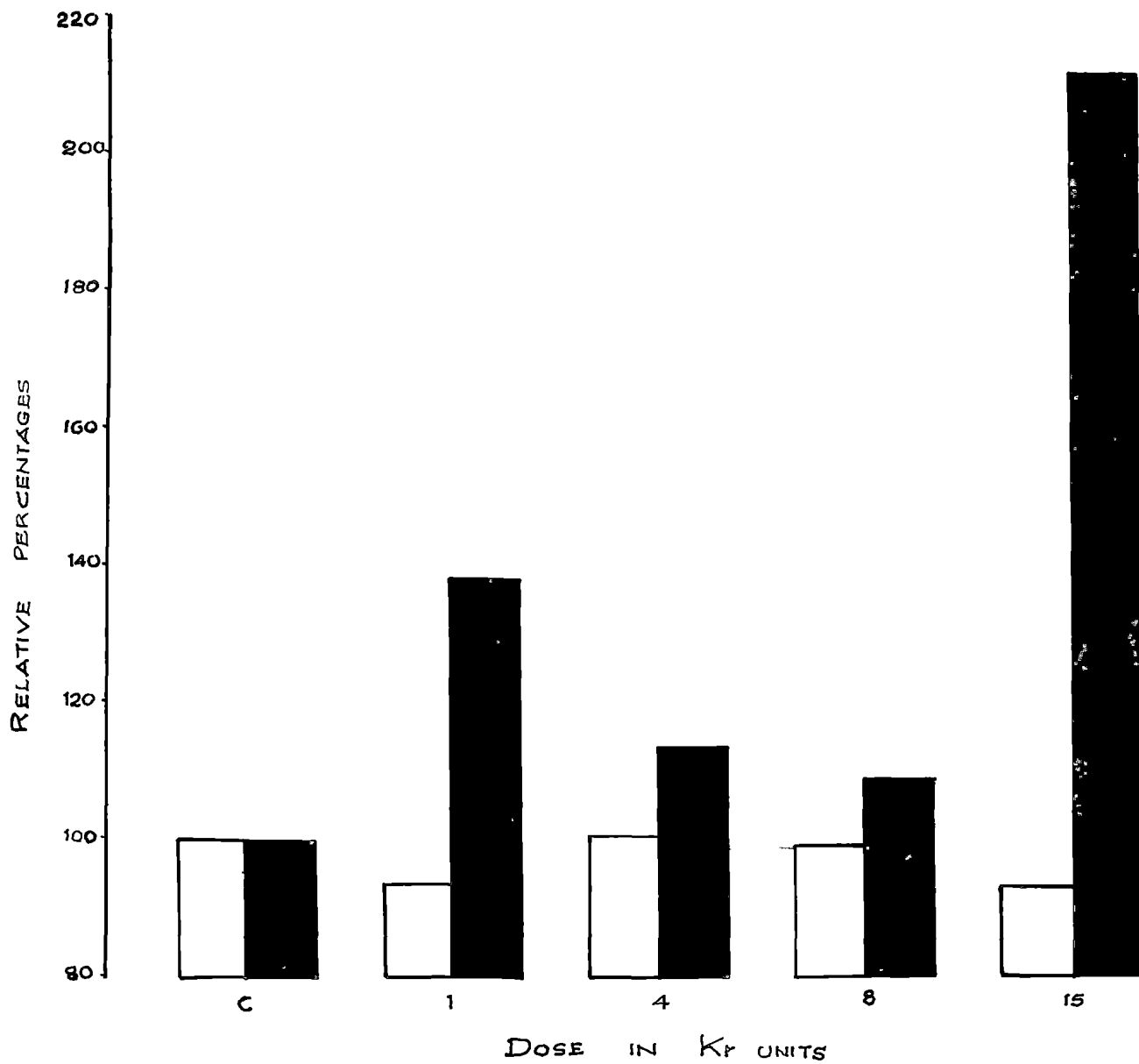
A graphical representation of the data is presented in Figure III.

TABLE - 3

DATA SHOWING EFFECTS OF X-IRRADIATION IN INDUCING VARIATIONS IN
DURATION OF FLOWERING AND POLLEN STERILITY (M₁ GENERATION)

Treatment	Duration of flowering (No.of days)				Pollen sterility (Percentages)			
	Mean	Relati- ve per- centage	Range		Mean	Relati- ve per- centage	Range	
			Mini- mum	Maximum			Mini- mum	Maximum
Control	77.60	100.0	65	90	14.61	100.0	5.46	46.52
1 Kr.	72.65	93.6	60	85	20.11	137.6	4.92	44.52
4 Kr.	77.95	100.4	72	89	16.58	113.5	6.92	66.48
8 Kr.	76.35	98.4	72	83	15.79	108.1	4.55	38.42
15 Kr.	71.90	92.6	63	80	30.87	211.3	9.88	75.00

Fig. III. **Graph showing duration of flowering
and pollen sterility.**



DURATION OF FLOWERING
 POLLEN STERILITY

FIG III

Pollen sterility:

Chromosomal aberrations are possible events in X-irradiation and meiosis will end up in the formation of a higher frequency of abnormally formed and sterile pollens. The effects of the four different doses of X-rays employed in the present study were measured in terms of percentages of pollen sterility. The data is presented in Table-3 and Appendices VIII and IX.

The effects of X-irradiation on the different individual plants were not uniform as can be seen from the data. Therefore variations in pollen sterility could be observed within the treatments.

It was a plant in the highest dose of 15 Kr. which suffered the highest percentage of (78%) sterility in the whole of the experiment.

The analysis of variance projected a significant variance ratio for treatment effects, and the means were therefore compared against a critical difference of 8.16. In the study, a statistically significant effect was yielded only by the 15 Kr. treatment, which was higher than all the other treatments and the control. The effects of 1 Kr., 4 Kr. and 8 Kr. were not significantly different from that of the control.

A linear increase could not be observed between doses and pollen sterility. A graphical representation of the mean pollen

sterility is given in Figure-III.

Morphological abnormalities:

A wide range of morphological abnormalities could be observed among the M_1 plants. Only glaring variations that could be attributed to effects of X-irradiation are presented here.

(i) Dwarf plants:

In the treated populations a striking morphological effect that could be observed was the presence of a number of dwarf plants. Their existence was discernible even from the early period of growth in the main field. They were undersized when compared to normal stature, but not too puny in appearance. In addition these dwarfs were superior in yield. Illustration No. I shows a typical dwarf.

The frequencies of dwarf plants in the four treatments were different. There were 5 dwarf plants in the 1 Kr. treatment, 2 in the 4 Kr., 4 in the 8 Kr. and 3 in the 15 Kr. Incidentally, in both the 1 Kr. and 15 Kr. treatments it was one of these short plants which gave the earliest blossoming. In more than 50% of the cases the leaves of these plants were narrower than the normals. In the 8 Kr. one of these plants showed a characteristic branching nature, in which, the branches took too wide an angle from the primary axis of the plant and also in the later branches, so that, branches grew almost parallel to the surface from a height of about 15 cm. It

was the shortest of all the plants.

(ii) Narrow leaved plants

Substantial reduction in the width of the leaves was observed in many plants.

Illustration No. II shows a typical narrow-leaved plant compared to a plant with normal leaves. This characteristic was observed in 7 plants in the 1 Kr. treatment, 11 plants in the 4 Kr., 4 in the 8 Kr. and 3 in the 15 Kr., treatments. In the low doses the frequency of the narrow leaved plants was found to be higher than in the higher doses. Two plants in the 8 Kr. treatment showed quite characteristic leaf types. One of these plants had dark green leaves, which were reduced both in width and length. Leaf nodes were also too close and as a whole a compact growth was exhibited. Very few fruits were borne on this plant. The second plant showed normal leaf types on branches produced on one side of the plant, while on branches of the otherside leaves were of oval shape. In chillies there are varieties with oval leaves, but they produce only oval or round fruits. However no fruit shape difference was observed in this particular plant. Illustration No. III shows these two plants.

(iii) Chimeras:

One plant in the 1 Kr. treatment and another in the 8 Kr. treatment produced chimerical branches. Photographs of Illustration No. IV show these plants.

In both plants the chimera arose as the first branch. The leaf on the axillary bud of which the chimeric branch arose also showed variegation of green and yellow. In the 1 Kr. treatment the chimeric plant was one of the two plants which blossomed the earliest. It gave 18.2% pollen sterility. Flower buds were yellowish but petals when opened were normal white. Fruits were comparatively smaller and few fruits had yellow streaks on their rind. Seed germination in the M_2 generation from fruits of variegated rind was 77%. It also gave 39.8% chlorophyll variants in the M_2 seedlings of which 27% were xanthas. Of the total seedlings 14% showed variegated leaves like the M_1 parent. Seeds from non-chimeric branches did not produce variegated seedlings.

The variegated leaves of the chimeric plant in the 8 Kr. treatment were crinkled. Flower buds were yellowish. Fruits were very small and showed yellowing on the rind. Pollen sterility was 25.6%. M_2 seed germination was 57%.

(iv) Fruit type variation:

Normally the K-1 variety of chillies bear pendent fruits. In the X-irradiated material, however erect types were also manifested. Photographs of Illustration No. V show such aberrant fruit types.

The number of plants showing erect type fruits were different in the four treatments. In the 1 Kr. and 4 Kr. treatments there were 4 plants each showing erectness of fruits. In the 8 Kr. there was one plant and in the 15 Kr. 3 plants. These plants showed no other abnormal behaviour and they were bearing normally. Not only that, one of these 1 Kr. plants gave the highest yield in the M_1 generation. This plant showed extraordinary vigorous growth with wide spreading branches, and flowered profusely. It yielded 190 grams of dry fruits, while the next higher yielder below this gave only 150 grams. Illustration No. VI presents this plant. The erectness of fruits did not show any corresponding reduction in fruit size in any of the plants.

Colour variants:

Eventhough the normal colour of the plant is green, petals of flowers white, and the rind colour of unripened fruits green, various grades of purple colouration were manifested in all these parts in the X-irradiated materials.

Out of the 617 plants raised, one plant in the 4 Kr. treatment was completely purple. This was discernible as soon as the M_1 seeds germinated. It showed normal growth. The stems, leaves, flowers and fruits showed intense purple colouration (Illustration No. VII). It blossomed on the 80th day. Pollen sterility was

15.77%. The size of the fruits were comparatively small. In the M_2 generation all its 134 progenies had purple colour, but the intensity of colour differed widely.

In most of the plants which showed colour expression, it could be observed only with flowering. The frequencies of such plants were, 2 in the 1 Kr. and 2 in the 15 Kr. treatments. In the 8 Kr. one plant showed purple fruit colour, but no flower colour was observed and even this purple fruit colour did fade and succumb to green colour in reaching maturity.

One plant each in the 1 Kr. and 15 Kr. showed identical colour effect. These plants were normal and green. The petals showed intense purple colour on their edges but less intense in the other parts. The fruits were fully purple or had purple streaks when immature but on attaining maturity the colour gradually faded and gone. These plants showed no other relevant effects.

Another plant in the 15 Kr. treatment had deep purple flowers, similar to those borne on the purple plant of the 4 Kr. treatment. The fruits were also of intense purple colour and this colour remained till ripening of the fruit. On other parts this plant had normal green colour.

Plants which were normal coloured on all parts in the initial stages began to show colour effects at the end of the

growing period. After about 140 days one plant in the 1 Kr. treatment showed dilute purple colour on the petals. The fruits borne out of these flowers were however green. Photographs of Illustration No.VIII show the variant expression in flower colour and fruit colour.

The inheritance of colour in the M_2 segregating generation also require mention. In the seedling stage the progeny frequency for purple colour of the M_1 purple parents were as follows:-

- 1 Kr. 7 seedlings out of 259 numbers.
- 4 Kr. 134 seedlings out of 134 numbers.
- 8 Kr. 0 seedlings out of 130 numbers.
- 15 Kr. 150 seedlings out of 320 numbers.

The M_2 progenies of certain normal M_1 parents have given colour expression in the following frequencies.

- 1 M_1 parent in 1 Kr. gave 1 seedling out of 106 seedlings.
- 5 M_1 parents in 4 Kr. gave 6 seedlings out of 134 seedlings.
- 1 M_1 parent in 8 Kr. gave 1 seedling out of 126 seedlings.
- 1 M_1 parent in 15 Kr. gave 1 seedling out of 88 seedlings.

It was the M_1 parents in the 4 Kr. treatment who gave a higher frequency of colour expression in the segregating M_2 generation. The above are not absolute figures, since only with the study of

flower and fruit colour in the M_2 generation can a true picture of colour inheritance be obtained. Such a study was not included in the present work.

Cytological studies:

In the present work both mitotic and meiotic studies were conducted. The root tips used for mitotic study were specially treated to obtain better results. Chromosome fragments were observed in the dividing cells revealing the effects of X-rays. Similarly, meiotic study revealed the presence of high frequencies of fragments, laggards and anaphase bridges. The results showed the occurrence of breakage-fusion-bridge cycle.

Photographs of illustration No. IX show few of the chromosome aberrations observed during the study.

M_2 GENERATION

Chlorophyll mutants:

Chlorophyll mutants were present in all the treatments. Table-4 presents observations and results.

It could be observed that 7 M_1 plants each in the 1 Kr. and 4 Kr. treatments produced chlorophyll mutants, 6 M_1 plants, in the 8 Kr. and 2 M_1 plants in the 15 Kr. produced such mutants.

TABLE - 4

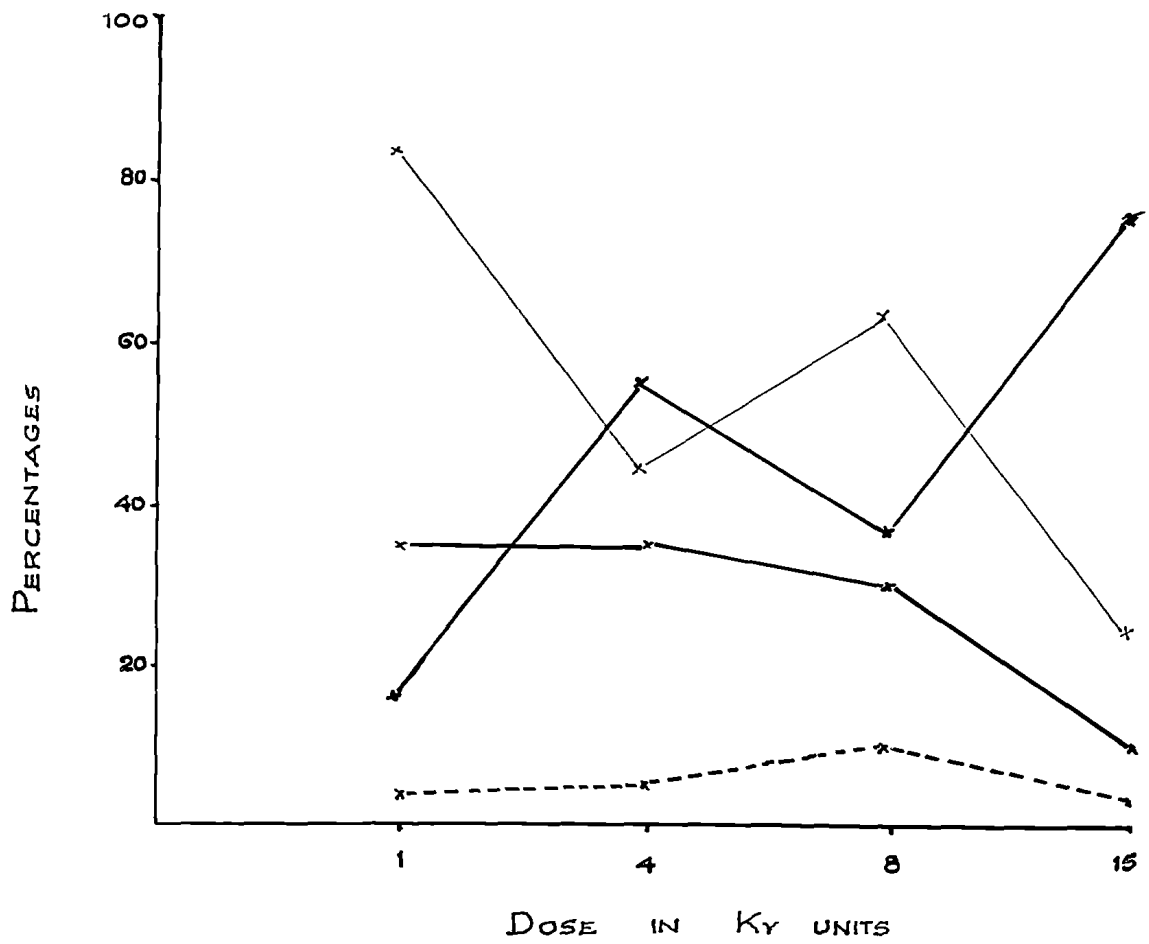
A. DATA ON CHLOROPHYLL MUTANTS M_2 GENERATION

Treatments	Total number of seeds sown	Number of normal seedlings	Number of mutants		Total
			Xantha	Chlorina	
1	2000	1014	2	10	1026
4	2000	1212	11	9	1232
8	2000	1063	11	19	1093
15	2000	1073	3	1	1077

B. CHLOROPHYLL MUTANTS ON M_1 - BASIS M_2 - BASIS
AND MUTATION SPECTRUM

Treatments	Mutants on M_1 - Basis			Percentage of mutants on M_2 Basis.	M_2 Mutation spectrum percentage of mutants	
	Number of M_1 plants	Percentage	Relative percentage		Xantha	Chlorina
1	7	35	100	1.1	16.6	83.4
4	7	35	100	1.6	55.0	45.0
8	6	30	85.7	2.7	36.6	63.4
15	2	10	28.6	0.3	75.0	25.0

Fig. IV Graph showing chlorophyll mutants
in the M_2 generation on M_1 - basis,
 M_2 - basis and the mutation spectrum.



- MUTATION %AGE ON M₁ BASIS
- - - MUTATION %AGE ON M₂ BASIS
- ▬ MUTATION SPECTRUM OF 'CHLORINA'
- ▬ MUTATION SPECTRUM OF 'XANTHA'

FIG IV

The mutants were clearly of two types. The clear yellow "xantha" seedlings were recognisable soon after germination. These did not put out new leaves and did not live more than a week after germination. No albinas were present.

The other type of chlorophyll deficient plants showed general yellowness on the leaves by the 4th leaf stage. These had slight yellow spotting also. Such plants were obviously "chlorinas".

The mutation percentage on the M_1 plant basis showed a decreasing tendency with increase in dose, from 35% to 10%. On M_2 seedling basis the percentages did not give any particular pattern.

The mutation spectrum shows that the percentage of "xantha" mutants increased with the dose increase.

A graphical representation of the data is given in Figure.IV.

DISCUSSION

D I S C U S S I O N

The biological effects of X-rays, which is a physical mutagen are due to its characteristic mode of action on the living cell. When ionizing radiations pass through matter, they dissipate their energy partly through the ejection of electrons from the outer shells of atoms and the loss of these balancing negative charges leaves atoms that are no longer neutral but are positively charged. Such charged particles are called ions. When an atom becomes ionized, the molecules of which it is a part probably undergoes chemical change. When this changed molecule is a gene or a part of a gene, and when the modified gene duplicates its new pattern, the result of the change is a mutation.

X-rays are electromagnetic radiations of extremely short wave length and are originated by interaction of high energy particles with more tightly packed electrons. The ionized atoms or ions triggered by X-rays are not randomly distributed in the irradiated substance, but tend to lie in tracks, the length of which is dependent on the initial speed of the ionizing particle i.e. its initial energy, as well as on its mass. The biological effects of X-rays are also dependent upon the secondary interactions developed by the preliminary effects. Generally immediate effects are chromosome aberrations, but radiation damages are also assessed in terms of

cell death, depression of mitotic activity, depression in the synthesis of DNA, a variety of morphological changes and the stickiness of metaphase and anaphase chromosomes. Altogether, the primary effects and the complex secondary biochemical events that result from X-irradiation are manifested in the material exposed to radiation and such effects are found to have definite relation to the intensity of radiation.

The present investigation in chillies (Capsicum annuum L.) was intended for evaluating such biological effects of various doses of X-rays in this particular material.

Germination

Survival

In the present study X-irradiation did not show significant deleterious effect on germination with the four doses tried. In the control the germination percentage was 88 where as, it was 85 in the 15 Kr treatment. In the lowest dosage of 1 Kr units the germination percentage was 80 and in the intermediate doses of 4 Kr and 8 Kr it was 81 and 76 per cents respectively. It shows that there was no dose-based increase or decrease in germination percentage.

The above result is in agreement with the observations made by many workers. Krishnaswamy and Rangaswamy Ayyangar (1941) reported that they could not find any reduction in germination in

X-irradiated Bajra. Datta and Datta (1953) in rice have made similar observations. A few examples of other workers who got similar results are Beard et al (1958) in maize and barley; Micke (1958) in Melilotus alba; Gottschalk and Scheibe (1960) in leguminous plants; Kundu et al (1961) in jute; Sjodin (1962) in Vicia faba; P.N.R. Nair (1964) in cow pea; etc.

The main effect of X-rays is on the chromosomes and any deleterious effect can manifest only with beginning of cell division. Although the cells which are affected deleteriously will be damaged due to abnormal mitosis, the genetic complement of a cell in which large numbers of breaks have been induced will naturally remain intact as long as the cell does not divide. Since the germination of seeds does not follow cell division there is no possibility of the X-rays to manifest its effect on germination. This inference is in line with the views of Schwartz and Bay (1956). They could observe normal germination even with 500 Kr units, but this high dose inhibited mitosis completely and the growth ceased after about five days. They suggested that germination and early growth were due to the elongation of the cells already present in the embryo at the time of irradiation.

It was also observed during the present study that there was a linear reduction in the survival percentage with increase in

dosage. There was a sudden fall to 72.5% in the 1 Kr unit treatment and between the 8 Kr and 15 Kr the linearity has almost levelled off with a difference of only 0.2%. The result therefore shows that there was an immediate effect on survival even at the low dosage of 1 Kr and that the linearity of dosage effect remained upto 15 Kr. The trend shows, that further increase in dosage will minimise survival percentage.

Similar linear relationship between dose and survival was reported by earlier workers such as Gustafsson (1941), Wohrmann (1961) and P.N.R. Nair (1964). But Yagyu and Morris (1957) observed in dormant tomato seeds that dosages upto 10 Kr did not show any reduction in survival percentage, but beyond this they could also observe a decrease in survival corresponding to dosage.

In the present study no such optimum safe levels could be observed with regard to survival. It is therefore probable that X-rays have deleterious effects on survival of chillies even at the low dosage of 1000 r units.

Seedling Height

Plant Growth

This investigation showed an early increase in seedling height in the lowest dosage of 1 Kr treatment, but the higher dosages did not show a corresponding increase. But all the four dosages did show

some increase in seedling height in the 25th day old seedlings. Some sort of a stimulative effect seems to have been existed in the X-irradiated plants.

The effect of X-rays on plant growth is a much disputed subject. Many works can be cited for and against the stimulative effects of X-rays. Miede and Coupe (1914) in Bananas and Lepidium recorded a stimulative effect of X-rays. Reports of Nevera (1920) Jungling (1920), Schwartz (1923), Geller (1924), Goodspeed and Olson (1928), Haskins and Moore (1935), Kersten *et al.* (1943), Muzin (1956), etc are but a few examples to cite in support of the stimulative effects caused by X-rays.

Natarajan, Sika and Swaminathan (1956) have attributed the stimulative effects of X-rays to the increase in cell expansion.

The adverse effects of high dosages of X-rays have been reported by several workers. The inhibition of plant growth was due to chromosomal changes and physiological disturbances according to Lea (1947), Catchside (1948), and Gordon and Weber (1950). From the present study it was found that dosages upto 15 Kr did not cause any growth retardation in cillies and instead such low and medium dosages did impart a stimulation to initial plant height.

The plant height recorded on the 60th day showed no significant variation between the different treatments. It seems that

the plants have overcome the initial growth difference and have kept up a balanced growth in due time.

Such normalization of transient growth effects was reported by P.N.R. Nair (1964). Workers like D'Amato (1961) have indicated that a strong intrasomatic selection occurs in the treated plants. This leads to elimination of damaged or abnormally formed cells and the normal ones proliferate. So normal growth should result in the later stages of growth. Therefore it may be said that the initial stimulative effect of plant growth was a transient effect of X-irradiation and that subsequent growth has normalised the situation.

Flowering

Fertility

The present study revealed that X-irradiation has caused significant earliness in flowering. The mean duration from sowing to flowering was the least for the highest dosage of 15 Kr. unit treatment. Some earliness was invariably found in all the treated cases, though no dose-based earliness could be observed. It shows that the precocity observed might probably be due to effects of X-irradiation. Haskins and Moore (1935) have recorded earliness in flowering in citrus induced by X-rays. Similar reports were also made by Ramakrishnan and Jacob (1955) in Sesamum. The accelerated vegetative growth must

have produced physiological effects to that the ripeness to reproductive phase was attained earlier and hence this earliness to flowering in the treated plants.

In studying the pollen sterility no relationship between increase in dosage and mean percentages of pollen sterility could be established. The 15 Kr treatment gave the highest mean pollen sterility percentage and the control gave the lowest percentage. The lower doses did not show any significant decrease in fertility.

A linear relationship between dosage and pollen sterility was reported by many workers like Frieslben and Lein (1943), Gustafsson (1944), Mackey (1951) and Wohrmann (1955). However contradictory results were obtained by Genter and Brown (1941), Hackbarth (1955) and Zacharias (1956), who reported that they could not observe any fertility difference between the control and treated plants.

The present work shows that X-irradiation did not affect pollen sterility percentage upto a dose limit of 8 Kr. In the 8 Kr treatment the pollen sterility percentage was only 15.79. But above this dosage, there was significant effect on pollen sterility. In the 15 Kr this was 30.87%, almost double that of 8 Kr. In chillies, therefore, as per the present result, there may be an optimum limit of resistance to X-rays, beyond which the pollen sterility will be substantially

increased. Higher dosages of X-irradiation produced more lethal chromosomal aberrations and this would have contributed to increased pollen sterility. The effects of intermediate dosages between 8 Kr and 15 Kr units will have to be explored to ascertain the exact " lift off " point.

Cytological Effects

Morphological Variations

In spite of the adoption of special techniques for the study of root tip mitosis in the M_1 generation no convincing result could be obtained. But chromosome breaks and fragments were observed in most of the treated specimens.

Meiotic studies did yield a number of chromosome aberrations such as fragments, laggards and anaphasic bridges.

Structural aberrations of chromosomes have been reported in X-irradiated materials by several workers such as Muller (1928), Rasch (1952), Sparrow (1962) and Revell (1965). Comprehensive analysis of the various aberrations have already established that X-rays cause terminal and interstitial deletions, formation of rings and polycentrics like dicentric and tricentric chromosomes. Evans (1967) concluded that the aberrations are only indirect secondary effects of lesions caused on the chromosomes by X-rays.

Several morphological abnormalities observed during the present study were common in any X-irradiation experiment. The occurrence of dwarf plants in the treated stocks were reported by Gelin (1954), Sarda (1959), Athwal (1961) and P.N.R. Nair (1964). There were 14 dwarf plants in the present experiment. The occurrence of dwarfs and leaf type variants such as narrow leaved plants according to Gordon and Weber (1950) was due to the low level of Oxygen content in the irradiated plants, which consequently results in physiological disturbances.

In the present study there were two chimeric plants in the treated populations, which were subjected to detailed study.

Similar chimeric plants were also reported by earlier workers like P.N.R. Nair (1964), Monti (1965), and Gichner (1966). The observed plants in this case were sectorial chimeras and these would have resulted by one of the cells of the stem primordia within the seed turned to an abnormal type by radiation. Such a possibility has been shown by Brumfield (1943) who, employing X-rays had induced sectorial chimeric tissues in roots of Vicia faba.

Few of the M_2 progenies (14%) produced on the variegated branches were variegated in nature. No such M_2 variegated progenies were obtained from seeds formed on the other branches of the chimeric plants or from other plants. The effect of maternal

tissue may possibly be the reason for M_2 variegated seedlings. But in the absence of controlled cross breeding studies, this possibly could not be established. But it did show that the production of chimeric behaviour in the M_1 plants was not a transient effect, as it was transmitted to the M_2 generation also.

The M_2 progenies of these chimeric plants showed a higher rate of chlorophyll mutants (39.8%), mostly lethal 'xanthas'. It shows that mutations on a genic level were also involved in these chimeric plants.

Several colour variants were observed in the M_1 and M_2 generations. Similar colour changes were reported by previous workers like Goodspeed (1930), Bruns (1954), Hoffmann and Zoschke (1955), Sjodin (1961) and P.N.R. Nair (1964).

It is important that in this investigation there was a plant showing full purple colour in the M_1 generation and its progenies were all purple in the M_2 generation, with varying grades of diluted colouring. In the M_1 generation there were other plants also which produced partially or fully purple flowers and fruits. The M_2 progenies of normal looking M_1 plants also developed differential purple colouration in differing frequencies. These colour manifestations, although show sure changes in the genic level, present very complicated patterns of expression and inheritance. Only by further breeding studies can any satisfactory

explanation be given on this phenomenon.

Chlorophyll Mutants

In the M_2 generation chlorophyll studies revealed the occurrence of " xantha " and " chlorina " types of seedlings, the former type increasing in frequency with increase in dosage of irradiation.

Gustafsson (1940) reported similar mutants in barley. Other workers like Blixt (1961), P.N.R. Nair (1964) and Popovic and Zecevic (1965) have also reported occurrence of several types of chlorophyll mutants in the segregating generation.

The increase in the frequency of " xanthas " with higher dosages might possibly be due to the severity of mutagenic effect of X-rays and these are sure lethals; they do not live beyond a few days.

Mutation percentage shows a decrease on the M_1 basis, with increase in dosage. This is probably due to a more severe elimination of lethals and structural mutants in the M_1 generation in the higher dosage treatments, which reduce the frequency of mutants of a less severe kind like the chlorophyll mutants in the M_2 generation in these treatments.

SUMMARY

S U M M A R Y

This thesis work was oriented towards the study of the M_1 parameters, and the chlorophyll mutation spectrum in the M_2 generation of the X-ray irradiated seeds of K-1 variety of Chillies (Capsicum annuum L.).

Dry pareline seeds of chillies were subjected to X-ray treatment at doses of 1,000, 4,000, 8, 000 and 16,000 units, employing a Superficial Therapy Medical X-ray unit.

Germination tests showed higher percentage of germination in the control than in the treatments.

A reduction of survival percentage was noticed in the treated cases. A definite inverse linear relationship existed between increase of dosage and survival percentage.

X-irradiation showed an initial stimulative effect on plant growth as measured by height of plants.

There was also earliness to flowering in the treated plants, though it was not correlated to dosage.

The 15 Kr treatment caused most severe damage in terms of pollen sterility. The other three treatments did not affect fertility

Interesting morphological abnormalities were observed like dwarfs, narrow leaved plants and chimeras. A number of coloured ✓

plants were also present.

Cytological studies further revealed the deleterious effects caused by X-rays on the chromosome level.

In the M_2 generation there were chlorophyll mutants of the "xantha " and "chlorina " types.

The study as a whole has helped to gather definite understanding on the various biological effects of X-rays on the K-1 variety of chillies.

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APPENDICES

APPENDIX - I

IMPORTANT FEATURES OF THE K - 1 VARIETY OF CHILLIES
AS GIVEN BY ITS ORIGINAL BREEDERS

1	Length of fruits	(cm)	6.62
2	Girth of fruit	(cm)	3.20
3	Weight of fruit with calyx	(gm)	0.76
4	Number of seeds/fruit		73
5	Colour of fruit		Shining dark red
6	Pungency - capsaicin per 100 gm of dry chillies on moisture free basis	(gm)	1.75
7	Average yield per hectare	(kg)	1904

APPENDIX - II

DATA SHOWING SEEDLING HEIGHT OF 25 DAYS OLD SEEDLINGS
(M_1 GENERATION)

(SELECTION FROM ENTIRE POPULATION ON RANDOM BASIS)

Serial number of plants	Seedling height in cm				
	Control	Treatments in Kr. units			
		1	4	8	25
1	6.8	9.8	7.5	8.9	7.6
2	6.3	9.1	7.5	5.9	7.3
3	5.3	9.3	7.8	8.7	8.5
4	5.8	9.4	7.4	5.6	7.9
5	6.9	8.8	7.5	8.9	8.3
6	7.2	8.5	7.6	8.9	8.2
7	6.4	9.3	7.3	8.6	7.9
8	6.6	8.9	7.6	7.3	7.7
9	6.2	8.8	7.1	8.7	8.0
10	6.7	9.9	6.8	8.3	7.9
11	6.7	9.2	7.2	9.2	8.0
12	6.2	9.5	7.1	8.1	8.6
13	7.1	9.7	6.3	8.0	8.0
14	7.4	8.9	8.2	7.6	8.5
15	8.8	10.2	6.6	9.2	7.6
16	6.5	9.9	7.4	8.5	8.1
17	6.9	9.7	7.9	8.0	8.2
18	7.1	10.5	7.3	8.0	8.2
19	5.6	10.4	7.3	7.6	7.9
20	6.3	10.7	7.9	7.3	8.6

APPENDIX - III
ANALYSIS OF VARIANCE TABLE

Source	Sum of squares	Degree of freedom	Variance	F ratio
Total	128.55	99
Treatments	102.41	4	25.61	94.8**
Error	26.14	95	0.27	..

** Variance ratio highly significant at 1% & 5% level.

Critical difference = 0.38

APPENDIX - IV
 DATA SHOWING PLANT HEIGHT OF 60 DAYS OLD PLANTS
 (in cm) (M_1 GENERATION)

Serial number of plants	Treatments in Kr. units				
	Control	1	4	8	16
1	17.0	21.0	16.0	13.0	16.0
2	7.0	16.0	6.0	16.0	19.0
3	9.0	19.0	13.0	10.0	15.5
4	17.0	11.0	14.5	12.0	9.5
5	26.5	12.0	9.0	15.5	25.0
6	12.5	9.5	5.5	16.0	15.0
7	14.0	9.0	9.0	16.0	16.0
8	17.0	10.0	15.0	15.0	26.0
9	23.0	23.0	14.5	21.0	6.0
10	27.0	20.0	12.0	21.0	21.0
11	13.0	16.0	17.5	10.0	23.0
12	11.0	19.0	17.0	15.0	16.0
13	23.0	15.0	17.0	22.0	19.0
14	16.0	15.0	12.0	31.0	20.0
15	14.0	18.0	10.0	20.0	13.0
16	10.0	20.5	15.0	20.0	14.0
17	5.0	26.0	15.0	9.0	17.0
18	6.0	21.0	11.0	12.0	9.0
19	5.5	16.0	22.0	28.0	17.0
20	7.0	19.0	26.0	17.0	11.0

APPENDIX - V

ANALYSIS OF VARIANCE TABLE

Source	Sum of squares	Degree of freedom	Variance	F ratio
Total	3407.56	99
Treatments	183.88	4	45.97	1.35
Error	3223.63	95	33.93	..

Variance ratio is not significant

APPENDIX - VI
 DATA SHOWING DURATION IN NUMBER OF DAYS FROM
 SOWING TO FIRST FLOWER OPENING
 (M₁ GENERATION)

Serial number of plants	Control	Treatments in Kr. units			
		1	4	8	15
1	69	78	77	78	68
2	85	72	75	78	76
3	86	66	84	78	78
4	70	82	73	70	75
5	67	76	80	72	65
6	83	78	72	79	72
7	79	77	75	72	76
8	74	83	75	75	68
9	71	60	80	83	73
10	66	60	80	73	63
11	72	75	73	73	73
12	85	72	75	73	72
13	65	85	72	76	75
14	80	73	76	75	65
15	73	72	87	72	72
16	80	69	76	79	79
17	82	63	89	83	76
18	90	69	84	79	79
19	90	75	78	76	63
20	85	66	73	80	80

APPENDIX - VII
ANALYSIS OF VARIANCE TABLE

Source	Sum of squares	Degree of freedom	Variance	F ratio
Total	4720.59	99
Treatments	639.94	4	159.98	3.72 ^{**}
Error	4080.65	95	42.95	..

^{**} Variance ratio significant at 1% and 5% levels

Critical difference = 4.12

APPENDIX - VIII
 PERCENTAGE OF POLLEN STERILITY
 (M₁ GENERATION)

Serial number of plants	Percentage of pollen sterility				
	Control	Treatments in Kr. units			
		1	4	8	15
1	12.25	18.42	21.68	4.55	12.02
2	12.43	22.50	11.62	6.34	29.45
3	16.26	21.82	66.48	38.42	54.38
4	7.77	34.71	18.02	9.47	21.00
5	18.40	28.17	22.05	11.56	9.88
6	15.35	44.52	12.15	20.55	10.46
7	10.21	11.58	7.28	36.27	50.73
8	13.95	22.98	16.10	14.42	11.01
9	6.98	18.20	15.77	10.65	46.17
10	11.89	12.11	10.75	7.69	75.00
11	5.46	9.76	12.20	25.56	35.57
12	21.49	32.61	13.92	9.17	34.63
13	13.60	19.12	20.91	10.44	58.86
14	9.40	20.87	13.14	38.32	20.59
15	12.90	17.26	15.07	7.20	11.79
16	46.52	10.99	11.26	11.99	14.89
17	14.79	13.62	9.69	15.13	60.60
18	16.43	6.75	8.78	16.22	10.00
19	18.78	31.32	6.92	11.18	23.20
20	7.44	4.92	17.85	10.57	27.10

APPENDIX - IX
ANALYSIS OF VARIANCE TABLE

Source	Sum of squares	Degree of freedom	Variance	F ratio
Total	19486.29	99
Treatments	3514.16	4	878.54	5.22**
Error	15972.13	95	168.12	..

** Variance ratio is significant at 1% and 5% levels

Critical difference = 8.16

ILLUSTRATIONS

* All colour photographs in Kodacolor.

ILLUSTRATION - I

**A dwarf plant in the 1 Kr. treatment
in the M_1 generation.**



ILLUSTRATION - II
(ABOVE)

**A plant with narrow leaves compared to
a normal plant.**

ILLUSTRATION - III
(BELOW)

**Two plants showing leaf shape
variation.**



ILLUSTRATION - IV

**Photographs showing variegated branches
of chimeric plants.**



ILLUSTRATION - V

**Erect type fruits borne on treated plants
are compared to normal pendent fruits.**

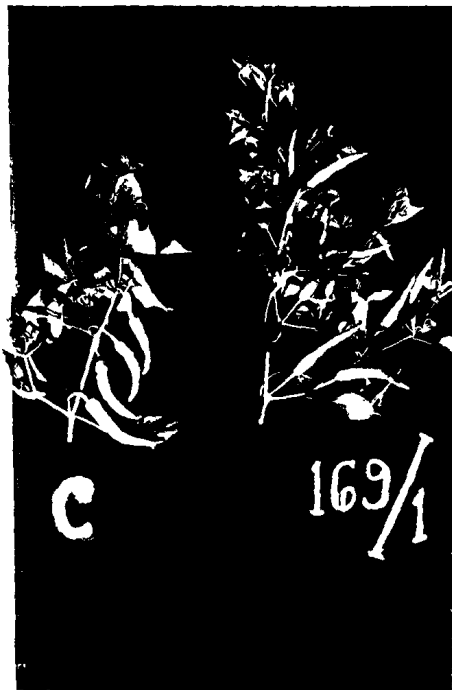


ILLUSTRATION - VI

**A high yielding plant with erect
type fruits.**

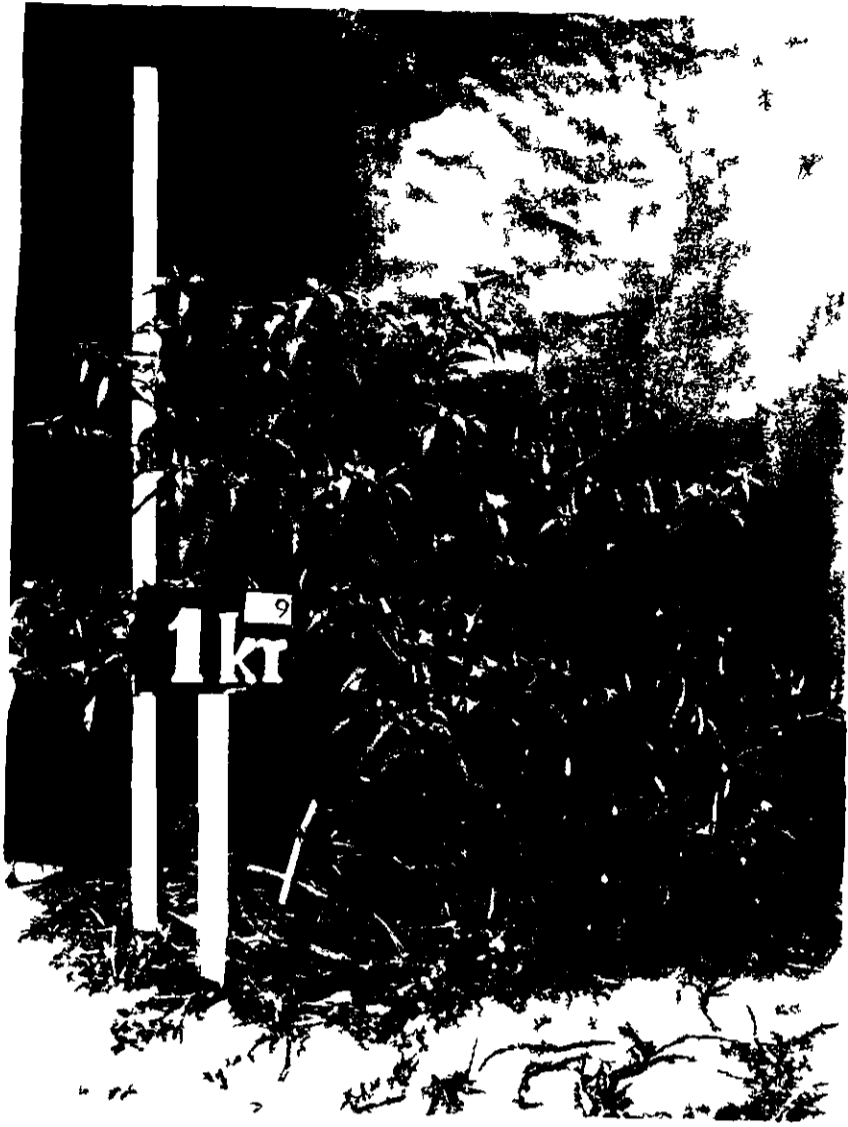


ILLUSTRATION - VII

A purple coloured plant



— — — — —

ILLUSTRATION - VIII

(ABOVE)

A single purple coloured flower

(MIDDLE)

Flowers showing different purple shades

(BELOW)

**Fruits showing different shades of purple
colour.**

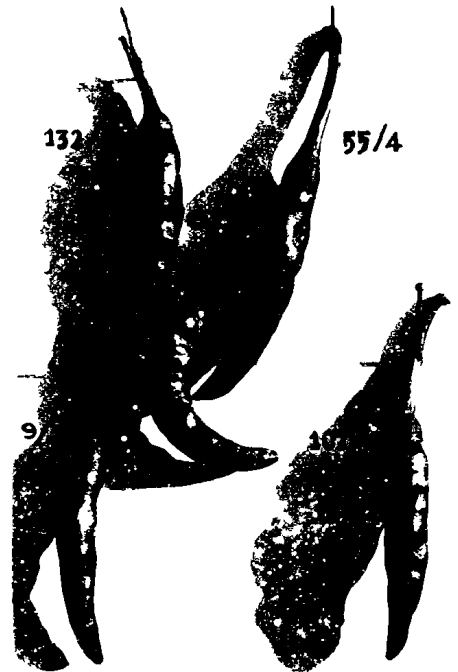


ILLUSTRATION IX

Chromosome Aberrations.

