The Effects of Cobalt 60 Gamma Radiation on
Microsporogenesis and Male Gametophyte
Development in Tomato (*Lycopersicon*
esculentum Mill.)

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CHAPTER I

INTRODUCTION

The physiological reactions of living organisms and living cells to ionizing radiations have long been of interest to biologists. The subject has been studied from many aspects, but their growing use for the induction of heritable genetical and cytological changes calls for further study of the effect of irradiation on dividing cell behavior.

It has been established both genetically and cytologically that the chromosomes may be altered under the influence of various radiations. These alterations may involve the mutation of one or more genes, the deletion or inactivation of portions of chromosomes, or the fragmentation and subsequent translocation of chromosome segments.

Many of these changes are permanent and may be perpetuated in further cell-divisions, being finally transmitted to the progeny of the treated organism through the gametes. On the other hand, destructive changes may be induced, resulting in the death of the cell and the destruction of the chromosomes. These changes are of no genetical consequence.

The haploid phase of the plant reproductive cycle offers some distinct advantages to genetic analysis. Here inheritance is not complicated by dominance relationships and the segregations are much less involved than in disomic inheritance. The greatly simplified problem of irradiating the inflorescences, rearing large populations and opportunities of tetrad analysis are among the reasons which support the usefulness of germ-cell irradiation.

Tomato pollen is an excellent material for the analysis of irradiation effects. The influence of radiation on microspore development, nuclear differentiation as well as the chromosome structure can be conveniently studied. Extensive quantitative analyses, which primarily concern the mechanism of chromosome breaks and reunions induced by radiation have already been reported (2,8,13,77). Production of male sterile mutants by using pollen from irradiated buds for commercial hybrid seed production has been recognized as a possibility (25). The present investigation deals with the effects of Cobalt 60 gamma radiations on microsporogenesis and male gametophyte development, in tomato (Lycopersicon esculentum Mill.). The following studies have been made:

1. Irradiation effects on flower buds prior to anthesis:
 - (a) Studies on cytological abnormalities;
 - (b) Studies on pollen from treated buds prior to anthesis.

2. Irradiated pollen at various dosages for:

- (a) Studies on germination behavior in vivo and in vitro;
- (b) Fruit and seed production;
- (c) Studies on pollen abortion in X_1 individual plants.

These studies were made in an effort to determine the effect of irradiation on nuclear phenomena in the process of microsporogenesis and male gametophyte development.

CHAPTER II

LITERATURE REVIEW

The idea that the hereditary changes could be induced in animals and plants by irradiation has been known since the classic work of Muller (47) and Stadler (68). Relatively little work was done to use radiation as a tool for plant breeding until 1950. It was believed by many geneticists that radiation caused lethal or destructive genetic changes. The present more optimistic attitude towards the use of radiations for plant improvement has been due to the persistent, successful efforts of European workers. Also contributing towards this attitude has been the greater availability of radiation sources and generally increased knowledge of hereditary materials. The accounts of programs to induce beneficial transformations come from various countries, e.g. Canada (39,63); Germany (22,32,70,71); India (11,12,46); Russia (57,75); Sweden (18,19,29); and others. Plant breeders in the United States have recently started to apply radiations in their search for genetic variability. The results of various workers Gregory (28); Frey (23,24); Konzak (36,37) indicate the usefulness of this new tool.

Goodspeed (27), Rick (50,51,54) and Stadler (68) established that variation could be induced by irradiation of microspores. Even though somatic selection is relatively fast in detecting mutations, the highest sensitivity to radiations in corn was shown by Singleton (64) to occur during meiosis with at least 25 per cent of the grains being injured. It was further reported by Singleton (64) that the stage of greatest mutability occurred fully 10 days after the stage of highest radio-sensitivity, when the mutation rate for endosperm character exceeded 3 per cent per gene. Singleton, et al. (65) demonstrated the observable spontaneous microspore mutation rates to be 50 to 100 times higher than the accepted megaspore rates, which were less than 10 per million gametes. There was a specific period of several hours for each species between the first meiotic division and the first nuclear division of the megaspore. However, in the microspore, all mutations that occurred throughout the period prior to fertilization could be observed. The period from meiosis to fertilization was about 2 weeks.

Normal gametogenesis in the tomato, including the early development of floral organs and derivation of the sporogenous layers, has been described in detail by Smith (66). Sporogenous tissue was differentiated very early in the development of the anther. As seen in a transverse

section of the anther, it appeared as a crescentic mass, several cells in thickness. The pollen mother cells were readily distinguished from other cells by their very dark staining cytoplasm. Their nuclei gradually enlarged until, in prophase of the first meiotic division, they nearly equalled the volume of the cytoplasm of the cell. The 2 meiotic divisions proceeded very rapidly, giving rise to a 4 nucleate pollen mother cell. The 4 nucleate stage and the subsequent 1, in which 4 microspores were delimited by cell walls, persisted for a much longer period than the meiotic divisions. After the uninucleate microspores were released from the old pollen mother cell wall, they quickly assumed a spherical shape and enlarged until each finally reached a volume comparable to that of the original pollen mother cells. The microspores became refractory for study of nuclear behavior early in this final period because their cytoplasm changed to a dense, granular condition. According to Smith (66), a single mitosis occurred during this interval resulting in a mature pollen grain containing a generative and a tube nucleus.

Many papers (26,35,44,60,62) have been published dealing with investigations to describe the effects of ionizing radiations on dividing cells and to identify the stage most sensitive to these radiations. Death, retardation of the cell division, and the production of aberrations

in the chromosomes have been used to study the effects of radiation. Every stage of division, including interphase, has been reported to be the most sensitive stage in various animal and plant materials studied (26).

Sax and Swanson (61) and Koller (33,35) have reported the primary or physiological effects of radiation on pollen grain development. From their work, it can be inferred that the duration of the pollen grain division can be shortened or lengthened by irradiation and by small changes in temperature. Even though the cell populations from individual locules of an anther vary considerably, an index expressing the proportion of post-metaphase/metaphase stages between anthers of the same flower bud was accurate enough to test the effects of radiation. No attempt was made in their work to classify the stages of the first microspore division other than pre-metaphase, metaphase and post-metaphase. They concluded from their experiments that irradiation suppressed prophase; that premeiotic cells were not affected; that prolonged metaphase was observed as long as 48 hours after irradiation and that all stages of division were retarded with "the effect on metaphase being the most obvious."

In retarding the rate of division or delaying the onset of meiosis or mitosis in cells the radiations might act on the cytoplasm, the nucleus, or both. When cytoplasm

was the seat of action of the radiations in the studies of Duryee (17), it was found that if the cytoplasm of enucleated frog eggs was given a dosage of 50,000 roentgens and the unirradiated nucleus was then returned to the cytoplasm, the same sort of chromosomal abnormalities appeared as occurred when the entire egg was irradiated. On the other hand, the nucleus, irradiated with the same dose in salt solution and then put back in untreated cytoplasm, showed no such defects. Other studies made on changes in cytoplasmic properties have also indicated marked effects. Thus Mukerji (46) found that the mitochondria were destroyed and the golgi apparatus was swollen. Scott (62) has summarized the main changes seen in the cell as a decline in glycolysis or no change at all; an increase in acidity; and an increase in viscosity. To what extent such changes can be correlated with retardation of cell division is still uncertain.

Nuclear changes following irradiation have been more intensively studied than cytoplasmic effects and extensive literature upon the subject is available. Sax and Swanson (61) have discussed the desirability of recognizing 2 fundamentally different types of effects of ionizing radiations on nuclei; (a) permanent changes in the morphology of the chromosomes, such as breaks, deletions and gross alterations, as well as point changes or

mutations; and (b) temporary cessation of mitosis and clumping of chromosomes in metaphase and anaphase. Lea (41) has discussed and analyzed the literature on the types of effects listed under (a). Under certain conditions reconstitution of the chromosome split occurred, e.g., under the action of centrifugation, at higher temperatures as contrasted to low, under the influence of ultraviolet radiation, and also under conditions in which nucleic acid was condensed on the chromosomes (31). Otherwise the breaks were permanent. Lea regarded the bulk of evidence as pointing towards breakage resulting from the action of the single ionizing particles (target theory). A single particle might break 2 chromosomes at the same time (isochromatic breaks) if the chromatids were lying close together and the pathway of ionizing particle was sufficiently long.

Two types of changes in chromosome chemistry have been postulated in order to explain some of the physiological effects of ionizing radiations: (a) depolymerization of the nucleic acid, leading to stickiness and clumping of chromosomes (15); and (b) interference with nucleic acid production or its conversions (45). Whatever the final analysis, scientists are inclined to attribute the various effects of radiations as resulting from their action on the enzymes engaged in syntheses. The possibility of a

selective action on enzymes has been suggested by the sensitivity of sulfhydryl enzymes (1).

The results of several workers (3,4,59) using *Tradescantia* as the experimental material, have emphasized the necessity of considering the physiological expression, not only as an effect of radiation in itself, but also in relation to chromosome breakage. It has been reported that several factors may be related to breakage frequency. These are unspiraling, clumping errors in reproduction and abnormal centromere behavior. Also such cellular disturbances as inhibition in development, stimulation and modifications in the chemical process occurring in the cell may influence the frequency of breakage. Beatty and Beatty (3) studied the physiological effects occurring in the different stages of the first microspore division of *Tradescantia* which altered the frequency of chromatid aberrations. Since much of the information was derived from irradiation-produced chromosomal aberrations, the studies of the physiological effects were extended to the developmental stages of the microspore--the time irradiation induced chromosomal aberrations became manifest.

Goodspeed (27) has described the effects of treating pollen mother cells of *Nicotiana tabacum* with X-rays and radium when the treatment was applied during the late archesporial stage or during early prophase. He found that

the treatment gave rise to abnormalities involving fragmentation, non-disjunction and non-conjunction of the chromosomes. These changes first became apparent at anaphase of the first division. He concluded that the chromosomes were structurally but not visibly altered as the result of treatment, and that these alterations only became apparent when the chromosomes were subjected to the anaphase forces.

The relationship between nuclear development, chromosome aberrations, and microspore sterility has been studied by Sax (58,60). His findings have shown that the susceptibility of the chromosomes to X-ray treatments was the greatest at meiosis and presumably at meiotic prophase. Since all the chromosomes found in a tetrad of resting microspores were already differentiated at late pachytene of meiosis, it appeared that the meiotic chromosomes were much more susceptible to X-ray breakage than the chromosome in resting nuclei of the microspores. In this study of Sax, the chromosomes at meiosis have been reported to be found at least 10 times more susceptible than chromosomes at the resting stage in the microspore. The prophase stage of mitosis was more susceptible to X-rays than the resting stage, but at prophase about half the breaks were chromatid breaks, while X-rayed resting nuclei showed only chromosome breaks at metaphase and anaphase. The mitotic prophase

stage was found to be about twice as susceptible to X-ray treatment as the mitotic resting stage.

The irradiation of meiotic cells produced a high degree of microspore sterility, but some microspores did develop. These microspores, even though they included the more viable cells, showed a large proportion of breaks. Every chromosome might have been broken, but if no fragments were lost, the microspores developed normally. Occasionally diploid microspores were produced after irradiation, and these also had many chromosome aberrations. These microspores were produced from meiotic cells which were irradiated at interphase or during the second meiotic division (59).

It has been demonstrated that ionizing radiations applied to microspores of *Tradescantia* shortly after meiosis induced mutations which were expressed in abortion and changes of size in the subsequently developed pollen grains (54). Evidence existing in genetic literature has suggested that many characters may be expressed in the pollen. For example the genes determining small pollen size in Zea mays were discovered by virtue of the fact that the small pollen is less viable. Other respects in which genetic autonomy occurred in pollen include chemical composition, semi-sterility and self-sterility alleles.

Interstitial deficiencies have been found to occur

simultaneously, or they can be readily induced by radiation. Ultraviolet radiations have given rise chiefly to terminal deficiencies in maize (14). On the other hand Barton (2) has shown that ultraviolet radiation can induce interstitial losses in tomato chromosomes. He has raised the question whether there exists a species specificity as to the type of aberration that will arise following exposure to various types of radiations.

Since deficiencies involved the loss of genetic material, it would be expected that deficiencies would have deleterious effects on an organism. This effect would depend upon the amount of genetic material and its quality. Creighton (14) has described one such deficiency in maize that survived in the haploid state, i.e., passed through male gametophyte. The great majority acted as gametophyte lethals in that they caused pollen abortion. Deficient gametes, however, could survive to take part in fertilization in animals but the haploid generation in plants served as a very effective screen for the removal of such gametes. Deficient chromosomes could survive more readily through the female side in plants. On the male side, if they were not entirely eliminated by the formation of unviable pollen grains, the pollen grains that contained the deficiencies could not compete successfully with normal grains.

Mature pollen from a single plant clone of the tomato variety Valiant was irradiated with 4,000 roentgens by Clayberg (13). An X_1 generation of 199 plants was grown in the field. Preliminary estimates of pollen abortion in this study gave an interplant range of from 5 to 80 per cent abortion with 80 per cent of the plants having no greater than 20 per cent aborted pollen. There were 23 plants in the range 40 to 80 per cent abortion. Meiotic studies of diakinesis in 20 of these latter plants revealed only 4 reciprocal translocations. Barton (2) who also used a dosage of 4,000 roentgens in his studies, obtained 8 reciprocal translocations in a random sample of 48 plants for an induction rate of 17 per cent as compared with 2 per cent in Clayberg's findings.

Genetically determined pollen abortion, whether conditioned by chromosomal deficiencies, duplications, translocations or by gene mutations has been frequently reported in the literature. There are reports of similar control of microspore and pollen size (54). However, pollen size might be determined by the sporophyte producing the pollen rather than by genetical control in the microspore itself. Male sterility is thus an inherited abnormality. But this abnormality can be successfully employed in the commercial hybrid-seed production. Rick (55) has pointed out the advantages of using male sterile

mutants: emasculation was unnecessary; there was no contamination from self-pollination; they were frequently easy to identify in the field because of their greater vegetative growth (this is particularly true of tomatoes); and since they usually were determined by a single recessive gene, they constituted 50 per cent of a back cross progeny and were therefore easy to obtain in sufficient numbers. Male sterility has now become an asset as a method which can eliminate the necessity of hand emasculation.

CHAPTER III

MATERIALS AND METHODS

Gamma radiations

The Cobalt 60 gamma radiation facility of the University of Florida Agricultural Experiment Station was used for treatment of selected experimental materials. This irradiation unit is one of the largest in the United States. A detailed description has been reported by Teas (73). An aerial and sectional view of the facility and the details of the Cobalt 60 irradiator have been presented in Figure 1 (after Teas).

Selected experimental materials

Because of its importance as an economic plant, the tomato has been the subject of numerous cytological investigations. The pachytene and later meiotic chromosomes of the tomato offer interesting material for cytological and cytogenetic studies because of their morphology. The chromosomes of the tomato are strikingly differentiated into regions differing in diameter and staining capacity. In addition, the differential regions seem to show differential behavior during meiosis with respect to pairing, location of chiasmata and contraction.

Fig. 1

AGRICULTURAL EXPERIMENT STATION
IRRADIATION FACILITY
UNIVERSITY OF FLORIDA

• AERIAL VIEW OF FACILITY
WITH SECTION OF PIT WALL REMOVED

SECTIONAL VIEW OF
FACILITY
RADIATION PIT IN OPERATION

DETAIL OF CO⁶⁰
IRRADIATOR

The structure of the tomato chromosome is most clearly evident at the pachytene stage of meiosis. The chromosomes typically show the following structural characteristics: (1) a centromere, (2) chromatic regions on either side of the centromere and of varying extent for each arm, (3) achromatic distal regions, and (4) small terminal knobs, or telochromomeres, which appear to terminate all chromosome arms.

Bonny Best and Roma varieties of tomato have been used in the present study. Bonny Best is a widely adapted early variety with round, deep, scarlet, solid fruits. Its vines are vigorous and productive. Roma, on the other hand, is a warm weather variety which was selected for mid- or late spring plantings. It has exceptional fruit setting potential although the fruits are rather small. Both of these varieties have an extended blooming period and the buds in meiotic stages of development may be found from time to time for considerably long periods. Cytological studies of microsporogenesis were made on Bonny Best variety only but both Bonny Best and Roma were used to study the effects of irradiated pollen on fruit and seed set and on abortion of the pollen of the subsequent generation.

Irradiation of the tomato stock

Tomato microspores in various stages of development were irradiated in the inflorescences intact with the mother

plant. Plants were placed at predetermined distances around the irradiator and by varying the length of exposure, dosages from 600 to 1,400 roentgens (r) were obtained. All 1 to 14 mm size buds were immediately tagged.

For irradiation of the mature pollen, whole flowers of the Bonny Best and Roma varieties, before and after anthesis, were collected. Flowers, in small plastic vials, were irradiated after being placed in a galvanized iron container. Mature pollen was collected from the treated inflorescences and the non-treated controls. The pollen from a single flower was considered a unit collection and only 1 sample was taken for either pollination or germination studies per collection. Pollen was treated with various dosages from 800 to 100,000r.

Studies on anther development

The length of time for development of the different stages in microsporogenesis and gametophytic development were determined in Bonny Best variety. The measurements were carried out by marking buds with india ink and making smears each day from other buds of the same size from the same plant or from the two halves of the same anther when possible. Although the division seemed to occur in both microspores and microspore mother cells at all hours of the day, it was generally easier to obtain the meiotic divisions between 8 and 11 a.m. and microspore divisions between 3 and 6 p.m.

The development of the anther for each of the 600r, 800r, 1,000r and 1,400r irradiated buds was studied and compared with unirradiated controls of the same age and in similar stages of development. The preparations consisted of fixed and sectioned anthers or anthers smeared directly in aceto-orcein. Both of these techniques were useful, but smears were found to be particularly helpful as an aid to the study of pollen mother cells and male gametophytes. Sectioned preparations were necessary for the study of the nonsporogenous tissues and their temporal and spatial relations with the sporogenous tissue.

It was essential to establish the developmental ages of the anthers before the treated plants could be compared with the normal type. Timing of the final stages of the development including anthesis of the flower could be followed accurately in the differentiation of the endothelial cells that regulated the opening of the dehiscence slit. In advanced stages these cells elongated radially and retained an active condition longer than other sterile cells of the anther. By examining these tissues it was possible to classify anthers without much difficulty into 14 stages (stage II to XV; Fig. 2) of development between the early differentiation of pollen mother cells and anthesis.

Radiation effects on sporogenesis

It was difficult to obtain reliable data from quantitative analysis of gamma ray effects on a cell population owing to the developmental differences between cells at the time of irradiation. But it has been shown by Koller (33) that in spite of the developmental differences the proportion of the dividing pollen grains in metaphase and post-metaphase stages was similar in sister anthers of *Tradescantia*. Therefore only the dividing cells and the relative proportion of the division stages were utilized when analyzing the effects of irradiation. The observations were made on aceto-orcein smears of microspore mother cells and microspores of Bonny Best tomato at various stages of the nuclear cycle.

Beginning with the older buds, an anther from each bud was examined until 1 with the pollen mother cells in first meiotic metaphase was found. This bud was left on the plant and all the others removed. The remaining buds were numbered according to size and their position on the inflorescence. At different periodic intervals a bud was removed and examined to determine the approximate location of the buds with pollen mother cells in various stages of the meiotic prophase. These buds were then exposed to gamma rays. The remaining anthers from the bud that had shown the prophase figures were smeared soon after

irradiation. An anther from the bud immediately above was then examined. If anaphase and metaphase figures were found, the bud was removed and smeared and the next bud examined until one with its pollen mother cells in pachytene stage was found. This was usually 2 to 4 buds above the first 1 with anaphase I figures. The bud was examined 24 hours later when most of the cells were in the first meiotic anaphase. When the inflorescence was approaching its maximum growth, usually about 11 to 14 days after irradiation, it required approximately 48 hours for cells in pachytene to reach the first anaphase.

After mounting, the preparation was heated until a few bubbles appeared. This treatment resulted in a better differentiation between nucleus and cytoplasm. After the slide had been heated, it was inverted on filter paper and slightly pressed to remove the excess fixing fluid. A mixture of gum mastic and paraffin was used for sealing. When kept in a cool place such preparations remained in good condition for several weeks. These were studied with the microscope under oil immersion for chromosome fragmentation and other aberrations.

The pollen was collected after anthesis for examination of the aborted pollen from buds of irradiated plants. A sample of this pollen was mounted in aceto-orcein on a slide and covered by a cover glass to prevent any distortion

of the pollen. By using aceto-orcein it was possible to determine pollen abortion and to estimate the size of normal healthy grains in the same collection. In this stain normal grains remained turgid and completely expanded as compared to the shrivelled aborted grains.

Pollen germination trials *in vitro*

Two germination media, one containing 15 per cent sugar-75 ppm boron-2 per cent agar and the other containing 20 per cent sugar-100 ppm boron-2 per cent agar solutions were used for pollen germination and pollen tube growth. The hanging drop technique for pollen germination was employed and the cultures were stored at 24°C. and 85 per cent relative humidity. Pollen was not considered germinated unless the pollen tube lengths were 5 times the diameter of the pollen grains.

Pollination and pollen tube study *in vivo*

Shortly after irradiation, the pollen was applied to stigmas of previously emasculated Bonny Best flowers. Simultaneously some stigmas were dusted with untreated pollen and others were left unpollinated. About 20 flowers were pollinated at each dose level and a total of approximately 300 pollinations were made. The plants were grown in a greenhouse maintained at a night temperature of 70°F.

At intervals of 6 hours, until 84 hours after pollination, flowers were collected from different levels of radiation treatments. The pistils were scalded for 3 minutes in water at 75°C. They were killed by immersing for several hours in 50 per cent alcohol containing 6 per cent formalin. The strand of conducting tissue was separated from the cortex and stained in a mixture of aqueous acid fuchsin and light green. The strand of tissue was cleared overnight in 80 per cent lactic acid and mounted in lactic acid (7). Another technique after Smith (66) was also employed wherein some pistils were killed, fixed and embedded in paraffin. Longitudinal sections of 10 and 12 micron thickness were cut, stained in iron-alum haematoxylin and resorcin blue and mounted on slides. The number of pollen tubes ending in each region greater than 5 times the pollen diameter within the pistil was recorded.

The percentages of self-pollinations of Bonny Best and Roma varieties that set fruit were determined in the following manner: each day from the time the first bloom appeared on the plants, 2 inflorescences (whenever available) were tagged with the date and the dosage of the irradiated pollen to be used for self-pollination. The self-pollinations on each of these inflorescences were counted and examined weekly until fruit had set or abscission had occurred. All data were taken and recorded on the basis

of the individual plants. This was continued for about 85 days after the first bloom.

As they matured, fruits from individual plants were harvested separately for seed extraction. Seeds were extracted by peeling off the epicarp of the fruits and allowing the remainder to ferment for 36 hours. Fermented pulp was then thoroughly washed and the seed dried and stored. These were later sown and plants grown to maturity for pollen abortion study of the X_1 generation.

Examination of the aborted pollen in X_1 generation

The mature pollen of X_1 generations of the Bonny Best and Roma varieties, obtained from the seeds resulting from normal megagametes and microgametes from irradiated pollen were examined in aqueous solution containing 0.3 gram Iodine, 1.0 gram Potassium iodide and 100 ml water. The percentage of visibly aborted grains was determined by counting the number of defective grains which were empty or small. Some contained starch grains indicating immaturity.

CHAPTER IV

EXPERIMENTAL FINDINGS

Microsporogenesis and male gametophyte development in normal buds of the Bonny Best tomato

Examination of the sporogenous tissue at an early stage revealed a mass of cells with dense, deeply stained contents and large nuclei (Fig. 2;I). By periclinal division a single row of primary sporogenous cells formed in the center of the archesporial cells. These continued to divide, and formed a mass of deeply stained cells with a large nuclei (Fig. 2;II & III). At the first meiotic division the chromosomes passed to opposite sides of the microspore mother cell, and the daughter nuclei were formed near the outer cell wall (Fig. 2;IV & V). This was followed by the formation of the dyad (Fig. 2;VI) showing the characteristic position of the nuclei. Both of the second meiotic division spindles were usually in the same plane and at right angles to the plane of the first meiotic spindle. Again the daughter chromosomes passed as far as possible to opposite poles, so that the four nuclei were near the periphery of the tetrad (Fig. 2;VII).

As the tetrad nuclei passed into the resting stage,

Fig. 2.-Stages of normal microspore development.

they took a position near the center of each cell. At a later stage the one-nucleated microspores separated and became independent cells. The nucleus, which at this time was in an early prophase stage, lay nearer the inner wall; at first closer to one end of the cell but later in a more central position (Fig. 2;VIII,IX & X). During these prophase stages large vacuoles appeared in the cytoplasm.

The chromosomes lay flat on the cell equator at the metaphase stage of the microspore division (Fig. 2;XI). The vacuoles were conspicuous on both sides of the dividing chromosomes. As the chromosomes passed to the poles, those near the inner or flattened wall of the microspore remained compact, while those near the outer wall elongated even before a nuclear wall could form. The compact nucleus was cut off by a thin wall resulting in a cell with very little cytoplasm. The other nucleus increased greatly in size and passed into the resting stage. This was the vegetative or tube nucleus (Fig. 2;XII,XIII & XIV). The vegetative nucleus seemed to begin disintegration even before the pollen tube was formed.

The small or generative nucleus never passed into a typical resting stage. The staining reaction was retained as it enlarged. The thin cell wall surrounding this nucleus was apparently broken as the nucleus rapidly elongated to form a slender sickle shaped body (Fig. 2;XV).

The pollen tube germinated at one end of the microspore. The generative nucleus passed into the pollen tube, where it divided to form the two male gametes.

Duration of microsporogenesis and male gametophyte development

The development of the mature pollen of Bonny Best variety has been summarized in Table I.

Table I. Duration of microsporogenesis and male gametophyte development in Bonny Best tomato

Level of bud development	Days of development beyond initial 2mm length of bud	Microscopic events	Visible changes
Less than 2mm	0-4	Pollen mother cell prophase	---
2-3mm	5-6	Meiosis	---
4-5mm	7-8	Growth of microspore	Anthers white
6-7mm	9-10	Vacuolation of microspore	---
8-9mm	11	Microspore division	Color appears on petals
10-11mm	12-14	Differentiation of nuclei; elongation of generative nucleus	---
12-14mm	15-16	Mature pollen	

The time required for the differentiation of the pollen mother cells from the primary sporogenous tissue was hard to assess in the smear preparations. However, the pollen mother cell prophase, in buds less than 2mm size, had an extended period taking about 36 hours. The differentiation of the prophase stage into very early, early, mid-, and late prophase stages was also difficult to distinguish for an accurate estimation of their duration. The remaining meiotic stages of prometaphase, metaphase, anaphase and telophase required about 24 hours for completion. Following meiosis, in buds of 2 to 3mm size, the resultant microspores remained associated as tetrads for 24 to 30 hours. After separation, as was observed in buds of 4 to 5mm size, there was an increase in the size of the microspore. The nucleus was centrally located, loosely reticulate and occupied about two-fifths the width of the microspore. The duration of this stage was between 24 to 36 hours. Vacuolation was easily recognized, in 6 to 7mm buds, by the more compact nuclei and corresponding microspore size, particularly in length. This stage lasted for about 36 hours. The next stage in microspore development was quite distinctive due to the movement of the nucleus to one end of the microspore, during the formation of a central vacuole. The duration of this stage was approximately 24 hours. The next stage was characterized by a distinct vacuole and nucleus flattened

against one end. The cytoplasm was arranged around the periphery of the microspore. Finally, the preparation from the buds of 12 to 14mm size, showed an increase in size of both nucleus and microspore. The enlarged, spherical nucleus began to move away from its position at the end of the microspore, projecting into the vacuolated area. Serration on the outer microspore wall was evident at this stage. The last 2 stages appeared to take 36 to 48 hours for completion. Thus anthesis occurred about 16 days beyond the 2mm stage of the bud development.

Development following irradiation of anther

The effect of various dosages (600r, 800r, 1,000r and 1,400r) on microsporogenesis was somewhat similar in all the treatments except 1,400r in buds shorter than 2mm. At the high dosage, degeneration of the anthers occurred before a distinct sporogenous tissue could progress to the stage of meiosis. The walls of the sporogenous cells collapsed and degenerated even before the prophase stage was initiated.

The 800r treatment was selected as being representative of the other treatments and results are reported for buds treated at this level. Even though no direct measurements were made, microsporogenesis appeared to be prolonged in the 4-nucleate stage of the pollen mother cells or in the immediately subsequent tetrad stage.

Development was normal up to meiotic prophase; after that meiosis appeared to be delayed to varying degrees.

Evidence of abortion appeared at practically all stages of meiosis from prophase to intermediate stages of microspore development. Cells that were affected in early stages were either resorbed or so greatly modified that they could not be recognized as typical cells at anthesis. Aborted microspores still adherent in tetrads were seen in fully one-third of the anther smears. Microspores aborted in later development also persisted to anthesis in recognizable form. Although the stage at which actual degeneration of anther cells occurred varied with the rate of microsporogenesis of cells within a locule, the anther cells degenerated at the same time. Thus some cells were in various stages of meiosis while others had reached microspore stage at the time of degeneration.

The development of the irradiated tapetal cells were characterized by several deviations from similar cells which were not irradiated. Early differentiation of the irradiated tapetal cells at 800r appeared normal but enlarged at a slower rate and finally reached a maximum size which was only half that of the tapetal cells in the normal type. This delay in development was also manifest by their slow degeneration, even though at anthesis most of the walls of the tapetal cells were still intact.

Irradiation effects and consequent nuclear changes in
microspore development

After the formation of the uninucleate microspore at meiosis there was approximately an 8-day period before the pollen grain division. With this latter mitosis, the microspore became binucleate pollen grain, and remained in that condition until the division of the generative nucleus. This division took place after the pollen tube had almost reached its maximum development.

It was observed in the preparations of microspore mother cells, made from the buds treated with 800r, that the beginning of the microspore division was marked by the movement of the nucleus to a central position. In most observations no visible change took place in the structure of the nucleus until it reached this position. The very early prophase lasted approximately 36 hours. Preparations that did not show any later stages of division than very early prophase readily showed some cells in which the spherical nucleus began to move away from its position at the end of the microspore, projecting into vacuolated area. However, preparations showing later stages of division usually did not show any stages earlier than very early prophase. All cells in very early prophase did not seem to move into the next stage at the same time. More than 36 hours were required for all the cells to move out of

very early prophase although the remaining stages of division required 24 hours for completion at 70° F. This reservoir of cells in very early prophase was depleted by 3 to 4 per cent of the cells moving into early prophase. The end of the first microspore division was recognized by the presence of 2 nuclei in the microspore.

Both chromosome and chromatid aberrations found at metaphase were readily distinguished and this permitted a direct indication of the nature of the chromosomes at the time of exposure to radiations. Whole chromosome aberrations commonly included small acentric fragments and dot deletions. More rarely, centric or acentric rings and chromosome exchanges resulting in dicentric chromosomes or simply translocations were also noted. Chromatid aberrations, on the other hand, included chromatid exchanges and, at certain stages small chromatid deletions.

In cases where there was degeneration, the cytoplasm and the nucleus at first seemed to be closely associated, and their entire degenerating mass held stain firmly. Shortly thereafter both the nucleus and cytoplasm lost their capacity to hold stain. As a result, the early degenerating microspores were distinguished at anthesis by their shrunken, irregular shape and by their more or less colorless appearance when stained with aceto-orcein. Furthermore, at anthesis in degenerated anthers such

microspores comprised a considerable portion of the so-called "pollen grains." This fraction was characterized as "visible aborted grains."

In addition to this early degeneration, the microspores were subject to degeneration at any period during their maturation up to the meiotic division of the pollen nuclei. This was indicated by their variation in shape, appearance of their cytoplasm, and their staining properties. The division of the microspore nucleus to form the vegetative and generative nuclei did not proceed regularly and extra chromosomal fragments occurred as a result of this irregular division of the microspore nucleus.

Considering that the proportion of the dividing microspores in metaphase stages was very similar in the anthers of the same bud, an estimate of the dividing cells and the relative proportion of division stages was made while analyzing the effects of irradiation. Data have been reported in Table 2. It is evident that following irradiation with 800r, there was a decrease in the number of microspores in prophase. The lowest level was found at 4 hours after treatment (33.2 per cent) after which the number of microspores in prophase again started to rise. Another effect of this treatment was an increase in the number of microspores in metaphase.

Table 2. Frequency of stages at different times after irradiation with 800r
(Bonny Best tomato)

Treatment	Hours after treatment	Total number of microspores	Per cent of dividing cells in		
			Prophase	Metaphase	Post-metaphase
Control	1	305	68.3	11.9	19.8
"	2	288	69.6	12.2	18.2
"	4	315	73.8	10.8	15.4
"	6	211	72.1	11.3	16.6
"	24	267	70.2	11.9	17.9
"	48	243	75.3	10.3	14.4
800r	1	214	70.1	16.3	13.6
"	2	279	60.9	21.8	17.3
"	4	323	33.2	27.5	39.3
"	6	284	69.7	17.7	12.6
"	24	302	81.5	10.4	9.1
"	48	192	78.1	11.1	10.8

The effect of irradiation on various stages of pollen grain mitosis has been shown in Figure 3. It appears that 4 hours after irradiation, when the "prophase-suppressing" effect is greatest, the relative proportion of the post metaphase stages is the highest. The number of microspores is very high which indicates that the "metaphase-prolonging" effect of radiation is active.

Chromosome aberrations at various stages of micro-sporogenesis

The period during which the chromosome sensitivity was studied extended from the third to the sixteenth day after irradiation of the 2mm size buds. This provided stages from just prior to the pollen grain mitosis to the prophase of the generative nucleus division, and thus included both chromosome and chromatid aberrations. The effects of irradiation on earlier meiotic stages were difficult to distinguish except occasional "clumping" of the chromosomes which might have resulted in prolongation of the metaphase stages.

Chromosome aberrations were used as a measure of radiation damage. Following irradiation (800r) of buds of 2 to 4 mm size, the cells were examined at anaphase I. The observed effects may be placed into two groups, namely fragmentation and achromatic spots (similar to secondary constrictions) in the chromonemata. These buds were in the

Fig. 3.-Frequency of grains in pollen grain mitosis showing suppression of prophase after treating with 800r (var. Bonny Best).

meiotic prophase at the time of irradiation. In Table 3 are recorded frequencies of fragments observed mainly at the first meiotic anaphase in buds of the same inflorescence removed at various times after treatment with 800r.

Table 3. Frequencies of induced fragments following metaphase I at various times after irradiation with 800r; Bonny Best pollen mother cells

Time after irradi- ation	Total pollen mother cells	Frag- ments Total X 100	Achro- matic Total X 100	Total aber- rant Total X 100
1 hour	413	0.60	0.00	0.60
1 day	254	47.10	0.00	47.10
2 days	281	94.00	1.10	95.10
3 days	92	61.50	1.70	63.20
4 days	82	54.40	1.30	55.70
5 days	289	44.10	0.20	44.30
6 days	199	94.30	0.30	94.60
7 days	378	56.16	0.06	56.20
8 days	132	8.30	0.10	8.40

The number of chromosomal abnormalities observed at various intervals after irradiation and compared with different stages of microsporogenesis showed 2 distinct maxima (Fig. 4) at second and sixth day after irradiation. Most of the cells in the second day fixation were in late meiotic prophase or metaphase I stages indicating that they were in early prophase at the time of irradiation. The cells of the sixth day fixation were in metaphase II

Fig. 4.-Induced aberrations in meiotic chromosomes during an eight day period following irradiation of pre-meiotic buds at 800r (var. Bonny Best).

stage. The data indicate that the sensitivity is approximately equal (94 per cent) at both these stages. Following the second day observation (with most of the cells in meiotic prophase) there was a steady decline until metaphase II, when the percentage of abnormalities again rose almost equal to that of the prophase. The small percentage of abnormalities observable after 7 days, illustrates the futility of pursuing this study beyond this time. Pollen mother cells, 1 hour after irradiation, resulted in only 0.60 per cent total abnormality which may be considered as spontaneous or naturally induced breakage. It is improbable that such low fragmentation would be the immediate result of radiation treatment because of the short interval between treatment and observation. The eighth day figures, when most of the cells were in interphase, were considered unreliable because of the elimination of fragments in preceding stages. It would appear that the best times for the observation of abnormalities are 2 and 6 days following irradiation at which time the cells are in meiotic prophase and metaphase II respectively.

Pollen abortion induced by irradiation of the buds of various ages and containing various meiotic and microspore developmental stages

In order to permit observations throughout the developmental cycle, inflorescences were irradiated while

still on the plant. Three levels of radiation 600r, 800r, and 1,400r were delivered at a uniform distance from the irradiator by varying the time of exposure. All buds from 1 to 14 mm size were tagged and mature pollen collected as they arrived at anthesis after the treatment. The percentage of pollen abortion induced by different dosages at different bud sizes is reported in Table 4 and Figure 5.

It is evident that the irradiation of cells in early meiotic stages (less than 4mm size) produced the highest degree of pollen abortion (82.8 per cent at 1,400r). Most of the pollen mother cells at this stage were either pre-meiotic, in early meiotic prophase, or had arrived at metaphase I. Occasionally diploid pollen grains were observed which could have resulted from failure of division mechanisms. The age of the bud at the time of irradiation, as reflected by abortive pollen, appeared to be more important than the dosage. However, there was a constant increase in pollen abortion with increasing dosage. The normal non-irradiated plants showed 3 per cent pollen abortion under the same growing conditions.

Effects of irradiation on pollen germination and pollen tube growth in vitro

Bonny Best and Roma flowers were irradiated with 800r; 20,000r; 50,000r and 100,000r. Irradiated mature pollen was dusted on 2 germinating media and the slides

Table 4. Pollen abortion induced by irradiation of Bonny Best tomato buds of different sizes

Bud size at the time of irradiation	600r			800r			1,400r		
	Total pollen	Aborted pollen	Per cent aborted pollen	Total pollen	Aborted pollen	Per cent aborted pollen	Total pollen	Aborted pollen	Per Cent aborted pollen
Less than 2mm	57	41	71.9	30	23	76.6	57	43	75.4
2-3mm	76	55	72.3	81	63	77.7	35	29	82.8
4-5mm	49	32	65.3	73	51	69.8	68	42	61.7
6-7mm	67	37	55.2	59	36	61.0	69	43	62.3
8-9mm	73	28	38.3	78	43	55.1	53	31	58.4
10-11mm	41	12	29.2	63	34	53.9	68	39	57.3
12-14mm	45	8	17.7	46	12	26.0	39	15	38.4

Fig. 5.-Pollen abortion resulting from irradiation of the buds of different sizes.

were scored after being kept for 3 hours at 74°F. The recorded data are presented in Table 5 and Figure 6.

Radiation treatments (E) and (D) in Table 5 almost completely inhibited the germination of the pollen, whereas, treatment (C) retarded it considerably. The pollen grains in treatments (C) and (D) tended to stick together and occurred as a clustered mass. They appeared unhealthy and shrivelled but did pick up a faint aceto-orcein stain. Treatment (E) pollen exhibited no viability at all. No attempt has been made to compare the 2 germination media for lack of sufficient data. Column 5 represents the percentage of pollen tubes larger than 5 times the diameter of the pollen grain. This count was felt to be necessary because many germinated pollen grains had very small tubes and had ceased growing. Column 5 figures include the percentage of pollen tubes 3 hours after dusting on the growing media. Any addition in number of pollen grains with larger tubes after this period has not been recorded.

The germination of the pollen grains irradiated before pollen grain mitosis is reported in Table 6. The buds were irradiated 8, 9 and 10 days before dehiscence and the pollen was collected at anthesis for germination trials. Dosages of 100r, 400r and 800r were included in this study.

A perusal of Tables 5 and 6 shows that while 800r did not considerably affect the germination percentage when

Table 5. Effect of irradiation at anthesis on tomato pollen germination

Treatment	Medium 1.				Medium 2.			
	15 per cent sucrose + 75ppm Boron (2) (3) Total	(4) Per cent pollen apparent germi- nation	(5) Per cent P.T.* 5D	20 per cent sucrose + 100ppm Boron (6) (7) Total	(8) Per cent pollen apparent germi- nation	(9) Per cent P.T.* 5D		
(A) Control								
1. Bonny	613	348	56.7	413	281	68.0	33.4	
Best	541	351	64.8	321	231	71.9	36.7	
2. Roma								
(B) 800r								
1. Bonny	355	175	52.2	339	251	64.5	31.3	
Best	281	177	62.9	372	255	68.5	34.5	
2. Roma								
(C) 20,000r								
1. Bonny	264	53	20.0	561	140	24.9	6.3	
Best	401	80	20.0	216	56	25.9	8.1	
2. Roma								
(D) 50,000r								
1. Bonny	326	7	2.2	411	9	2.3	-	
Best	259	8	3.2	503	17	3.4	-	
2. Roma								
(E) 100,000r								
1. Bonny	439	-	-	371	-	-	-	
Best	391	-	-	512	-	-	-	
2. Roma								

* P.T. 5D : Per cent pollen tubes longer than 5 times pollen diameter.

Fig. 6.-Effect of irradiation at anthesis on tomato pollen germination.

Table 6. Germination of pollen grains irradiated before
pollen grain mitosis
(Bonny Best tomato)

Gamma Dose	Time of irradiation in days before dehiscence	Total number of pollen grains	Per cent of germinated pollen grains
100r	8	360	48.2
	9	291	37.6
	10	353	24.3
400r	8	288	19.4
	9	402	13.5
	10	335	9.8
800r	8	311	3.7
	9	345	2.4
	10	322	2.1

pollen was irradiated after pollen grain mitosis, the same dosage greatly affected the germination when administered before pollen grain mitosis.

It was observed that the pollen grains which did germinate had a differentiated generative nucleus. About one-third of such pollen grains had 1 or 2 small, deeply stained micronuclei. The tubes of pollen grains which had supernumerary nuclei or lightly stained micronuclei, or an undersized vegetative nucleus either did not grow at all, grew very slowly, or burst. The germination of pollen grains without differentiated generative nucleus or with supernumerary nuclei was also arrested. The cytoplasm

of such pollen grains became vacuolated, and stained deeply with orcein. It was found that the cytoplasm in the short pollen tubes may be completely isolated from that within the pollen grain. The extreme condensation of cytoplasm in non-germinating pollen grains indicated that the cytoplasmic movement was suppressed.

Morphologically normal, non-germinating pollen grains were encountered among these which were irradiated before mitosis. Owing to the high percentage of such pollen grains, it is reasonable to assume that the failure of their germination may be due to lethal gene mutations. It is further maintained that the degree of fertility of pollen grains irradiated before pollen grain mitosis can not be measured directly by the frequency of the morphologically normal pollen grains in which the micronuclei are absent.

Germination of irradiated pollen and pollen tube growth in vivo

Bonny Best pollen irradiated with 4,000r; 15,000r and 30,000r was used for pollination of untreated normal flowers. These dosages were selected to determine the highest workable dosage capable of giving maximum germination in vivo. The data presented in Table 7 show the effect of these treatments on germination and pollen tube growth inside the stigmatic tissue. Pollinated stigmas were collected every 6 hours and were embedded and sectioned at a later time. Data have been graphically presented in Figure 7.

Table 7. Pollen tube growth for different durations in the styles pollinated with pollen irradiated with different dosages; Bonny Best tomato

Hours after pollination	Control			4,000r			15,000r			30,000r		
	Per cent		Total	Per cent		Total	Per cent		Total	Per cent		Total
	Total	P.T.		Total	P.T.		Total	P.T.		Total	P.T.	
6	340	19.1	614	31.9	335	3.8	699	1.1				
12	220	35.0	600	36.0	705	6.9	640	1.0				
18	427	40.0	302	39.0	635	16.0	416	2.0				
24	320	41.6	335	42.0	527	14.0	214	1.9				
30	360	42.1	439	47.9	221	14.9	619	3.2				
36	687	42.9	639	50.9	538	13.0	391	4.1				
42	473	47.9	311	41.2	267	19.1	356	5.0				
48	325	52.0	250	45.9	418	22.9	246	6.3				
54	245	51.0	207	60.9	554	24.8	339	5.6				
60	478	49.0	316	63.0	306	20.1	288	5.1				

Fig. 7.-Effect of irradiation on pollen germination in vivo

The percentage germination of pollen was similar in untreated pollen and pollen irradiated at 4,000r for most of the observations made at 6-hour intervals. At 15,000r germination was low, less than half that of the control at all intervals. However, a tendency to increase up to 60 hours after pollination was observed. At 30,000r, not only was germination restricted and delayed but it never reached more than 6.3 per cent during the entire period of 60 hours.

Effect of pollen irradiation on fruit set and seed production

Whole flowers of variety Bonny Best were irradiated in small plastic vials in the center of the irradiator. Dosages from 800r to 100,000r were administered by varying the duration of exposure. The percentage of fruit set and mean number of seeds obtained per pollination with the treated pollen are given in Table 8. Figure 8 illustrates the effects of irradiation treatment in seed production and its efficiency in possible mutation production.

It is evident from Table 8, that fruit can develop after pollination with pollen irradiated at dosages as high as 50,000r. However in the one fruit that was obtained at this dosage, there were no seeds, indicating that fertilization did not occur. Parthenocarpic development of this fruit might have occurred independently of pollination. The possibility is entertained that the pollen

Table 8. Effect of pollen irradiation on fruit set and seed production

Gamma dose	Number of flowers pollinated	Fruit set		Total number of seeds per dosage	Number of seeds per pollination	Mean number of seeds per fruit set
		Number	Per cent			
Control	30	19	63.3	1713	57.1	90.2
1,000r	30	13	43.3	947	31.6	72.8
4,000r	30	10	33.3	459	15.3	45.9
7,000r	30	8	26.6	136	4.5	17.0
15,000r	30	2	6.6	14	0.5	6.7
30,000r	20	1	5.0	3	0.2	3.0
50,000r	20	1	5.0	0	0.0	0.0
100,000r	10	0	0.0	0	0.0	0.0

applied, though incapable of true fertilization might have provided a stimulus for the fruit development. Fruit set was reduced to about half the level of untreated material at 4,000r. Data show that the average number of seeds per fruit was also reduced to half at 4,000r.

Pollen abortion in X_1 plants originating from normal megagametes fertilized by microgametes from irradiated pollen

Twelve X_1 plants were randomly selected from seedlings obtained from crosses made between normal megagametes and microgametes from irradiated pollen. These plants were raised to maturity and pollen samples were collected from individual plants separately to determine the amount of abortion induced. The percentages of visibly aborted pollen are reported in Table 9 and Figure 9.

In this series there is a consistent increase and statistically significant difference in pollen abortion. The mean values for pollen abortion are plotted against dose in Figure 9. The relation between dose and pollen abortion is approximately linear. It does deviate markedly from squared or near-squared relationship found for chromosomal aberrations induced in such irradiation treatments as is reported by Tmofeeff-Ressovsky (75).

Table 9. Percentages of X_1 pollen abortion observed in sporophytes resulting from normal megagametes and microgametes from irradiated pollen

Plant number	Bonny Best			Roma				
	4,000r	2,000r	1,000r	Control	4,000r	2,000r	1,000r	Control
1	11.36	4.91	4.83	2.89	8.53	7.14	4.87	4.80
2	10.29	8.51	4.25	3.92	7.24	9.09	3.12	2.40
3	21.73	3.36	5.12	2.40	13.04	7.01	6.12	2.81
4	12.50	6.06	3.65	3.19	8.21	6.25	3.84	2.89
5	11.53	4.34	2.15	5.55	11.76	5.55	4.76	3.70
6	11.11	4.76	4.28	2.09	10.78	5.88	3.44	3.19
7	12.13	2.63	6.25	2.23	6.26	3.15	5.88	4.16
8	5.06	5.17	5.31	3.57	5.61	5.55	5.74	2.77
9	8.69	6.57	3.17	1.96	8.19	6.77	4.34	3.38
10	7.70	4.81	4.28	2.56	7.40	6.38	4.87	3.03
11	7.31	2.94	5.55	3.22	10.71	5.63	5.55	4.08
12	7.60	3.38	2.94	2.63	7.31	5.12	5.54	4.16
Mean	9.91	4.78	4.31	3.01	8.75	6.12	4.83	3.44

Table 10. Analysis of variance of pollen abortion
after Arcsin transformation for proportions

Source of variance	d.f.	Sum of squares	Mean sum of squares	F
Replications	11	45.39	4.126	1.516
Varieties	1	2.73	2.730	1.003
Error A (Var. X Rep.)	11	29.92	2.720	---
Treatments	3	759.27	253.090	47.915**
Treatment X Variety	3	35.03	11.676	2.210
Error B	66	348.64	5.282	---
Total	95	1220.98	---	---

** Significant difference ($P > 0.01$) according to F test.

Fig. 9.- X_1 pollen abortion induced by parent microspore irradiation

DISCUSSION

The suggested denaturation and flocculation of proteins and depolymerization of nucleic acid (41,67) represent the gross physical effect of both ultraviolet and ionizing radiations. It is therefore logical that the inactivation of the hormones and enzymes or structural changes in genes (mutations) may be related to protein denaturation. Since radiations are known to cause degenerative changes in both proteins and nucleic acids, alone or in combination, it is reasonable to assume that such changes may somehow be responsible for both chromosome breakage and mutation.

Ionizing radiations interact with cell nuclei to produce a physiological effect in the immediate vicinity of the ionization track. The ability of radiations to penetrate into the tissue is a fundamental property upon which all their biological effect is dependent. Therefore, in planning radiation experiments it is necessary to know the tissue-penetrating capacity of the radiation used. From the practical point of view, the less penetrating radiations are often less satisfactory. In 1937, Sax (58) concluded from a number of experiments that the ionizing radiation of high intensity is more effective in producing

chromosome aberrations in *Tradescantia* microspores than the same dose given at low intensity. The frequency of 2-hit chromosome aberrations was two and one-half times greater when the same total dose was given in 1 minute than in 16 minutes. The same general conclusion has been supported by the work of Rick (52,53), Faberge (20), Koller (35), Thoday (74) and Bora (6). Therefore the Cobalt 60 gamma radiations have been selected for this study because of their high intensity and deeply penetrating properties.

Irradiation of microspores with 800r throws light on 2 important phases: the degree and type of differentiation (1) within chromosomes and (2) between nuclei. Evidence has been established during the last few years that all the latter effects of radiation can be classified as due to alterations in the organization of the chromosomes. However, suppression of the pollen grain mitosis, detectable by quantitative analysis (Table 2), is actually the result of qualitative changes induced in those processes which are introductory to the onset of prophase, and hence primarily concerned with chromosome organization (33). It is postulated by Koller (35) that previous to prophase several changes must take place within the resting nucleus. These changes, according to him, are related to gene multiplication, nucleic acid polymerization and molecular spiralization. The arrest of any of these processes

results either in suppression of division or in the prolongation of the "resting" stage.

Koller (35) further suggests that while the "resting-stage-prolonging" or "division-suppressing" effect can be detected only by a quantitative analysis, the effect of radiation on the metaphase chromosomes is shown by qualitative changes. Both prolonged congression and stickiness are believed due to disturbance in nucleic acid synthesis; the amount of nucleic acid being increased and deposited on the chromosomes more rapidly than under normal conditions (15).

Thus the physiologically degenerative changes set in by premeiotic irradiation do not proceed at the same rate in all meiotic division products. This can be further supported from the observations following a given dosage level which may show:

- (1) A proportion of shrivelled microspores indicating early morphological degeneration;
- (2) The apparent occurrence of physiological degeneration as indicated by a percentage of non-germinable pollen grains;
- (3) Apparent physiological degeneration after germination as evidenced by a reduction but not complete inhibition of seed production.

The second phase, namely the nuclear division, has been reported to be preceded by an increase in the amount of nucleic acid within the cell and the nucleus. It is utilized in building up the structure of the chromosomes (9). It is known that the pollen grains have a different chemical organization and metabolism from that of other cells, because the chromosome of the generative nucleus retain the nucleic acid charge between the pollen grain and pollen tube mitosis, and no nucleic acid is required by the vegetative nucleus for the chromosome organization (33). The localization of nucleic acid and the change in the chemical metabolism of cytoplasm may be considered as adaptations of primary importance which enable the pollen to fulfill its function.

The data obtained on the quantitative effects of radiation (Tables 2 and 3), indicate that the end result of these effects is determined or greatly influenced by the combination of various factors. The developmental differences between sister anthers alone can jeopardize the reliability and comparability of quantitative analysis. Though no direct observations were made it was noticed that the environmental differences particularly in temperature, exaggerate the developmental differences, hence further diminish the reliability of the analysis. It is obvious that developmental differences, prophase suppression and

prolongation of metaphase which follow irradiation, should be taken into consideration also when "secondary effects" are quantitatively analyzed. Secondary effects observed in pollen grains of sister anthers at given time after irradiation cannot be used alone as the most reliable and only criterion to determine the actual developmental conditions which were present in a cell at the time of irradiation.

The observed degeneration of pollen grains following treatment of premeiotic buds with 800r was a frequent occurrence. Although many pollen grains were morphologically perfect, they failed to germinate on the stigma or the agar medium. These pollen grains were capable of deep staining but this fact alone did not indicate germinability. It was observed that physiological degeneration may set in during any of the final stages of development.

These morphologically perfect but non-germinable grains are produced as a result of less severe injury. Such grains develop in functional condition up to a brief period just previous to anthesis. Irradiation at this stage may cause enzymatic disturbances in the grains which prevent their germination. It is not to be understood in this connection that all the grains in a mature anther that failed to dehisce normally at anthesis with the production

of "dusty" pollen necessarily contained only germinable grains. Although the percentage of morphologically perfect but non-germinable grains was fairly high, a considerable number of germinable grains was present. Since in mature anthers a very high proportion of grains may also be germinable it is not possible by casual observation of mature yellow anthers to predict the presence of germinable or non-germinable grains. In view of the fact that the degeneration occurred at various stages from telophase of the second division up to and including mature male gametophyte stage, it is not surprising that single locules containing this entire range of degenerated microspores and pollen grains were observed. Degenerated microspores and sterile pollen grains were found lying side by side within anthers which failed to reach the deep yellow color characteristic of mature anthers at anthesis.

Chromosome breakage at various stages of microsporogenesis has been presented in Table 3. The differential susceptibility of nuclei at different stages of development is common for both radiation induced mutation and induced chromosome aberrations (44). This differential susceptibility is attributed to differences in pH (77), to water content (30) and to differences in amount of chromatin around the gene string (44). The differential susceptibility of meiotic and mitotic nuclei is therefore difficult

to establish in recognition of these factors but this susceptibility appears significant. As Goodspeed (27) has suggested, the cellular activity seems to play a part in radiation susceptibility. As applied to the chromosomes, it appears that the period of the greatest sensitivity to irradiation is correlated with greatest activity in coiling mechanism (60). At this time the chromosomes appear to be under strain, and some of the breaks will be prevented from rejoining in the original position, and adjacent breaks in adjacent chromatids or chromosomes will join in new associations (48).

Apart from the factors known to be significant, it would appear that the state of contraction of the chromosome may be important in relation to sensitivity since the most sensitive stage is one at which the chromonemata are shortest (67). Conversely, interphase is a stage at which the chromonemata are considered to elongate.

Lewis has indicated that genetic damage may also vary with the stage of meiosis and is high near meiotic metaphase. This is also a stage of high sensitivity to breakage. It is not clear from the consulted literature whether the stage of low breakage sensitivity (interphase) is also low in sensitivity to radiation induced mutation.

It is possible that an unknown proportion of initial breaks undergo restitution, i.e., the broken chromosome

reunite in the original way. At prophase, even resting stage effects are largely postponed to appearance at anaphase during chromatid separation. Similarly metaphase and anaphase effects are not released until the interphase when the nucleic acid charge is lost; they appear at the second division.

Koller (34) has shown that the frequencies of open or simple breaks that have undergone reunion, are reduced when the irradiation is done at low intensities. According to Sax (59) restitution of the broken chromosomes is increased at low intensities, and this is the cause of the reduction in aberration frequency. Koller further observed that pollen grains undergo various metabolic disturbances, which affect chromosome behavior and alter the rate of mitosis itself. Sax (58) also indicated the possible importance of the "physiological effects" which could be responsible for the reduced breakage yield. The cytological phenomena observed in pollen grains show that both increased restitution and physiological disturbances occur together and interact. Radiation at low intensities may change the sensitivity of the chromosomes as a consequence of which the number of initial breaks arising later are reduced.

Koller's (35) studies of the effects of low intensity radiation confirm that chromosome injuries in tomato pollen can not be considered and analyzed merely as mechanical

effects and as the direct result of single events of ionization. They are produced in a physiological system which is in a continuous flux during the developmental cycle and which is reacting continuously with environmental factors.

When the pollen grain mitosis was examined after treating meiotic stages with 800r, majority of the pollen grains were damaged. In addition, a large number of cells had aborted. The real damage done therefore was several times greater than the one directly recorded. The same dose of 800r when observed on the pollen grains with resting stage, neither caused excessive damage nor real abortion. The real breakability was, therefore, much greater at meiosis than it appeared to be and vastly greater than in the following pollen grain resting stage.

It can be supposed that the sensitivity of different stages to breakage was a simple entity. All breakage that was measured was really breakage minus restitution. But how important the restitution variable was, can be known only by comparison of the effects of different types of irradiation using analytical methods which separate breakage from reunion.

The considerable variation in suppressing germination when differentiated pollen grains are treated, indicates that there is a fundamental change in the structure of the

chromosomes of the "resting" vegetative nucleus. While chromosomes of the ordinary resting nucleus have a small amount of nucleic acid attached, chromosomes of the vegetative nucleus of the pollen grains are almost completely free, and remain so throughout the cycle. It is not improbable that this difference is responsible for the failure of radiation to suppress germination in some cases, and the effect may be delayed and manifest in the physiological deterioration of male gametophyte development.

On the other hand, the chromosomes of the generative nucleus between the telophase of the pollen grain and pollen tube division are heavily charged with nucleic acid. Their reduplication (splitting), which occurs not later than the approximate time of anther dehiscence (33), must have taken place in the presence of the nucleic acid attachment. The behavior of the generative nucleus shows that not only breaks and reunions can take place, but gene and chromosome reduplication are also possible in chromosomes which are heavily charged with nucleic acid.

To study the effect of irradiation on pollen germination, pollen grains were irradiated before and after mitosis. The germination frequencies have been reported in Table 5 and 6 respectively. The data show that at lower dosage of 800r, the germination was not affected when

pollen grains were irradiated after pollen grain mitosis and corroborate the findings of Paddubnaja (49) and Newcombe (48). Germination of pollen grains was not prevented even when a very high dosage (20,000-50,000r) was used. It was also seen that while radiation was effective on the chromosome of the generative nucleus, at the same time it was ineffective on the vegetative nucleus. It may be assumed that the release of the total, or almost total, nucleic acid charge of the chromosomes may alter the organization of the vegetative nucleus to such a degree that it becomes radioresistant.

On the other hand, germination of the pollen grains which were irradiated before pollen grain mitosis is greatly affected (Table 6). It was observed that the pollen grains which germinated normally had a differentiated generative nucleus. Many of these pollen grains had deeply stained micronuclei. Several morphologically normal, non-germinating pollen grains were encountered. Owing to the high percentage it was assumed that the failure of the germination of some morphologically normal pollen grains may be due to lethal gene mutation.

The germination of the irradiated pollen grains showed that irradiation at lower dosages does not directly kill the cell, but death may come about through changes induced in the chromosomes. If these are gross structural

changes, they may be identified at the succeeding division as breaks, reunions and acentrics. Division following radiation is the usual way by which the radiation effects become observable. Genic unbalance, an accompaniment of the induced structural or gene changes, is responsible for the death of the daughter cells. The induced damage to the chromosomes may be very extensive. The derangement of the nucleic acid cycle may cause not only a long but complete suppression of division. There is also a further possibility that radiation can also induce lethal gene mutation; thus the death of the cell after radiation may come about without division. But in all these instances the primary effect of radiation is always in the chromosomes.

Germination of irradiated pollen in vivo has been presented in Table 7 and graphically represented in Figure 7. Germination percentages following 4,000r indicate a stimulatory effect when compared with those of the control. Deviations from this situation at 42 and 48 hours intervals and also a lower percentage at 2 previous instances would indicate that some imperfectly understood factor was operative; e.g., pollen selected might have been inferior even before it was irradiated. Also there may be variation in the stigmatic surface. These factors may also account for other discrepancies encountered in these data.

Data obtained on the effect of pollen irradiation on fruit and seed set (Table 8) show that a workable dosage lies between 1,000 and 7,000r. This range of lower dosage is desirable because the expected gross chromosomal changes beyond 7,000r will be difficult to perpetuate. Lower dosages on the other hand may cause heritable germ plasm changes that may be within the practical range and provide enough economic variability. Dosages in the range of 6,000 to 7,000r would therefore seem to be optimum. It is possible that below this range mutation frequency will be so low as to be impractical for detection and separation; and above which the reduction in the seed supply, caused by gross chromosomal abnormality, might be contributing to impracticability.

Unpublished results of Lorz with *Lilium* (43) showed the same result but the range in lily was much lower. No seeds were set in excess of 9,000r. From Table 8 and accompanying graph, it would seem that the most practical dosage for future mutation production through irradiation of pollen would be about 7,000r (about 5 seeds per 7,000r treated pollination; Fig. 8). With repeated pollination at this dosage, it would be possible to obtain large enough population for screening purposes. It can be assumed that the lower dosages would decrease the expectancy of mutations and it seems definite that higher dosages would decrease the seed supply to the point of unworkability.

Pollen abortion in subsequent generation following irradiation of the mature parent pollen has been presented in Table 9 and Figure 9. The evident relationship between increased dose and increased pollen abortion is approximately linear which suggests it to be a mutational response. It does deviate markedly from the squared or near-squared relationship found for chromosomal aberrations induced during the same period as is observed by Tmofeeff-Ressovsky (75). It is in this respect that the response in pollen abortion resembles mutation and not chromosome aberrations (54). In the present work no attempt was made to separate the effects of point mutations and chromosomal aberrations with respect to pollen abortion. But it seems that both are operating simultaneously.

It is unlikely that this very significant association of increased abortion with the higher dosages could be caused by some change in the environment other than irradiation since it appeared in both varieties and all the treatments. It is also apparent that most mutations affecting abortion of pollen were intragenic changes of varying consequences. That they are probably not the results of chromosomal changes is due to the opportunity for the elimination of these major structural alterations in the progressive development of the generation following irradiation.

Koller (35) emphasized that mutations affecting pollen abortion require the completion of mitosis before they gain phenotypic expression, or as suggested by Stern (69), the resulting stage following the microspore mitosis might not be long enough to permit mutations induced during this period to gain expression. Another perhaps more likely explanation relates to the nuclear condition of post-meiotic microspore. Any recessive mutation induced subsequent to the stage at which chromosomes are effectively split into chromatids in respect to radiation action can not be expressed because of the presence of its dominant allele in the same cell. Obviously this masking effect can occur only if both the tube nucleus and generative nucleus exert a controlling influence on pollen development. They differ in appearance and staining reaction, yet both continue to function, at least until anthesis. While comparing the 2 methods of pollen treatment, namely the premeiotic treatment or the mature pollen treatment, it will be well to consider that even though a higher dosage for mature pollen may be equivalent to correspondingly lower dosage in premeiotic stages, it may be better to irradiate mature pollen for mutation production for the following practical reasons: (1) No greater expense is involved in the treatment; (2) The treatment requires less time because the pollen can be treated inside the irradiator

at its highest intensities; (3) Flowers are more easily handled than the whole plant; and (4) Within limits irradiated pollen can be stored if needed at a later time.

It is possible that many point mutations occurring in early meiotic development might be eliminated on fragments resulting from the higher dosages. The products of irradiation of pollen on the other hand would not be subject to this hazard since the pollen has already passed through the stages at which the elimination could occur and there is only one division left, that of generative nucleus into two sperm nuclei, at which time fragments bearing point mutations might be eliminated.

If the mechanism of fragmentation induction is due to ionization, the possibility that actual fragmentation may occur after division is entertained and occurrence of such post division fragmentation should not necessarily create a lethal effect on the point mutation.

CHAPTER VI

SUMMARY

1. Developmental differences within anthers and between anthers of the same flower bud of tomatoes are described.
2. The time required for the development of the different stages in microsporogenesis and gametophytic development was determined in Bonny Best variety of tomato. The duration from pollen mother cell prophase to mature pollen was found to be 16 days.
3. The exposure of tomato inflorescences to gamma ray dosages of 600r, 800r and 1,000r resulted in degeneration of the anthers, meiotic chromosomal aberrations, and consequent pollen abortion. There was a corresponding retardation of development exhibited by the tapetal cells.
4. Buds exposed to 800r dosages were selected for further cytological studies because a 1,400r dosage caused such deterioration of buds 2mm or less in size that it was not possible to follow their further development effectively.
5. It was established that the sensitivity to ionizing radiation was more dependent on bud size than upon the dosage within the range employed.

6. The "prophase suppressing" effect was greatest 4 hours after irradiation, when the relative proportion of the post-metaphase stages was the highest.
7. Radiation was still effective on metaphase of pollen grain mitosis 48 hours after irradiation.
8. Prolongation of the metaphase stage appeared to be associated with a "clumping" of the chromosomes.
9. Chromosome fragmentation was used as a measure of radiation damage. Early prophase and metaphase II stages were found to be most sensitive, providing approximately equal amount (94 per cent) of fragmentation.
10. The irradiation of cells in early meiotic stages produced the highest degree of microspore sterility (82.8 per cent). There was constant increase in pollen abortion with the increasing dosage whereas the radiation effect decreased with increasing bud size.
11. Abnormal germination occurred when generative nucleus was undifferentiated, tube nucleus was undersized and several micronuclei were present.
12. Failure of germination of morphologically normal pollen grains suggested that radiation induces either gene mutation or larger chromosomal changes of the vegetative nucleus.

13. Irradiated pollen could effect parthenocarpy at dosages as high as 50,000r but few to no seeds were produced. However seed set was reliable evidence of fertilization between 7,000r to 15,000r.
14. The fertilization of untreated eggs by male gametes from irradiated pollen at 1,000r; 2,000r and 4,000r resulted in X_1 individuals which exhibited higher percentages of pollen abortion than the plants having no history of irradiation.
15. The amount of pollen abortion in these X_1 plants increased in a linear fashion with increasing gamma dosage. X_1 plants from 1,000r averaged 4.31 and 3.44 per cent and that for 4,000r averaged 9.91 and 8.73 per cent for the Bonny Best and Roma varieties respectively.
16. The results indicate that the 4,000 to 7,000r range is probably an optimum threshold value which would take into account a practical balance between mutation frequency which increases directly with the dosage; and seed production efficiency which decreases with the increasing dosage.
17. The results reported here appear to indicate that the greatest practical efficiency from irradiation of any stage in a haploid cycle of the tomato plant development would be derived from the irradiation of the microspores although premeiotic stages of microspore development are more radiosensitive.

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BIOGRAPHICAL SKETCH

Mahendra Singh was born on November 11, 1931, in Kanpur, India. After his high school graduation from Bishambhar Nath Sanatan Dharm Intermediate College, Kanpur, in 1949, he took his college education at Government Agricultural College, Kanpur, India.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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