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THE EFFECTS OF RADIATION ON CHATHAM TOMATO SEEDS

SHIH-AN YU

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THE EFFECTS OF RADIATION ON CHATHAM TOMATO SEEDS

BY

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INTRODUCTION

Since the discovery of the use of X-rays in inducing mutations in 1927 by Muller (21), radiation of various types has been used as a tool in bringing about mutations in both animals and plants. Although most mutants are unfavorable, some favorable ones do occur. Investigations with various crops have indicated that irradiation may speed up the appearance of favorable gene mutation which would furnish additional material for plant breeders. Through irradiation of various crops, research workers have obtained beneficial mutations, such as stem-rust resistance and stiff straw in barley by Shebeski and Lawrence (23), stem-rust resistance in oats by Konzak (13), leaf-spot resistance and higher yield in peanut by Gregory (9), etc.

The tomato breeding program is one portion of the "Vegetable Breeding" project at the New Hampshire Agricultural Experiment Station. One of the serious problems with the tomato in this area is its susceptibility to early blight disease (Alternaria solani (E. & M.) Jones & Grout). Since there is no breeding material immune to this disease available at the present time, and since disease-resistant mutants have been induced in other crops by irradiation, an experiment was initiated attempting to induce a mutation that could be used in tomato breeding for such disease resistance.

This project furnished an opportunity to study the effect of irradiation of varying kinds and amounts on tomato

seeds, including the frequency of mutations, their nature and inheritability.

LITERATURE REVIEW

The effects of radiation on plants have been studied ever since the discovery of X-rays by Roentgen in 1895. Early research workers emphasized only morphological and physiological responses. During recent years, radiation of various types has been used in inducing beneficial mutations in improving agricultural crops. There is a very large number of publications discussing the effect of radiation on plants. The papers reviewed below pertain primarily to tomato but include some of the other more important contributions to the general subject.

Tomato Experiments

Johnson (11) irradiated John Bear tomato plants with X-rays at a distance of 30 cm. with no filter. The duration of irradiation was from 20 minutes to 30 minutes. She reported that the first noticeable effect on the plants was a peculiar warty or pebbly appearance of the young leaves, which became variegated when older. Irradiation of seedlings and young plants also caused other marked leaf abnormalities, including absence of half the length of leaflets, absence of the blade on one side, twisted leaflets, a greater development of lateral branches, and radiophyloxia. Irradiation of young floral buds caused malformations, including increase in the number of corollas, fusion of the calyx lobes, twisting of the corolla lobes, and formation of triple flowers. Fruit development was somewhat delayed, the fruits that developed to maturity were

misshapen and had few seeds or no seeds, and the internal pattern of the fruit was lacking.

Seeds, growing tips, and young, developing fruits were irradiated by Lindstrom (16) with Monel Radium needles. The dormant seeds were treated for 20 to 48 hours, the growing tips for 20 to 30 hours, and the young developing fruits for 5 to 15 minutes. He reported that the variations which occurred were due to chromosomal disturbance. He also stated that the irradiation of young tips was the most effective way to induce variations. Six inheritable recessive mutations were induced. Four arose from radiation of young tips---rough leaves (rl), virescent (v), yellow foliage (l), and sterile (st); two arose from irradiated seeds---yellow seedlings (ys) and rolled cotyledons (ro).

Dormant seeds of the variety Earliana were treated by MacArthur (17) with X-rays from a Coolidge tube. The target distance was 25 cm. and no filter was used. Seeds were exposed 9, 19, 27, 36, $46\frac{1}{2}$, and 60 minutes, the maximal dosage given being 14 "erythema doses" (each approximately 1200r units). He reported that in the X1 generation (immediate generation from irradiated seeds) and the X2 generation, the germination and fertility remained normal. Eight chlorophyll-deficient seedlings were found from 1200 irradiated seeds in the X1 generation. In the X2 generation he observed that 43 or 12.4 % mutations were produced from 346 progenies. The induced mutations were various in nature, involving color of the foliage and stems, shape of the leaves, branching habit, and

growth rate. The seeds that had been irradiated for one hour (approximately 1200r) produced 15.9 % mutants. The number of mutations induced was clearly related to dosage, for the 60-minute exposure caused 15.9 % mutations and 46½ minutes 8.3 %. All the mutations induced were recessive and monofactorial. He concluded that it seems possible that gene mutations are relatively more frequent with moderate and sublethal doses. Three of the mutants have been named and placed on the linkage map: mottled leaves (m) in group I, bushy (bu) in group VI and narrow cotyledons in group X. Two of the mutants discovered by MacArthur were tested later by Butler (5), who found that rosette (ro) showed a deficiency in its monohybrid ratio and linkage with dwarf in group I. Propeller (pr) showed good monohybrid ratio but no linkage was revealed.

Young (27) reported that a white-flowered mutation (y) was induced by X-ray treatment of Marglobe tomato seeds. This induced mutation was proved to be a simple recessive factor to the yellow-flower (Y).

Marshak (19) studied the effect of fast neutrons on root tips of young tomato seedlings. He found that in root tips that were irradiated, the percentage of normal anaphases (those showing no chromosome abnormalities) decreased to a minimum at three to nine hours after treatment with various doses of fast neutrons. Root tips irradiated with 100 "n" units of fast neutrons had 69.5 % normal anaphase cells, 49 "n" units 85.0 % and 0 "n" 98.8 % when examined three hours

after irradiation.

Barton (1) made comparative studies of the effects of X-rays and ultraviolet radiation on chromosomes of tomato. He found that the majority of the changes induced by X-rays were translocations with a relatively small incidence of deficiencies, while in the ultraviolet-irradiated sample a higher proportion of deficiencies was found. Chromosome breakage observed in the F1 plants was more highly localized in the centromere regions, where most of the chromatic region of chromosomes is located. If the breakage was localized due to a centromere effect, a greater proportion of the breakage should be in the parts of the chromosomes proximal to centromere region; however, since the breakage was distributed at random within the chromatic regions, the localization of breakage would seem to be due to some function of the chromatic region as such, rather than to the centromere.

Yagyu (26) studied the effects of X-rays and thermal neutrons on dormant seeds. The variety Puck was studied regarding chromosomal abnormalities in root-tip mitosis and seedling injury in the immediate and second generations from irradiated seeds. The dosage range for X-ray was from 600r to 1600r and for thermal neutrons from 12.83 to 77.00×10^{12} thermal neutrons per square centimeter. He found that chromosomal abnormalities occurred as bridges and fragments in the anaphasis stage of mitosis in the root tips of germinated seed. The occurrence of bridges per cell was 0.75 for combined X-ray doses and 0.98 for the combined thermal-neutron

doses. The fragments per cell were 2.32 for the combined X-ray doses and 3.70 for the combined thermal-neutron doses. There were 62.25 % of abnormal cells in the X-ray series and 80.79 % in the thermal-neutron series.

Yagyu also reported that there was an increase in chromosomal abnormalities when the post-irradiation period prior to germination was increased. When the dosages of radiation were increased, there was a corresponding decrease in growth of seedlings from irradiated seeds. In the immediate generation 2.3 % had abnormalities in the X-rayed group while in the thermal-neutron group 14.1 % had abnormalities. In the X₂ generation 4.0 % of the seedlings had chlorophyll mutations and 7.0 % morphological mutations; in the N₂ generation 16.7 % had morphological mutations and 10 % chlorophyll mutations. He concluded that the thermal-neutron radiation was less damaging to the tomato plants and gave a greater number of genetic changes than did the X-rays.

Lesley and Lesley (15), in a study of the effects of seeds treatment with X-rays and P³² on tomato plants of the first, second, and third generations, reported the following observations: Treatment with 10,000r X-rays for 33 minutes or soaking seeds in P³² solution (the initial dose per seed ranged from 2.7 to 10.8 microcuries of P³²) caused formation of unequal pairs, segmental interchanges, weakened pairing, inversion, and elongation and stickiness in meiosis in R₁ plants from treated seeds of tomatoes. From P³² treatment

a small proportion of seedlings and mature plants were variegated, pale grey-green, or albino in the R2 generation. Also yellow (r) and tangerine (e) flesh color of fruit, aurea, anthocyanless, male-sterile, brachytic, and other mutants occurred in the R2 generation from beta radiation of P³². From the X-ray treatment, a mutant resembling wiry "w" was obtained. Both X-ray and P³² treatments caused much pollen-sterility in the R1 and R2 generations. They claimed that some of the sterility was clearly due to cytological causes and some due to genetic caused.

Mertens and Burdick (20) irradiated tomato seeds of Lycopersicon pimpinellifolium and L. esculentum with X-rays (doses of 6,000r, 12,000r, 18,000r, 24,000r 48,000r and 60,000r) and thermal neutrons (exposures of 2.96×10^{13} , 8.64×10^{13} , and 12.00×10^{13} neutrons per cm²). The R1 plants were backcrossed to controls, and various earliness traits were evaluated in the backcross progenies. Two lines of tomato have been produced by X-ray irradiation that are earlier than the control lines. They suggested that similar mutations might be induced, recovered, and identified, if (1) seed-irradiation is used; (2) R1 plants are used to backcross to unirradiated controls; (3) backcross progenies are evaluated in replicated experiments; (4) mutations are sought affecting traits which show heterosis in tomato hybrids.

Young (29) irradiated Manatee tomato seeds with either high-intensity gamma rays from cobalt 60 (4,000r to 64,000r)

or thermal-neutrons for fifteen minutes to four hours. He stated that the strongest dosages of irradiation killed most of the seeds. No plants with resistance to southern blight (Sclerotium rolfsii) were found. Treatments with thermal-neutrons for four hours caused 18.2 % mutations to appear in the R2 plants, and one hour caused 4.2 % mutations. Treatments with 16,000r and 32,000r of gamma-rays caused 1.8 % mutations, and 64,000r caused 0.7 %. Mutations involving chlorophyll changes, leaflets with parallel veins, elongate curled leaflets, entire leaflets, etc. were caused by thermal-neutron treatment. Gamma-rays caused chlorosis, dwarfing, sterility etc. All the mutations were deleterious.

Other Crops

Horlacher and Killough (10) irradiated dormant cotton seeds with X-rays. They observed that, with an exposure of 60 minutes, the germination percentage of one-year-old seeds was not lowered but the germination percentage of two-year-old seeds was lowered markedly. The first noticeable effect was the production of numerous dwarf plants. Other morphological effects were leaf variegations, changes in leaf shape, and abnormal cotyledon numbers.

Wheat seeds were irradiated by Benedict and Kersten (2) with soft X-rays for different lengths of time. They reported that seedlings from seeds irradiated for five seconds showed an increase both in diastase and in sugar content, but seedlings from seeds irradiated for a longer time showed a decrease in these two substances. The percentage of water in

seedlings at the end of the germination period indicated that the irradiated seeds could not take in water as readily as could the controls.

Genter and Brown (8) treated dormant and germinating seeds of the Michelite variety of Phaseolus vulgaris with X-rays. They found that in the X1 generation survival decreased as dosage increased. The seeds that were germinated thirty-six hours prior to irradiation for twelve minutes were injured more than the seeds that were germinated eighteen hours prior to irradiation for fifteen minutes and the dormant seeds irradiated for thirty minutes. The seeds receiving heavy dosages produced seedlings that were severely retarded and grew slowly. The seeds that had light dosage treatments were retarded in growth in the initial phases.

In the X2 generation, 152 out of a total of 1534 progenies showed mutations. Sixty-seven percent of the mutants showed chlorophyll abnormalities. Forty-two percent of the chlorophyll mutants died in the seedling stage. Fifty-seven percent of the surviving plants were dwarfs. Other morphological characters affected were branching; size, shape, and texture of leaves; fertility; and earliness of maturity.

A comparison of the effect of thermal neutrons and X-rays on barley and maize seeds was made by Schmidt and Forlik (22). Barley seeds were irradiated for periods of two, four, eight, ten, twenty, thirty, forty and fifty minutes with a flux of 7×10^{10} thermal neutrons per cm^2 per second. Morphological effects of the two- and four-minute

periods on seedling development included the reduction of seedling heights, remarkable mottling of the first two leaves, a narrowing of leaves as compared with those of the control, and a significantly higher leaf number than in the control. Seeds X-rayed with 7,500r showed changes which were similar but not so pronounced as in the neutron treatment ---only a slight mottling of first two leaves and plant height slightly below that of control. Maize seeds were irradiated for two, four, and eight minutes with thermal neutrons with the same flux mentioned above and with 7,500r of X-rays. Some of the results were a lessening in the mean height of the seedlings causing them to be lower than the controls and a general reduction in height as the exposure time increased.

Other seedling effects of neutron irradiation of maize seeds were heavy mottling of the first two leaves, narrowing of the leaves, and other abnormalities. The only visible effect of the X-ray radiation of maize seeds consisted of slight mottling and narrowing of leaves and depression of growth.

MacKay (18) irradiated dry and pre-soaked barley seeds with X-rays (ranging from 5,000r to 25,000r) and fast neutrons (10,000 to 30,000 dis, 1 dis 0.6r). He found that the germination of dry seeds was not affected even after the heaviest dose but the retardation of initial seedling growth was conspicuous, giving a sigmoid relation to the X-ray dose and an exponential one to the neutron dose. Pre-soaking of the

seeds did not change this difference in the biological action of X-rays and neutrons. He explained that the lethality after pre-soaking in N1 fell considerably below that of X1 because of a more pronounced energy absorption due to the high moderating effect of water in the case of neutrons. The same types of induced chlorophyll mutations were found in both X2 and N2. Neutrons induced more chromosomal disturbance than X-rays did.

A comparison between the effects of irradiating dormant seeds of barley with X-rays and thermal neutrons as measured by survival and the frequency of chromosomal aberrations and mutations was made by Caldecott, Beard, and Gardner (6). Seedlings from X-rayed seeds had a greater variation than seedlings from seeds irradiated with thermal neutrons, which had a more uniform height effect as measured by the seedlings height. When survival is plotted against the dose, the slope beyond the inflection point is much sharper for thermal neutrons than for X-rays.

The frequency of chromosomal bridge in root tip cells and interchanges in pollen mother cells obtained with the highest non-lethal dose of the thermal neutrons was about 2.5 times the value obtained with 20,000r of X-rays. In the same way, the highest mutation frequency obtained with thermal neutron treatment, which did not cause a high degree of sterility, was about two times that obtained with 20,000r of X-rays. The author interpreted this difference, involving more chromosomal aberration and mutation induced and less

killing caused by thermal neutrons than X-rays, to be due to injury of the extra-chromosomal constituents of the cell by the X-ray radiation or X-rays' affecting the chromosomes in a cytologically undetectable manner.

Dormant sorghum seeds were treated with thermal neutrons by Kankis (12). He reported that the seedling height of young plants grown from irradiated seeds was less than that of the control, and the variability with a given X-ray dose was greater than that with the corresponding thermal neutron dose. In the mature plants all treated groups showed depressed stature when compared to the control. Also, all radiation dosages significantly delayed the beginning of anthesis and shortened the blooming period. The distribution of seedling mutations grown from intact XI and N1 generation heads of sorghum indicated that the reproductive tissue in the sorghum panicle was commonly derived from more than one cell present in the dormant embryo.

In all papers reviewed above, in general X-rays were considered less effective than thermal neutrons in producing mutations and chromosomal abnormalities.

MATERIAL AND METHODS

Seed Origin

The Chatham variety of tomatoes was selected because of its genetic purity, its earliness, and its desirable characters.

The original seeds were furnished by the Horticulture Department of the University of New Hampshire. The genetic purity of the seed was assured by growing and roguing the plants through three successive generations in a screened greenhouse at the University of New Hampshire, Durham, New Hampshire.

Seed Irradiation

The seeds were irradiated by Dr. Seymour Shapiro at the Brookhaven National Laboratory at Upton, New York. There the X radiation was made with the G. E. Maxitron operated at potential 250 kilovolts and 30 milliamperes. The machine delivered approximately 800r per minute. The thermal neutron radiations were made in the thermal neutron column of the nuclear reactor. A gold foil was used to accompany the seeds during the period of irradiation in the nuclear reactor. The flux, the number of neutrons that the sample received per square centimeter per second was calculated from the radioactivity induced in the gold foil. Before irradiation, seeds were stored in a room with sixty percent relative humidity for two weeks to stabilize the

moisture content of all seeds.

In 1956, a preliminary experiment was conducted at the Horticultural Farm of the University of New Hampshire. Seeds treated with X-ray dosages of 10,000r, 15,000r and 20,000r, and ten, twenty and twenty-five hours exposures at a flux of approximately 6.6×10^8 thermal neutrons per cm^2 per second were employed. The doses were found to be too low, since only a few plants were affected. There, in this experiment, conducted in 1957 and 1958, 30,000r and 50,000r X-ray dosages and twenty, twenty-six, thirty, and forty hours at a flux of approximately 7.0×10^8 thermal neutrons per cm^2 per second were employed. The total dose of the thermal neutrons per cm^2 per second given to the seeds in both experiments are as follow:

Date	Foil No.	Time	Flux	Total dose
5-16-56	229	15 hrs.	6.45×10^8	3.48×10^{13}
	258	20	6.69×10^8	4.82×10^{13}
	284	25	6.62×10^8	5.96×10^{13}
3-8-57	524	20	6.74×10^8	4.85×10^{13}
	478	26	7.02×10^8	6.56×10^{13}
	541	30	6.98×10^8	7.54×10^{13}
	496	40	7.04×10^8	1.00×10^{14}

Error = $\pm 15\%$

Flux = number of neutrons / cm^2/sec .

Total dose = flux x time

Germination Test

On April 15, 1957, irradiated Chatham tomato seeds were placed on moist filter paper in petri dishes which were placed in a germinator used for official state seed testing, one hundred seeds in each petri dish and two hundred seeds

for each treatment. A temperature of 86° F (30° C) during the day and 68° F (20° C) at night was maintained. A daily record of germination was made. As soon as the cotyledons were fully expanded the seedlings were transferred to three-inch pots to permit root expansion and subsequent growth.

Cultural Methods

For observation of the immediate generation. On May 4, 1957, 2000 seeds of each irradiation treatment and 2,000 untreated seeds were sown in flats in the greenhouse, 1,000 seeds in each flat. From May 17 to May 25 emerged seedlings were transplanted into three-inch pots which were placed on the greenhouse benches. A minimum temperature of 75° F (24° C) was maintained during the day and 65° F (18° C) at night. From June 4 on, the once transplanted seedlings were transplanted into the field whenever they reached proper size. The distance between plants was three feet and the distance between rows was five feet. No supports were used to hold the plants upright.

For progeny tests. Seeds for progeny tests were planted in flats in the greenhouse. Seedlings were transplanted into three-inch pots after two weeks of growth. Finally seedlings were transplanted into twelve-inch pots when flower buds appeared. The pots were placed on a bench in the greenhouse and supplied with supplemental illumination by 100-watt incandescent lamps. The photoperiod was maintained at approximately fourteen hours during the period of growth. A temperature of 75° F was maintained during the

day and 65° F at night. Bamboo stakes were used for supporting the plants.

Care of plants. Plants and seedlings were handled as for commercial culture except that no fungicide was applied in the field since this experiment was primarily devoted to the discovery of mutations for early blight resistance. Clean cultivation was maintained and irrigation used whenever needed. In the greenhouse Parathion was used as a fumigant in controlling insects and Maneb in controlling leaf mold (Cladosporium sp.).

Labeling and Recording

A bamboo stake was placed by each abnormal plant and tagged with a numbered metal label. Staked plants resulting from X-ray treatments were given X numbers such as X-1, X-2,---. There was no need to distinguish between X-ray treatments as only those from 30,000r X-ray treatment survived to reach the field. Staked plants resulting from thermal neutron exposure were given numbers preceded by the number of hours of exposure. Thus, for plants resulting from twenty-hours exposure, the plant number was preceded by 20. For thirty hours exposure, the number was preceded by 30 and so on for the various exposures. The abnormal characters of each numbered plant were recorded on 4 x 5 cards on which the location of the abnormal plant in the field was also indicated. No attempt was made to record the height measurement, size of leaves,

time and degree of flowering and fruiting of all plants.

Cross-Pollination Technique

Plants to be crossed were grown in a greenhouse screened to exclude insects. The flower buds were emasculated by removing the stamens with curved-tip forceps one day before the flower would have opened. All pollinations were made on the emasculated flowers the day they would normally have opened. Fresh pollen was taken from the pollen-donor plants by breaking the anthers with the points of the forceps and scraping the pollen out, and applied by gently rubbing the stigma with the points of forceps. Those flowers were tagged with the date and the pollen donor, and recorded tags were attached to the flowers of the seed parent immediately after each pollination. Usually two applications were made for each pollination.

Progeny Tests

Seeds from affected branches or plants were collected separately. Starting from September 25, 1957 until the end of March, 1958, progeny tests were made in the greenhouse. Due to space limitation, fifty progenies with thirty to fifty seeds for each progeny, were planted at two months intervals. Planting procedure and the care of seedlings was followed as described above. Progenies which grew normally and uniformly and in which no abnormal characteristics could be observed were discarded after two months of growth. If a certain progeny showed abnormal-plant segregation, a second planting

was made immediately to obtain a large sample for the purpose of determining more accurately the segregation ratio. All plants in progenies that had abnormal plants were transplanted into ten-inch pots for later observation. The characteristics and the segregation ratio of each progeny were recorded.

Tester Plants for Inheritance Studies

Two tester plants, daclry and dpsory, were used in the studies of inheritable mutations in this experiment.

daclry, which is characterized by six homozygous recessive genes on six separate chromosomes or linkage groups, has been often used as a tester plant in studies of tomato inheritance. d, symbol for dwarf character, is in the first linkage group, a symbol for green stem, is in the fifth linkage group, c, for potato leaf, is in the fourth linkage group, l, for lutescent, is in the sixth linkage group, r, for yellow flesh, is in the second linkage group and y, for clear skin, is in the third linkage group.

dpsory, which is characterized by six homozygous recessive genes on three chromosomes or linkage groups, has been also used in studies of tomato inheritance. d, representing dwarf, p, representing peach, s, representing compound inflorescence, and o, representing oblate-shaped fruit, are in the first linkage group. r, representing yellow flesh, is in the second linkage group and y representing clear skin, is in the third linkage group.

Inheritance Studies

From each fertile plant which displayed abnormal or mutant characters a selfed progeny of seedlings was raised in order to determine if the observed abnormalities were inheritable mutations. One hundred eighty-six progeny tests were made for this purpose. Forty-seven of these progenies displayed some abnormalities. Ten progenies were selected for further testing. Abnormal plants in these ten progenies were selfed, crossed with normal Chatham, and crossed with daclry. From these crosses F1 plants were raised and, as soon as they bloomed, backcrosses were attempted in each case to the mutant parent. In addition to testing the abnormal plants of the above ten progenies, all normal appearing plants of these progenies were also selfed and progeny tested.

Chi-square values were calculated for testing the goodness of fit for the segregation ratio whenever it could be applied. Because of the partially sterile nature and the lower viability of the mutants, a large enough sample could not be obtained in all cases.

Abbreviations

- R1 The immediate generation from irradiated seeds.
- R2 Second generation from irradiated seeds.
- R3 Third generation from irradiated seeds.

RESULTS AND DISCUSSION

Effects of Irradiation on Chatham Tomato Seeds

Effects of irradiation upon germination and survival.

The results of seed germination are summarized in Table 1. It will be noted that the germination of irradiated seeds was probably not affected by either type of irradiation when germination was in petri dishes in the germinator (Fig. 1). The percentage of the germination of irradiated seeds was about as high as that of the untreated seeds. The irradiation did affect the emergence of seeds when they were sown in flats in the greenhouse (Fig. 2). Some seeds germinated but failed to emerge from the soil. Increasing amounts of irradiation tended to delay germination and delay as well as, decrease emergence of seedlings.

There was a reduction in the percentage of survival as the dosage increased. In the extreme X-ray treatment of 50,000r all seedlings died before the cotyledons fully expanded. In the extreme thermal neutron treatment of 1.0×10^{14} thermal neutrons, of 2200 seeds treated only 1211 germinated and of these just 178 survived to grow into mature plants and these plants were weak and slow growing at first. Similar results of delayed germination and lack of survival from irradiated seeds were obtained by MacKay (18) with barley, Harlancher and Killough (10) with cotton, and MacArthur(17) and Mertens and Burdick (20) with tomato.

The early growth of the seedlings from thermal

Table 1. Effect of irradiation on germination and survival of Chatham tomato seeds.

1. Germination in the petri dish in the germinator.

Treatment	No. of seeds	No. of Germ.	% of Germ.	Mean days to Germ.	No. survived	% of survival
Control	200	195	97.5	5.26	168	84.0
30,000r X-rays	200	196	98.0	5.95	18	9.0
50,000r X-rays	200	183	91.5	6.50	-	-
4.85×10^{13} neutrons	200	194	97.0	5.56	180	90.0
6.56×10^{13} neutrons	200	193	96.5	5.95	157	78.5
7.54×10^{13} neutrons	200	194	97.0	6.51	116	58.0
1.0×10^{14} neutrons	200	197	98.5	6.71	18	9.0

2. Germination in the flats in the greenhouse.

Treatment	No. of seeds	No. of Germ.	% of Germ.	Mean days to Germ.	No. survived	% of survival
Control	2000	1852	92.5	7.80	1852	92.6
30,000r X-rays	2000	1462	73.1	8.11	557	27.85
50,000r X-rays	2000	212	10.6	10.22	-	-
4.85×10^{13} neutrons	2000	1870	93.5	8.99	1801	90.05
6.56×10^{13} neutrons	2000	1596	79.8	10.05	1549	77.45
7.54×10^{13} neutrons	2000	1736	68.8	10.31	1011	50.55
1.0×10^{14} neutrons	2000	1024	51.2	11.25	160	8.00

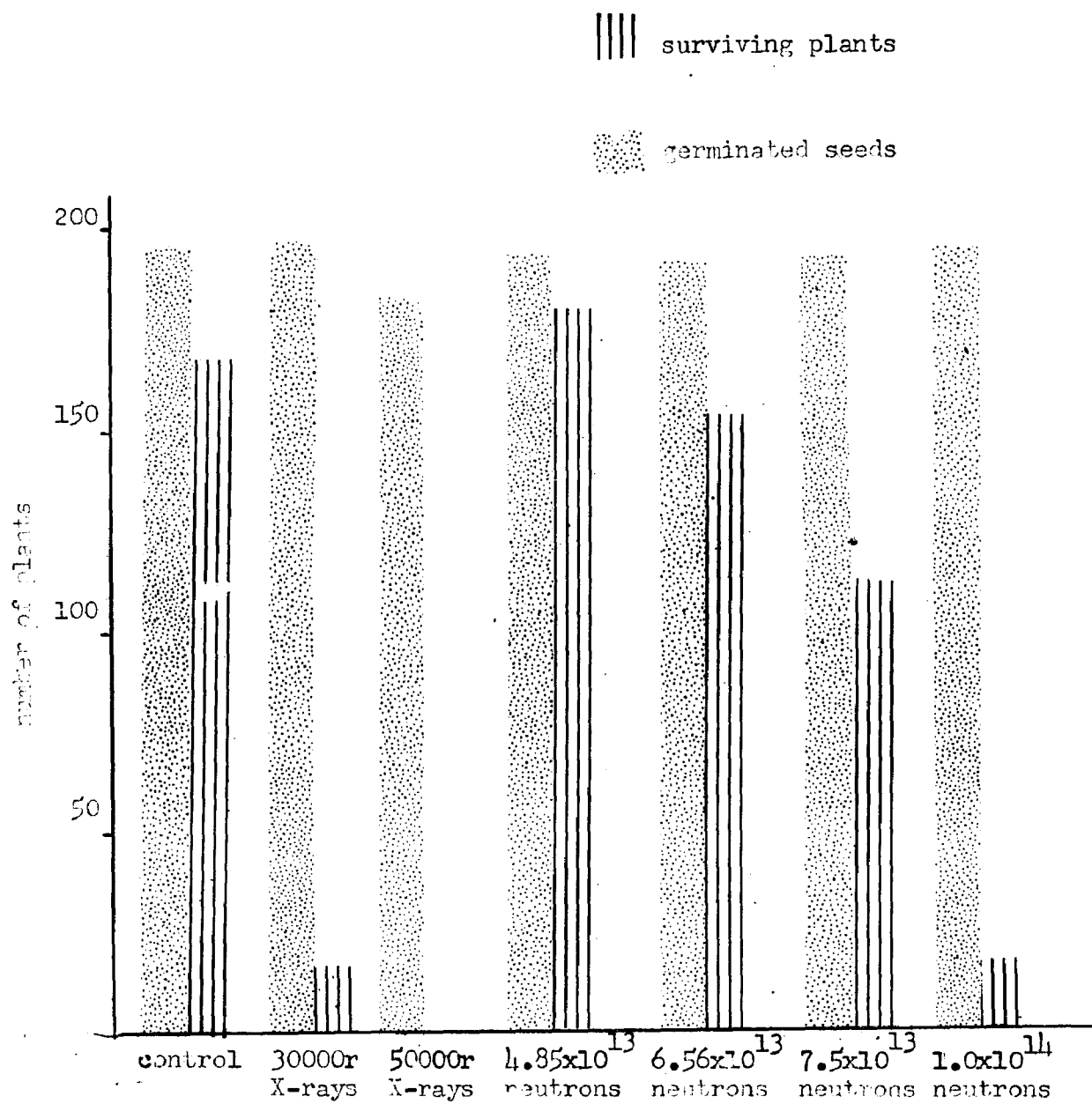


Fig. 1. Effects of irradiation on germination (in germinator) and survival

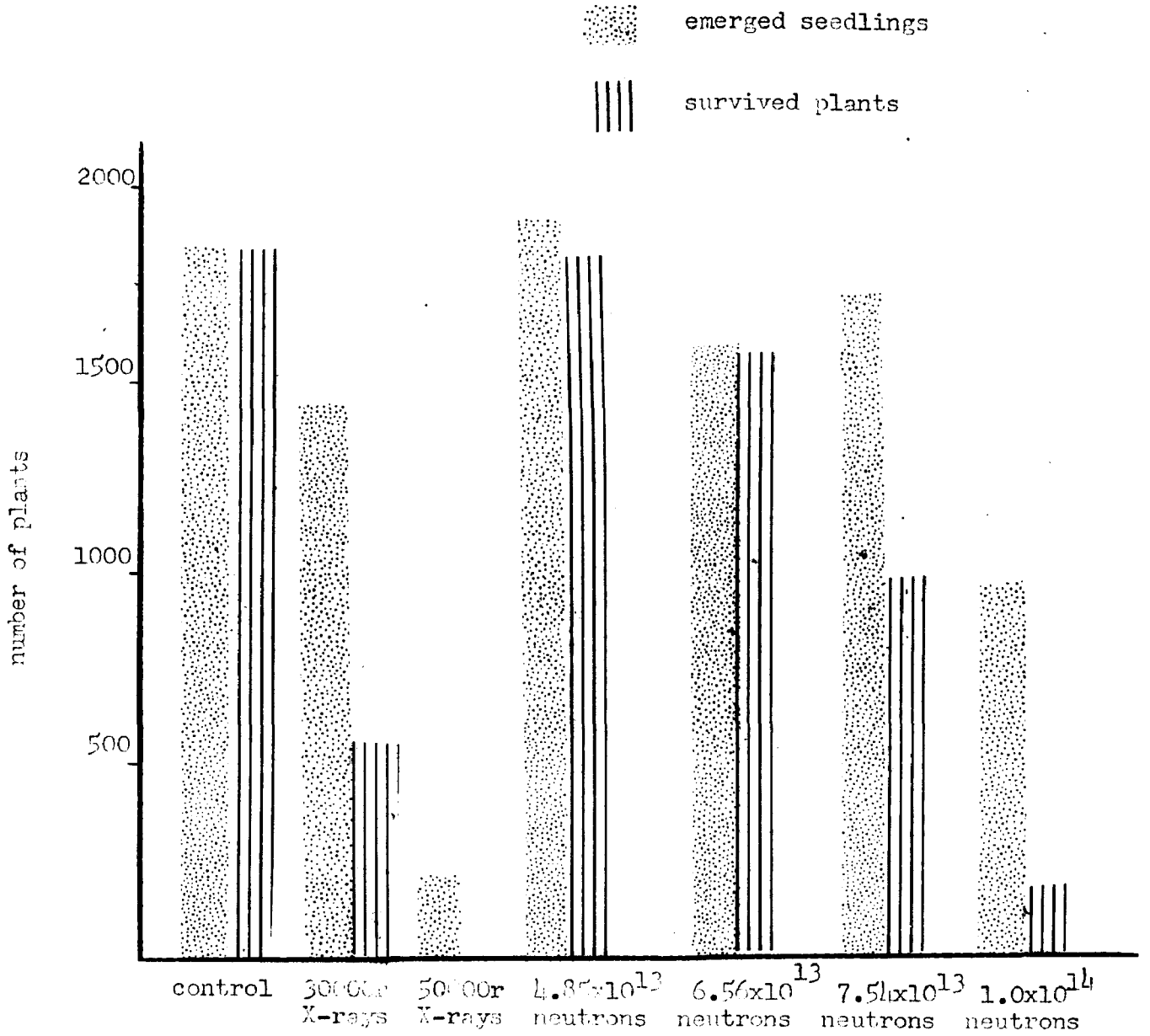


Fig.2. Effects of irradiation on emergence (in flats) and survival

neutron irradiated seeds was much more uniformly affected than that from the X-rayed seeds. This was reflected by the uniformity in height of seedlings obtained from the thermal neutron irradiated seeds and the variability in height of seedlings obtained from X-rayed seeds. Similar results has been reported by Caldecott, Beard and Gardner (6) with barley.

Effects of irradiation on later growth of surviving plants. Table 2. shows the relationship of surviving plants to numbers of treated seeds and the numbers, and the percentages of abnormal plants which resulted from each treatment. It will be noted that the 7.54×10^{13} thermal neutron treatment produced the highest percentage of abnormal of those plants that survived (Fig.3) but the 6.56×10^{13} thermal neutron treatment produced highest number of abnormal from irradiated seeds. It will be noted also that the percentage of abnormal plants to surviving plants is within a range of about two percent for three of the treatments (30,000r X-rays, 6.56×10^{13} and 7.54×10^{13} thermal neutron treatment).

The 50,000r X-ray treatment was eventually lethal to all surviving plants. The 1.0×10^{14} thermal neutron dosage seemed to be approaching the lethal treatment as few surviving plants resulted from the treated seeds; however, of the surviving plants the percentage of abnormal was not much below that of the three treatments mentioned above. The lowest thermal neutron treatment 4.85×10^{13} had only a moderate effect on the number of surviving plants as com-

Table 2. Effects of irradiation on surviving plants

Dosage	Control	30,000r X-rays	50,000r X-rays	4.85×10^{13} neutrons	6.56×10^{13} neutrons	7.54×10^{13} neutrons	1.0×10^{14} neutrons
No. of seeds planted	2200	2200	2200	2200	2200	2200	2200
No. of surviving plants	2020	575	-	1966	1706	1278	178
No. of abnormal plants	1	111	-	211	342	273	32
% of abnormal plants from irradiated seeds	0.05%	5.04%	-	9.59%	15.54%	12.41%	1.45%
% of abnormal plants from surviving plants	0.048%	19.30%	-	10.73%	20.04%	21.36%	17.97%
No. of whole plants affected							
morphological	1	6	-	4	11	15	7
chlorophyll	-	2	-	1	1	1	-
No. of plants with affected branches							
morphological	-	79	-	144	228	180	19
chlorophyll	-	24	-	62	102	77	6

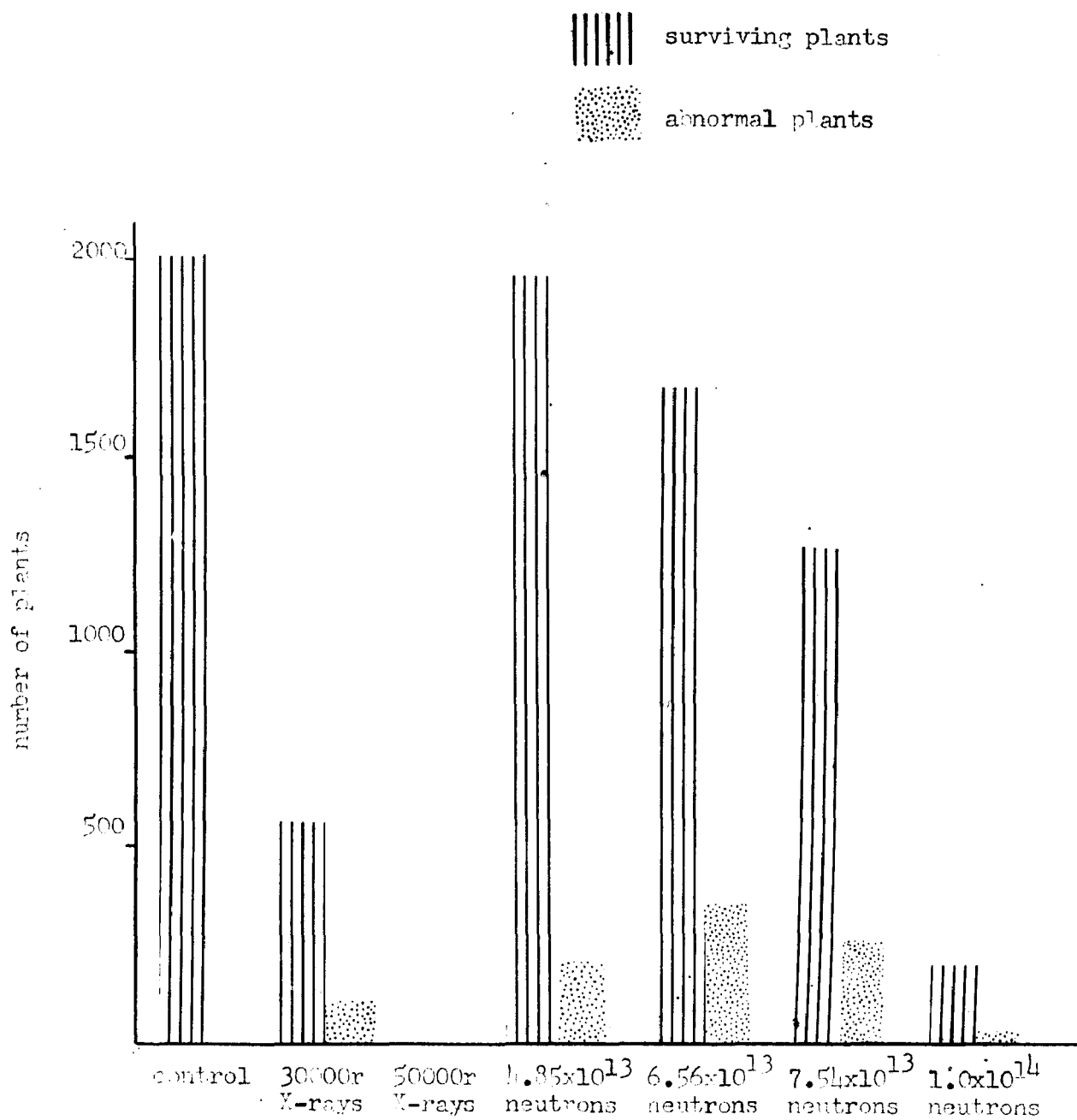


Fig. 3. Effects of irradiation on surviving plants

pared to the control and the percentage of abnormal to surviving plants resulting from the treatment was approximately half the average of the other treatments. This would seem to indicate that the 4.85×10^{13} thermal neutron treatment may not be strong enough to produce a desirably high percentage of abnormal plants.

Morphological effects. The occurrence of abnormalities is summarized in Table 3, and the description of abnormalities is on page 30-31. From the Table 3 it will be noted that the same abnormalities were induced by both types of irradiation. The 6.56×10^{13} thermal neutron treatment seemed to induce more morphological abnormalities and the 1.0×10^{14} thermal neutron treatment fewer than the other treatments. This latter effect is probably due to a low post-irradiation survival rate and is probably not significant. If failure of plants to survive is considered largely due to the effects of radiation, the total effects of 1.0×10^{14} thermal neutron treatment is significantly greater than that of the 6.56×10^{13} thermal neutron treatment.

Certain types of abnormalities occurred in seedlings from both treated and untreated seeds. However, the occurrence was sufficiently more frequent in the treatments to be noticeable to even a casual field inspection. These abnormalities included fasciated stem, fasciated flowers, dichotomous branching. Such abnormalities according to White (25) are common in over one third of the vascular plants with the

Table 3. Frequency of morphological abnormalities in the R1 generation

Kinds	30,000r X-rays		4.85x10 ¹³ neutrons		6.56x10 ¹³ neutrons		7.54x10 ¹³ neutrons		1.0x10 ¹⁴ neutrons	
	pl.	br.	pl.	br.	pl.	br.	pl.	br.	pl.	br.
curly leaves	1	10	-	10	1	30	1	24	-	2
irregular leaves	-	15	-	11	-	35	3	23	-	2
narrow leaflets	-	1	-	1	-	4	-	1	-	-
entire margined-leaves	-	-	-	2	-	1	-	-	-	-
leaf chimera	-	-	-	7	-	3	-	4	-	1
brown necrotic	-	2	-	2	-	9	-	4	-	-
grey necrotic	-	2	-	1	-	3	-	2	-	-
small leaves	-	3	-	3	-	5	1	3	1	-
glossy leaves	-	9	-	7	-	15	1	15	1	1
wilty leaves	2	8	1	41	2	33	2	42	-	4
mottled leaves	1	13	-	27	1	52	1	51	-	7
wrinkled leaves	1	9	-	9	2	16	2	-	-	1
weak plant	-	-	-	3	1	4	-	3	1	-
super-vigorous	-	-	-	-	-	1	1	-	-	-
depressed plant	-	1	-	-	-	-	-	-	-	-
wilty wrinkled mottled	-	-	-	14	-	9	-	4	-	-
dwarf plant	1	-	1	-	4	-	3	-	3	-
large leaves	-	6	-	6	-	8	-	4	1	1
Total	6	79	2	144	11	228	15	180	7	19

Description of morphological abnormalities

Curly leaves: leaf twisted with leaflets inrolled and / or circinate; leaf surface rugose*.

Irregular leaves: includes several forms of abnormal leaf shapes which are lumped together in this category. Included are: 1. twisted leaflets; 2. shoestring leaf; 3. half of leaf blade absent; 4. half of leaf blade normal and other half irregular; and other irregular leaf forms.

Narrow leaflets: leaflets proportionately much narrower than normal leaflets.

Entire-margined leaves: lobes of leaflets absent.

Leaf chimera: yellow or light green sectors on normal green leaves.

Brown-necrotic: young leaves with irregular brownish necrotic area, necrosis spreading as leaves mature, causing early death of leaves.

Grey-necrotic: leaves with numerous minute greyish spots; spots spread and cover whole leaf, eventually causing the death of the leaf*.

Small leaves: leaves normal in shape but half the size of normal leaves.

Glossy leaves: leaves dark green in color with shining smooth surface, nearly glabrous*.

Wilty leaves: leaflets infolding along the midrib.

* described in section on "Induced Mutation"

Mottled leaves: leaves normal green, mottled with light green or yellow.

Wrinkled leaves: leaf blades more or less rugose but not distorted.

Weak plant: entire plant weak, somewhat stunted but truly dwarf.

Super-vigorous: plant or branch exceptionally vigorous.

Depressed plant: stunted leaves distorted with indistinct chlorotic area.

Wilty-wrinkle-mottle: leaves exhibiting all three of these character (as described above) at once.

Dwarf plant: plant less than one foot in height with all parts depressed.

Large leaves: leaf blade thick and abnormally large.

exception of the halophytic and hydrophytic forms. Since these particular abnormalities were found in all treatments including the controls and in any case are of regular occurrence in the Chatham variety of tomato, they were therefore disregarded and not considered in the enumeration of abnormalities.

Chlorophyll changes. Chlorophyll changes were another common effect induced by irradiation. The same types of chlorophyll changes were induced by both X-rays and thermal neutrons and there seems no significant difference between treatments. For the sake of convenience, they were classified into six groups; light yellow, yellow, very light green, light green, grey-blue-green, and dark green. Except for two light yellow leaved branches from two different plants which produced normal fruits at the expense of normal branches, light yellow and yellow leaved branches usually died before they reached maturity. In general, most very light green branches also died when young, and surviving ones were very weak and sterile. The light green branches grew normally and behaved as normal branches, and some even resumed their normal color. Grey-blue-green branches, which had a large amount of pubescence, not only appeared to have less chlorophyll content but also were altered in internal structure, having no palisade layers, and were all sterile. Dark-green branches, that had smooth leaf surface with little pubescence were fertile; progeny results showed that this character was not inheritable. The frequency of chlorophyll

Table 4. Frequency of chlorophyll changes in the R1 generation

Kinds of changes	30,000r X-rays		4.85x10 ¹³ neutrons		6.56x10 ¹³ neutrons		7.54x10 ¹³ neutrons		1.0x10 ¹⁴ neutrons	
	pl.	br.	pl.	br.	pl.	br.	pl.	br.	pl.	br.
light yellow	-	1	-	1	-	7	-	5	-	-
yellow	-	-	-	4	-	10	-	3	-	1
very light green	-	5	-	4	-	22	-	14	-	-
light green	2	16	-	48	1	53	1	44	-	6
dark green	-	1	-	1	-	3	-	1	-	1
grey-blue-green	-	1	1	4	-	7	-	10	-	1
Total	2	24	1	62	1	102	1	77	-	9

changes is presented in Table 4.

Effects on fertility. In general, plants, that survived from irradiated seeds were delayed in blooming and fruiting as compared to the controls. Of a total of 5703 surviving plants from irradiated seeds of all the treatments, 12.56 % had sterile branches and 0.47 % were completely sterile. Among 969 affected plants 73.37 % had sterile branches, and 2.64 % were completely sterile. The observed data are summarized in Table 5.

Progeny tests of abnormal plants. One hundred and eighty-six abnormal plants which produced fruits with seeds in the R1 generation were progeny tested. Among these only forty-seven progenies showed abnormalities in the R2 generation, and of these only eight progenies had abnormalities similar to those of their mother plants as shown in Table 6. Only the following abnormalities were inherited: chlorophyll deficiency (No. 125, 194, 182, 488 and 504), curly (No. 83), and brown necrotic (No. 615, 813). In addition, some inheritable abnormalities appeared in the R2 generation which had not occurred in the R1 generation. The inheritable abnormalities in both generations will be discussed in more detail in the following section except the brown necrotic, the inheritance of which thus far has been irregular, needing further study.

Some plants with similar abnormalities in the R1 generation had different abnormalities occurring in their progenies. For instance, of 79 plants with curly leaves, only 4 plants produced fruits with viable seeds, and among

Table 5. Effects of irradiation on fertility of affected plants

	30,000r X-rays	4.85×10^{13} neutrons	6.56×10^{13} neutrons	7.54×10^{13} neutrons	1.0×10^{14} neutrons
No. of plants survived	575	1966	1706	1278	178
No. of affected plants	111	211	342	273	32
No. of sterile plants	7	7	4	7	1
No. of plants with sterile branch	82	151	252	214	12
No. of plants fruited	22	53	86	52	18
No. of fruited plants with seeds	20	50	64	43	9
No. of fruited plants with no seeds	2	3	22	9	9

Table 6. Results of progeny tests of 47 R1 abnormal plants

plant number	description of plant	description of offspring
2		
20	wilty, wrinkled-leaved branch.	2 dwarf plants, 5 normal plants.
33	mottled-leaved-branch.	27 suppressed-root plants, 19 normal plants.
38	mottled-leaved-branch	4 small plants, 10 normal plants.
43	curly-leaved plant	3 small plants, 7 normal plants
46	wilty, mottled-leaved branch	2 albino plants, 18 normal plants
48	mottled-leaved branch	1 dwarf plant, 17 normal plants
83	curly-leaved branch	2 albinos, 3 curly-leaved plants, 11 normal plants
89	wilty-leaved branch	2 dwarf plants, 6 normal plants
92	curly-leaved branch	9 lethals, 8 normal plants
115	branch with leaf chimera	1 wiry-leaved plant, 4 normal plants
125	yellow-leaved branch	all albinos
143	mottled-leaved branch	1 dwarf plant, 10 normal plants
162	wilty, mottled-leaved branch	2 lethals, 12 normal plants
176	irregularly-leaved branch	2 albinos, 3 normal plants
177	wrinkled-leaved branch	2 wiry-leaved plants, 8 normal plants
182	light green branch	6 light green plants, 3 normal plants
187	thread-leaved branch	2 lethals, 7 normal plants
189	wrinkled-leaved branch	12 lethals, 1 normal plant
194	yellow-leaved branch	all albinos
207	mottled, wrinkled-leaved branch	1 albino, 9 normal plants
220	mottled-leaved branch	1 lethal, 3 normal plants
248	curly-leaved branch	1 lethal, 3 normal plants
250	irregularly-leaved branch	4 plants with round leaflets, 10 normal plants, 2 grey-necrotic

Table 6. continued

plant number	description of plant	description of offspring
257	brown-necrotic	1 brown-necrotic plant, 6 normal plants
259	grey-blue-green-leaved branch	5 brown-necrotic, 5 normal plants
329	light green branch	2 lethals, 13 normal plants
392	mottled-leaved branch	5 lethals, 1 thick-leaved plant
436	dwarf plant	1 weak plant, 4 normal plants
485	light green branch	12 green-stemmed, 38 normal purple-stemmed plants
488	light green branch	12 albinos, 7 light green plants, 2 normal plants
490	light green branch	5 small plants, 11 normal plants
501	thread-leaved branch	2 albinos, 9 normal plants
504	light green branch	7 light green plants, 7 normal plants
508	wilty-leaved branch	1 tripinnate-leaved plant, 6 normal plants
542	thread-leaved branch	1 albino, 5 normal plants
615	brown-necrotic	2 brown-necrotic plants, 6 normal plants
715	curly-leaved branch	1 albino, 4 normal plants
736	curly, wrinkled-leaved branch	2 grey-necrotic plants, 3 normal plants
739	wilty-leaved branch	2 virescent plants, 9 normal plants
778	curly, mottled-leaved branch	1 brown-necrotic plant, 11 normal plants
789	light green branch	3 drooping-leaved plants, 1 normal plant
813	brown-necrotic	all brown-necrotic plants
953	glossy leaf surface	1 small plant, 1 normal plant
1001	abnormally-shaped fruit	2 wiry-leaves plants, 6 normal plants
A42	thick-leaved branch	1 dwarf, 16 normal plants
A56	glossy leaf surface	2 dwarf plants, 2 normal plants
B18	small-leaved branch	3 grey-necrotic plants, 5 normal plants

the 4 progenies only one showed the same abnormality as the mother plant, the other progenies exhibiting different abnormalities. One hundred and sixty-five plants in the R1 generation had chlorophyll deficiencies; this was inherited in only five cases.

Induced mutations

Inheritable mutations. From the progenies tested in the greenhouse, ten inheritable mutations were selected for more extensive study. These included nine recessive and one dominant mutations. Five followed the 7.54×10^{13} thermal neutron treatment; two, the 6.56×10^{13} thermal neutron treatment; one, the 5.85×10^{13} thermal neutron treatment; and one, the 30,000r of X-rays.

Among these ten mutations, seven affected morphological characters, one root development, one chlorophyll, and one the leaf tissue.

Two inheritable mutations occurred in the R1 generation. These were:

1. chlorophyll deficiency
2. curly-leaves

Eight inheritable mutations appeared in the R2 generation. These were:

- | | |
|----------------------|--------------------|
| 3. suppressed-root | 7. drooping-leaves |
| 4. green-stem | 8. slender-stem |
| 5. round-leaflets | 9. grey-necrotic |
| 6. tripinnate-leaves | 10. wiry-leaves |

1. Chlorophyll deficiency.

Albino lethal. Both abnormal plants No. 125 and No. 194 followed the 6.56×10^{13} thermal neutron treatment. They were plants with albino branches (Fig. 4) which grew to maturity nourished by the normal branches. A few fruits were obtained from the albino branches. Progeny tests in the greenhouse produced all albino seedlings. Since albino plants could not live alone, all albino seedlings died at an early age. The nature of the inheritance is unknown.

Light green. Abnormal plants No. 182, No. 488 and No. 504 all had light green branches in the R1 generation and produced light green plants in their progenies. No further study was made.



Fig. 4. Plant with albino branches

2. Curly-leaves. (Fig. 5) One plant from the seeds of 7.54×10^{13} thermal neutron treatment had an abnormal branch with mottled, wrinkled, and crisped leaves. The branch bore several fruits with normal seeds. This was labeled as abnormal plant No. 83.

Seedlings from this plant were 2 albino plants, 11 normal plants, and 3 plants with curly leaves. These 3 curly-leaf plants were labeled as 83-1, 83-2 and 83-3. Seeds from these curly-leaf plants produced an R3 generation of all curly-leaf plants that grew very slowly and were dwarf in form. Curly-leaf seedlings could not be recognized in the cotyledon stage. The leaves began curling downward when the plants reached about two to three inches high. The leaves were small, about three to four inches in length. The fruits on the curly-leaf plants, about one inch in diameter, were thirty to fifty days later in maturing than were fruits of normal plants. Seeds from three of the normal plants produced only normal plants, indicating they were homozygous. Seeds from the fourth normal plant produced two curly-leaf plants and nine normal plants, indicating that it was heterozygous. Curly-leaf was assumed to be due to a recessive gene. Progeny tests and crosses were then made. The results are described in Table 7.

83-1 was used as both male and female parent in crosses with normal Chatham and daclry. The phenotypes of the F1 plants of the crosses Chatham x 83-1 and 83-1 x Chatham were identical to that of normal Chatham. In the

Table 7. Results of progeny tests made in the study of curly-leaves.

Parentage	Generation	curly-leaf plants	non-curly-leaf plants
83	R2	3	11 normal plants 2 albinos
83-1, 83-2, 83-3	R3	all	0
83-1 x Chatham	F1	0	all
83-1 x daclry	F1	0	all
Chatham x 83-1	F1	0	all
daclry x 83-1	F1	0	all
Chatham x 83-1	F2	10	31
daclry x 83-1	F2	15	42

crosses of 83-1 x daclry and daclry x 83-1, the phenotypes of the F1 were similar to those of the F1 plants of Chatham x daclry.

The F2 generation from the cross of Chatham x 83-1 produced 10 curly-leaf plants and 31 normal plants, and the F2 generation of the cross of daclry x 83-1 showed 15 curly-leaf plants and 42 normal plants. (see Table 7). Both the F2 generations were close to a 3:1 ratio and there were no intermediates. Sixteen F2 plants from the cross of Chatham x 83-1 were selected at random, selfed, and backcrossed to 83-1. Among these sixteen plants, seven plants proved to be homozygous, because their selfed seeds and backcrossed seeds produced only non-curly, normal plants; nine plants proved to be heterozygous, because their selfed and backcrossed seeds produced both curly-leaf and normal plants.

These results confirm the hypothesis that the curly character is controlled by one simple recessive gene.

Small fruit was associated with curly-leaves in all cases. (Fig. 26)

No linkage for curly-leaves was indicated when tested with genes in group I, II, III, IV, V, VI, and VII as shown in Table 8.

Symbol cu₂ is proposed for this recessive curly-leaves to distinguish it from Cu proposed by Young (28) in designating a similar but dominant curly-leaf gene.

Table 8. F₂ segregation of curly-leaf mutant and tester genes from the cross of daclry x 83-1

linkage group	tester genes	parental classes		recombination classes		χ^2
		d-Cu ₂ -	D-cu ₂ -	D-Cu ₂ -	d-cu ₂ -	
I	d Exp.	9 (10.5)	9 (10.5)	33 (31.5)	6 (3.5)	2.38
Difficult classification of <u>d</u>						
V	a Exp.	12 (10.5)	11 (10.5)	30 (31.5)	4 (3.5)	1.25
IV	c Exp.	14 (10.5)	11 (10.5)	28 (31.5)	4 (3.5)	1.88
VI	l Exp.	15 (10.5)	11 (10.5)	27 (31.5)	4 (3.5)	2.89
II	r	16 (10.5)	12 (10.5)	26 (31.5)	3 (3.5)	4.14
III	y Exp.	10 (10.5)	10 (10.5)	32 (31.5)	5 (3.5)	1.16
VII	u Exp.	13 (10.5)	12 (10.5)	29 (31.5)	3 (3.5)	1.10
IV	sp Exp.	11 (10.5)	9 (10.5)	31 (31.5)	6 (3.5)	2.24

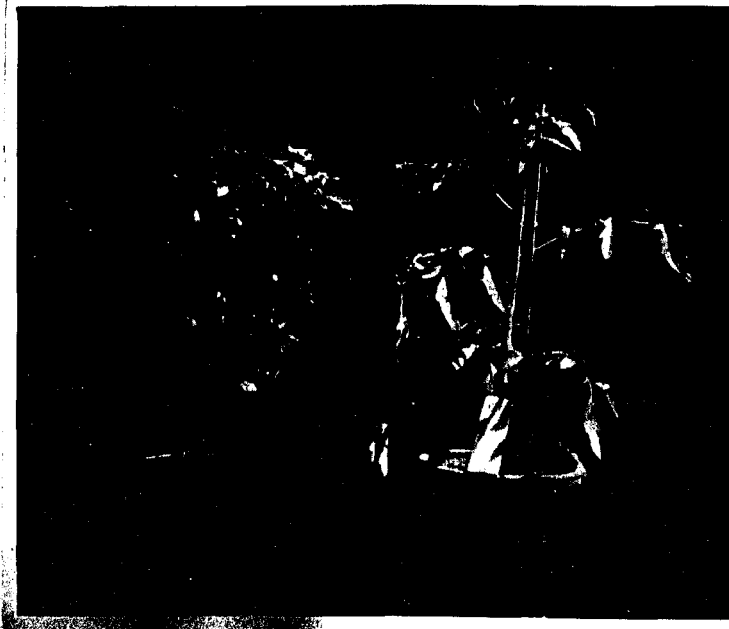


Fig. 5. A comparison of a curly-leaves mutant and a normal plant

Left: curly-leaves mutant

Right: normal plant (younger plant)

3. Suppressed-root. (Fig. 6) Abnormal plant No.33, from a 7.54×10^{13} thermal neutron treatment, was found to have a mottled-leaved branch from which three medium-sized open-pollinated fruits were obtained. A progeny test in the greenhouse gave no mottled plants in the R2 generation but did give three normal plants and ten small, retarded plants. A second planting produced 16 normal plants and 17 retarded plants. Seeds from the normal seedlings produced only normal plants. The retarded seedlings failed to grow after the cotyledons were fully expanded and the first two true leaves were noticeable. The undisturbed ones died after three months. A careful examination revealed that these plants had very few, small lateral roots and a small amount of root hairs. Thirty of these seedlings were grafted on thrifty plants of the Chatham and Window Box varieties. Of these, one grafted on Window Box (33-1) and two on Chatham (33-2 and 33-3) were successful. The one grafted on Window Box grew vigorously; those on Chatham also grew into plants of normal size and appearance. No difference could be observed between normal Chatham and these grafted mutants. Cuttings made from axillary shoots of the grafted plants failed to root (Fig. 7), although normal Chatham cuttings root readily.

Table 9 shows that seeds from selfed 33-1 produced 69 suppressed-root plants and 24 non-suppressed-root plants, which is approximately a 3:1 ratio. Seeds from each of the 24 non-suppressed-root plants produced only non-suppressed-root plants. These results suggest that 33-1 was heterozygous for.

Table 9. Results of progeny tests made in the study of suppressed-root

parentage	generation of progeny	suppressed-root plants	non-suppressed-root plants
33	R2	27	19
33-1	R3		
1st seeding		23	10
2nd seeding		18	7
3rd seeding		28	7
total		<u>69</u>	<u>24</u>
33-2	R3	67	0
33-1 x daclry	F1		
1st seeding		3	6
2nd seeding		4	4
total		<u>7</u>	<u>10</u>
daclry x 33-1	F1		
1st seeding		1	3
2nd seeding		2	1
total		<u>3</u>	<u>4</u>
Chatham x 33-1	F1		
1st seeding		9	10
2nd seeding		10	8
total		<u>19</u>	<u>18</u>
33-2 x Chatham	F1	18	0

a dominant suppressed-root character. Since its effect is lethal, its presence in plants not supplied with normal roots by grafting causes early death of the plants. If suppressed-root is assumed to be a dominant, one third of the 69 abnormal plants from the selfed 33-1 generation should be homozygous for suppressed-root. Seeds from selfed 33-2 produced 67 suppressed-root plants and no normal plants which indicated that 33-2 was homozygous and dominant for suppressed-root character.

To test the hypothesis of one dominant gene, 33-1 and 33-2 were used in reciprocal crosses with normal Chatham and daclry. Only a few seeds were thus obtained. The results are shown in Table 9. Crosses of daclry x 33-1 segregated in the F1 generation, producing 3 suppressed-root plants and 4 normal plants from two seedings. Eighteen normal plants and 19 suppressed-root plants were secured from the cross of Chatham x 33-1 from two seedings. Ten normal plants and 7 suppressed-root plants were obtained from the cross of 33-1 x daclry from two seedings. The seeds of the cross of 33-2 x Chatham produced an F1 generation of all suppressed-root plants, indicating the homozygous condition of 33-2 for the suppressed-root character.

The segregation in the F1 generation of the crosses of daclry x 33-1, 33-1 x daclry, and Chatham x 33-1 supports the hypothesis that 33-1 was a heterozygous plant and that the suppressed-root is dominant over non-suppressed-root. While a 1:1 ratio should be expected in the F1 generation of the crosses if it is controlled by one simple dominant factor;

the deficiency of suppressed-root plants in the F1 generation in the crosses could easily occur with these small samples.

The symbol Sr is proposed for this radiation-induced mutation. Further study would be desirable to test this hypothesis and to determine its linkage relationship.

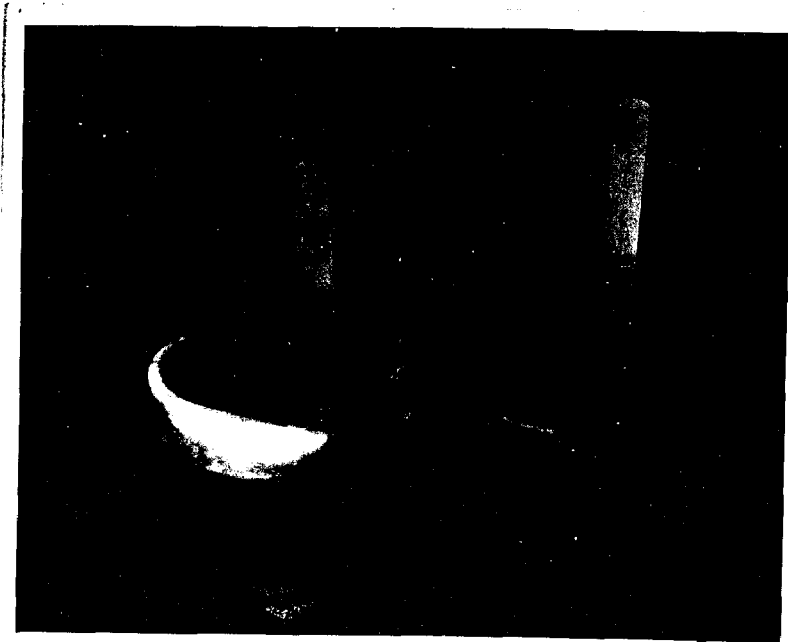


Fig. 6. Suppressed-root mutant

Right: suppressed-root mutant
Left: after grafting on normal roots, the same mutant at the same age



Fig. 7. A comparison of cuttings from a suppressed-root mutant and cuttings from a normal plant

Left: cuttings from suppressed-root mutant

Right: cuttings from normal plant made at the same time

4. Green-stem. (Fig. 8 and 10) One plant from the irradiated seed of 6.56×10^{13} thermal neutron treatment planted in the field in the summer of 1957 and labeled as abnormal plant No. 485 showed one branch with light green leaves. Two fruits were obtained from that abnormal branch.

Seeds from this branch produced 12 green-stemmed, weak, and slow-growing plants (485g) and 38 normal, purple-stemmed plants (485p).

Green-stem segregates were 1.5 to 2.0 feet in height, slow in growth and late-maturing. Stems and leaves were green in color, very brittle and fragile, with leaves wilting during the day. The fruits were small, $3/4$ to 1 inch in diameter and brownish red in color, and with many seeds in proportion to the size of the fruit. The flesh was brownish-green.

Progenies from the 12 green-stem plants produced only green-stemmed plants resembling the mother plants. Four plants were saved from the 38 purple stemmed plants. Three of them produced only purple-stemmed plants. The fourth purple-stemmed plant produced 16 purple-stemmed plants and 4 green-stemmed plants, indicating that the plant was heterozygous. Since the green-stemmed plants produced only green-stemmed plants in the next generation, green-stem would appear to be a single recessive gene.

In order to test this hypothesis, crosses were made between green-stem and normal Chatham. The results are summarized in Table 10. The offspring from both crosses of green-stem x Chatham and Chatham x green-stem were identical to

Table 10.. Results of progeny test made in the study of green-stem

parentage	generation of progeny	green-stem plants	purple-stem plant
485	R2	12	38
485g-1...-12	R3	all	0
485p-1,-2,-3	R3	0	all
485p-4	R3	4	16
485g x Chatham	F1	0	all
Chatham x 485g	F1	0	all
485g x Chatham	F2	25	76
daclry x 485g	F1	0	all
485g x daclry	F1	0	all
(daclry x 485g) x 485g	BC1	12	14
(daclry x 485) x daclry	BC1	35	31
daclry x 485g	F2	111	114

normal Chatham. Seeds from the cross of green-stem x Chatham produce an F₂ generation of 76 purple-stemmed plants and 25 green-stemmed plants, a typical monogenic segregation of 3:1. The second planting produced 62 purple-stemmed plants and 21 green-stemmed plants, also approximately a 3:1 ratio. These results indicate that the induced green-stem character is controlled by one simple recessive gene.

In order to determine whether this induced green-stem gene is the same gene as the previously known green-stem gene a, crosses were made between daclry, which contains the known green-stem gene a, and the induced green-stemmed plants.

The F₁ offspring from the crosses were all purple-stemmed plants similar to the F₁ generation of daclry x normal Chatham. This indicates that the induced green-stem gene is not the same as the one that produces green-stem in daclry. The occurrence of the purple-stemmed plants from the cross of daclry x green-stem might be explained as the interaction of two dominant genes, neither of them alone being capable of causing purple stem (Fig.9).

Backcrosses were made to both parents. Seeds from (daclry x green-stem) x daclry produced 31 purple-stemmed plants and 35 green-stemmed plants. Seeds from (daclry x green-stem) x green-stem produced 14 purple-stemmed plants and 12 green-stemmed plants. Both of the backcross generations showed segregation ratios of approximately 1:1 as would be expected from two different recessive genes. The F₂ generation from the cross of daclry x green-stem produced 114 purple-stem

plants and 111 green-stem plants. For a hypothesis of interacting inheritance of two factor pairs, a 9:7 segregation ratio in this case would be expected. From the calculation of goodness of fit for a 9:7 ratio, a $X^2=2.85$ value was obtained. If our hypothesis of two genes is correct, a X^2 value as great as this would be expected to occur between 5 and 10 times in 100 through errors of random sampling, according to Snedecor (24).

From these experiments it will be noted that the green-stem character fails to appear unless either a or the induced green-stem gene is presented in homozygous condition.

Small, brownish-red fruit (Fig.26) was associated with this induced green-stem character in all cases. It is not known whether this is the result of linked genes or another effect of the same gene. Further investigation is necessary to determine this.

The symbol a₂ is proposed for this irradiation-induced green-stem gene to distinguish it from the symbol a. Other genes giving green-stem, ag (4), aw (7), and a₃₂₅ (3), have been reported. ag, anthocyanin gainer, is obviously different from a₂. Tests are needed to determine whether a₂ is the same as aw or a₃₂₅.

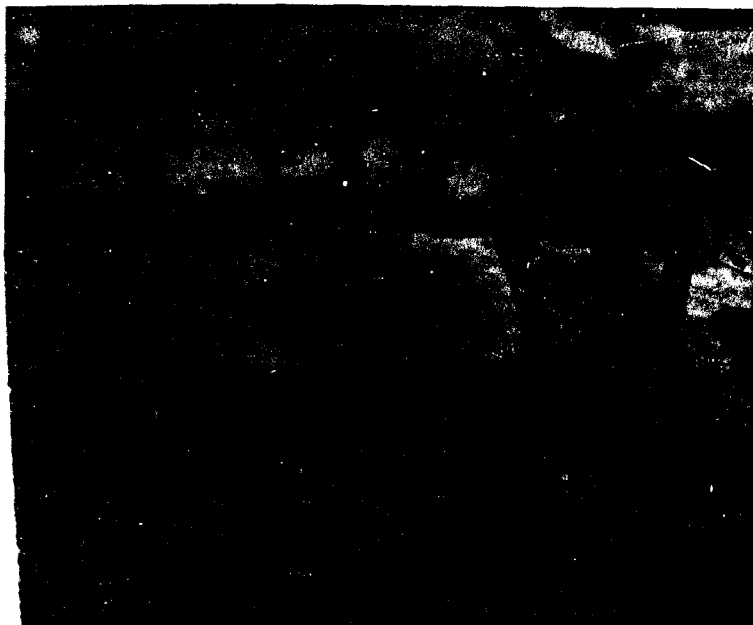


Fig. 8. A comparison of a green-stem mutant and a normal plant

Right: green-stem mutant

Left: normal plant at the same age



Fig. 9., A comparison of an F1 plant of
green-stem x daclry and its
parents

Left: green-stem mutant
Center: F1 purple stem plant
Right: daclry (green stem)

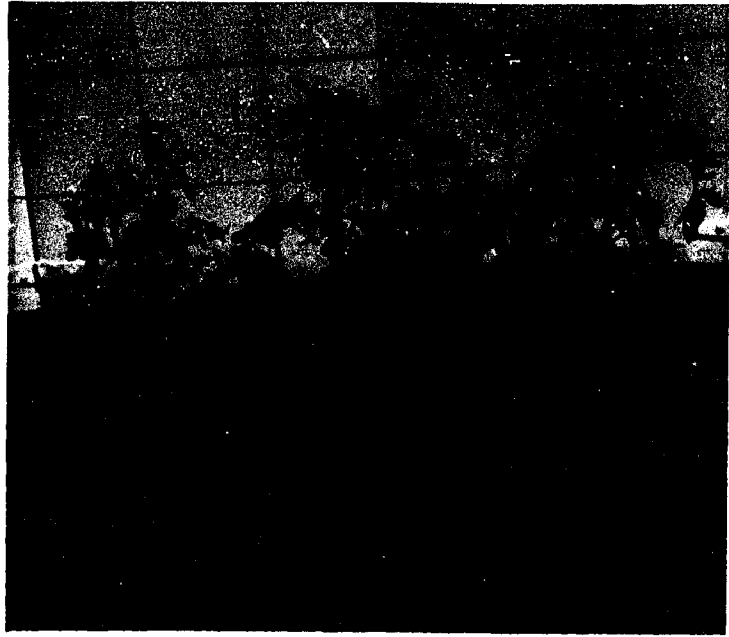


Fig. 10. Green-stem mutant in the field

5. Round-leaflets. (Fig. 11,12 and 13) One plant, No. 250, from the 6.56×10^{13} thermal-neutrons treatment produced a branch with abnormal leaves, fasciated flowers, and light green color.

Seeds from this branch gave 8 normal plants and 2 small plants with round leaflets (250-1 and 250-2) in the first seeding and 4 normal plants and 2 small plants with round leaflets (250-4 and 250-3) in the second seeding.

The most distinctive characteristics of the round-leaflets plants are that the leaves usually have 5 round leaflets with entire margins, and the plants have short internodes, are dwarf in form, and are slow-growing and late-maturing. They have two to three flowers in each flower cluster. The flowers are very small, about $\frac{1}{2}$ cm. in length. The number of petals and sepals are normal (5-7) but stamens and pistils are adhered, making emasculation impossible. Self-sterility is complete and no pollen is produced; however, parthenocarpic fruits are obtained.

Crosses were made between 250-1 and normal Chatham and daclry. Since no pollen could be obtained from 250-1, there was no reciprocal cross. Only a few seeds were obtained from the cross of 250-1 x daclry; the other crosses failed to set any fruit. Seeds from the cross of 250-1 x daclry produced an F1 generation resembling the F1 plants of Chatham x daclry. This indicated that the induced round-leaflets mutation is recessive.

Because no pollen was produced from round-leaflets

mutants, no backcross could be made.

Seeds were saved from 6 normal plants, and progeny tests in the greenhouse showed that 4 progenies gave round-leaflets segregates. The results are summarized in Table 11.

The F₂ generation of 250-1 x daclry had a segregation of 11 normal plants with cut leaves; 2 normal plants with potato leaves; 8 sterile intermediate plants with 5 non-lobed but dentate leaflets, large leaf blades, and longer internodes; and 1 plant with round leaflets resembling the mutants.

From the F₂ segregation of 250-1 x daclry and the segregation of 250-normal 1 and 250-normal 2, it appears that this characteristic may be controlled by two recessive genes. The round-leaflets character appears only when two recessive genes are in a homozygous condition in the same plant. If only one pair of the two recessive genes is present, the round-leaflets character fails to appear and the intermediate form is obtained. In such a case, a 9:6:1 ratio would be expected. From the calculation of goodness of fit, with the data from the F₂ segregation of 250-1 x daclry, a $\chi^2 = 0.26$ value was found and the observed χ^2 lay between p 0.80 and 0.50. This indicates that the deviation from 9:6:1 ratio was not significant. Due to the small sample, a definite conclusion can not be made. To confirm this hypothesis, further investigation is necessary.

Table 11. Results of progeny tests made in the study of round-leaflets

parentage	generation of progeny	round-leaflet plants	intermediate plants	normal plants
250	R2	4	0	12*
250-1,-2,-3,-4 all sterile				
250-normal 1	R3	1	2	14
230-normal 2	R3	1	1	15
250-normal 3	R3	1	0	15
250-normal 4	R3	1	0	15
250-normal 5	R3	0	0	15
250-normal 6	R3	0	0	25
250-1 x daclry	F1	0	0	35
250-1 x daclry	F2	1	8	13

* including 2 necrotic plants

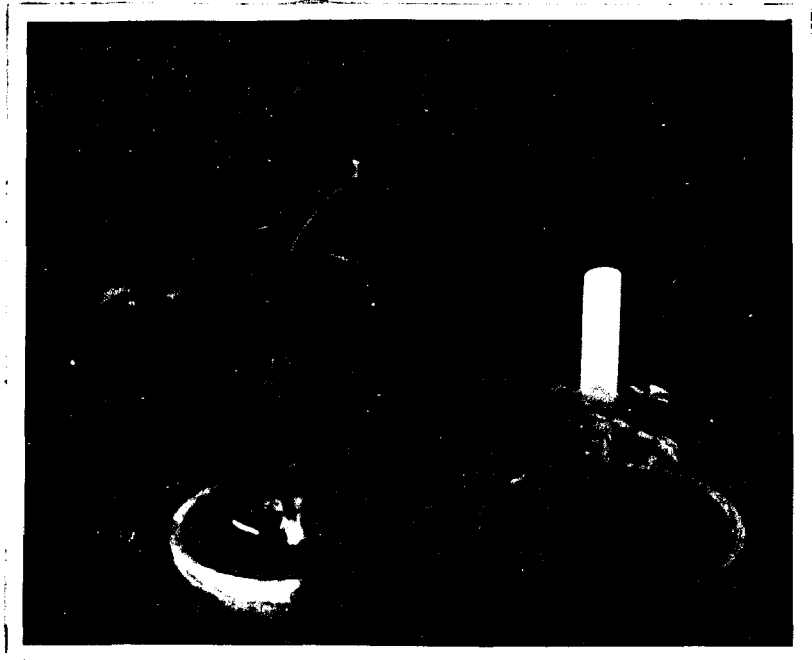


Fig. 11. A comparison of a round-leaflets mutant and a normal plant

Right: round-leaflets mutant

Left: normal plant at the same age

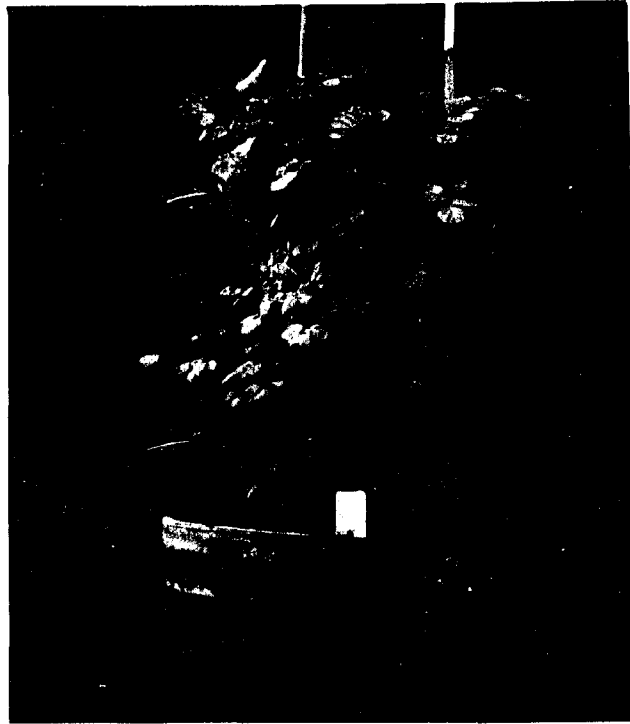


Fig. 12. Round-leaflets mutant at a later age



Fig. 13. Round-leaflets mutant in the field

6. Tripinnate-leaves. (Fig. 14 and 16) Abnormal plant No. 508, from the irradiated seeds of the 30,000r X-ray treatment, planted in the field in the summer of 1957, had light green stems and leaves. The plant grew normally and fruits were obtained from which seeds were secured.

A progeny test in the greenhouse gave 6 normal plants and 1 slow-growing plant with tripinnate leaves (508-1). This mutant has tripinnate leaves as compared to bipinnate leaves of normal tomato plant (Fig. 15). The mutant is slow in growth in the early-seedling stage but catches up later, reaching about normal size. This mutant seems to have more leaf area than a normal bipinnate-leaf plant. Blooming and fruiting are slightly delayed. The fruits are very firm and are completely under the protection of leaves. This might be a valuable characteristic in breeding for sunscald resistance.

Seeds from the mutant produced only tripinnate offspring, hence this character is inheritable. Crosses of 508-1 x Chatham, Chatham x 508-1, 508-1 x daclry, and daclry x 508-1 were made. The offspring from both the crosses of 508-1 x Chatham and Chatham x 508-1 were identical with normal Chatham and the offspring from the crosses of 508-1 x daclry and daclry x 508-1 were similar to the F1 plants of Chatham x daclry. No intermediate plants were found in any case. These results indicate that the tripinnate-leaf character is completely recessive to normal bipinnate leaf.

Seeds from the F1 of 508-1 x normal Chatham produced an F2 generation of 33 normal bipinnate-leaf plants and 4 tripinnate

leaf plants. F1 seeds of the cross of daclry x 508-1 produced an F2 generation of 68 normal bipinnate-leaf plants and 9 tripinnate-leaf plants. The results of the crosses are summarized in Table 12.

Out of 68 normal-appearing plants, from the F2 generation of the cross of daclry x 508-1, 34 plants were saved at random, selfed and backcrossed to 508-1. There was a deficiency of the mutants in heterozygous progenies in all cases. The expected 3:1 ratio from selfed seeds and the expected 1:1 ratio from backcrossed seeds were not obtained. However, among these 34 plants, 20 proved to be heterozygous and 11 proved to be homozygous. The ratio between the heterozygous and the homozygous progenies is approximately 2:1. This is expected ratio for a monogenic inheritance.

Although the deficiency of the mutants in the F2 is significant, the results of the progeny tests of the F2 dominants suggest that the tripinnate-leaf character is apparently controlled by one simple recessive gene and the deficiency of the mutants probably due to the lower viability of the seed. For this, the symbol tp is proposed.

Table 12. Results of progeny tests made in the study of tripinnate-leaves

parentage	generation of progeny	tripinnate- leaf plants	normal plants
508	R2	1	6
508-1 x Chatham	F1	0	46
508-1 x Chatham	F2	4	33
Chatham x 508-1	F1	0	42
508-1 x daelry	F1	0	28
daelry x 508-1	F1	0	31
daelry x 508-1	F2	9	68



Fig. 14. Tripinnate-leaf mutant

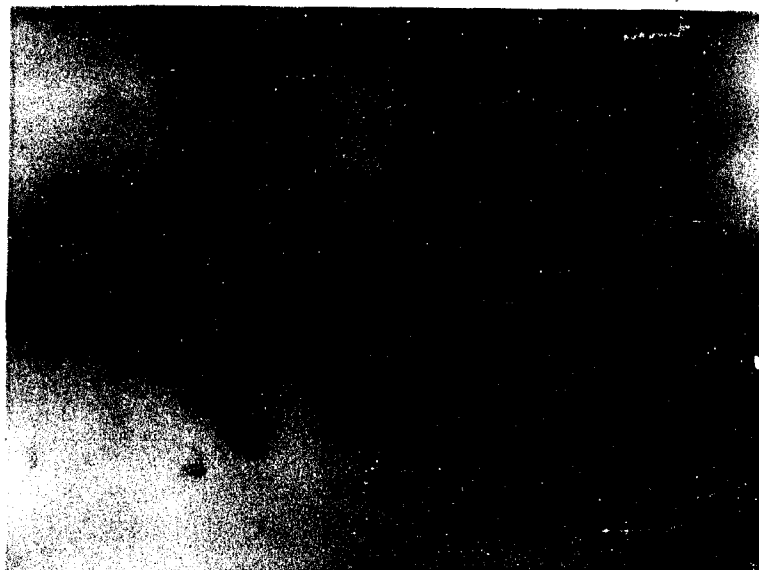


Fig. 15. A comparison of tripinnate leaf
and normal bipinnate leaf

Right: Tripinnate leaf

Left: Normal bipinnate leaf

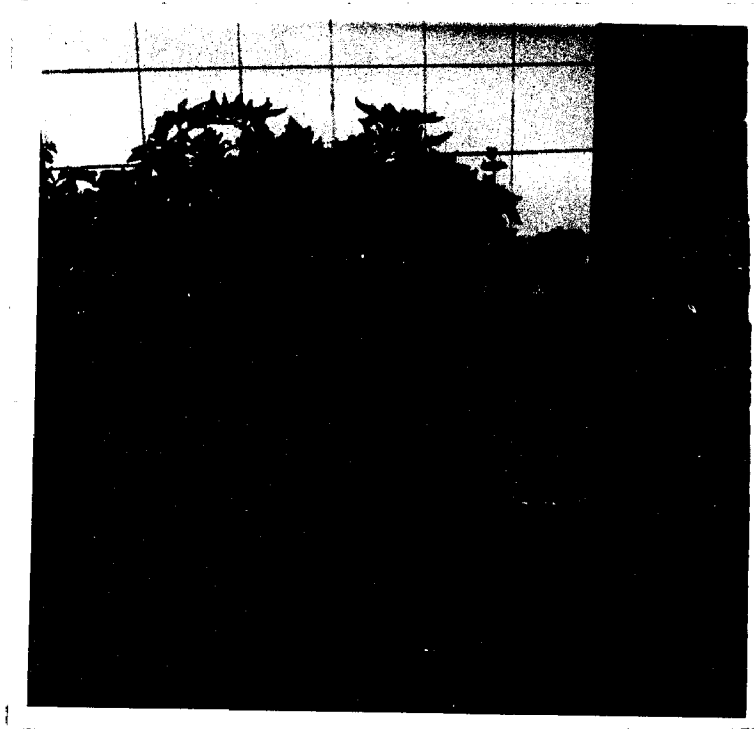


Fig. 16. Tripinnate-leaf mutant in the field

7. Drooping-leaves. (Fig. 17 and 18) Abnormal plant No. 789, which arose from the 4.85×10^{13} thermal neutron treatment, was a plant of normal appearance except for one branch of light color from which one fruit with four seeds of normal size were obtained. These produced in the greenhouse one normal plant and three drooping-leaf plants (789-1, 789-2 and 789-3).

The most distinctive characteristics of this mutant are as follows: the stem is very weak, slender, and prostrate unless supported; the plant is reduced in size and has few branches; the leaves are drooping and dark green in color, with a long midrib and only 5-7 leaflets. Sometimes the leaflets fold upward or downward as if a wilt gene were involved. The mutant is slow in growth, is late to mature, and bears normal-sized fruits with a slight trace of nipple character.

Offspring of each drooping-leaf plant consisted exclusively of drooping-leaf plants, indicating their homozygous nature.

In order to study the genetical behavior of this mutant, crosses and backcrosses were made and the results are described in Table 13. The F1 progeny of the following crosses were uniformly non-drooping: 789-1 x Chatham, Chatham x 789-1. 789-1 x daclry, daclry x 789-1, and 789-2 x dpsory. These results indicate that the drooping-leaf character is completely recessive.

The F2 generation of the cross of 789-1 x Chatham exhibited approximately a 3:1 ratio, with 19 drooping-leaf

Table 13. Results of progeny tests made in the study of drooping-leaves

parentage	generation of progeny	drooping-leaves plants	normal plants
789-1	R3	20	0
789-2	R3	20	0
789-3	R3	20	0
789-1 x Chatham	F1	0	20
Chatham x 789-1	F1	0	20
789-1 x daclry	F1	0	20
daclry x 789-1	F1	0	20
789-2 x dpsory	F1	0	20
789-1 x Chatham	F2	19	58
789-2 x dpsory	F2	10	31
789-1 x daclry	F2	41	132
(789-1 x Chatham) x 789-1	BC1	23	26

plants and 58 non-drooping-leaf plants. The seeds of the cross of 789-1 x daclry produced 41 drooping-leaf plants and 132 non-drooping-leaf plants, also approximately a 3:1 ratio. The F2 segregation ratio from the cross of 789-2 x dpsory produced 10 drooping-leaf plants and 31 non-drooping-leaf plants, a close fit to the expected 3:1 ratio. The backcross of (789-1 x Chatham) x 789-1 gave 23 drooping-leaf plants and 26 non-drooping-leaf plants, which is nearly a 1:1 ratio. These results indicate that this induced drooping-leaf character is controlled by one recessive gene. The symbol dp is proposed.

The segregation of the F2 plants from the cross of daclry x 789-1 is given in Table 14. From the table it will be noted that when tested with genes in group I, IV, and V, no linkage for drooping-leaf was indicated.

Table 14. F2 segregation of drooping-leaves mutant and tester genes from the cross of daclry x 789-1

linkage group	tester genes	parental classes		recombination classes		χ^2
I	d	d-Dp-	D-dp-	D-Dp-	d-dp-	1.34
	exp.	28 (32.44)	30 (32.44)	104 (97.31)	11 (10.18)	
		difficult classification of <u>d</u>				
V	a	a-Dp-	A-dp-	A-Dp-	a-dp-	1.19
	exp.	38 (32.44)	31 (32.44)	94 (97.31)	10 (10.18)	
IV	c	c-Dp-	C-dp-	C-Dp-	c-dp-	2.33
	exp.	39 (32.44)	28 (32.44)	93 (97.31)	13 (10.18)	

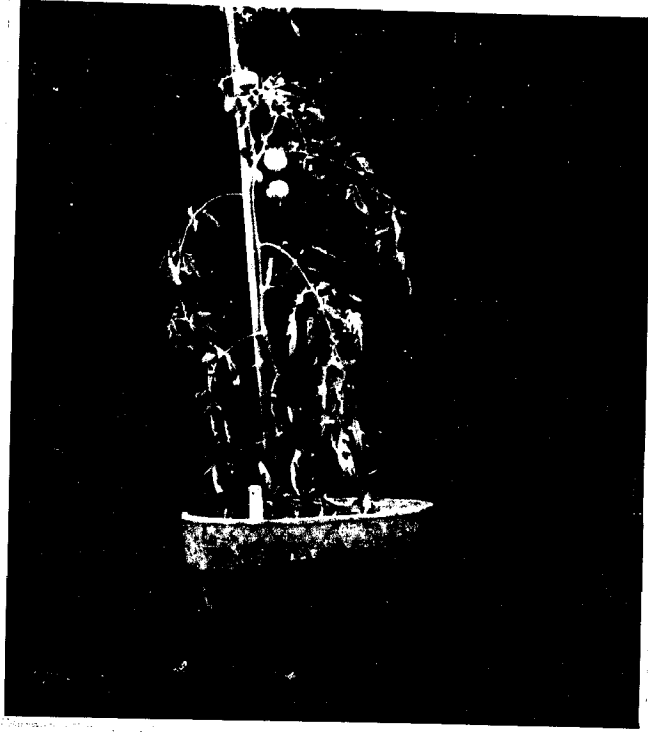


Fig. 17. Drooping-leaves mutant



Fig. 18. Drooping-leaves mutant in the field

8. Slender-stem. (Fig. 19) Abnormal plant No. 43 which arose from the 7.54×10^{13} thermal neutron treatment, had a branch with very curly leaves. Seeds from that mutated branch produced 7 normal plants and 3 dwarf plants which were labeled as 43-a, 43-b and 43-c. 43-a was a plant about 1 foot in height, with many branches and mottled leaves. It was unfruitful. 43-b died when it was in seedling stage. 43-c, with a slender stem and few branches, had a stiff, upright appearance. The leaves had proportionately longer petioles and 3 non-lobed leaflets; leaves with 4-5 leaflets occasionally appeared. Cotyledons and leaves were bluish-green in color. branching was absent while in the greenhouse, but a few branches were produced while the plant was growing in the field. Blooming and fruiting were much delayed. In general, all parts of the plants were reduced and the growth of the plant was slow. plant growth ceased after one or two small fruits were set and the plant died soon after the fruits were harvested. Each fruit, about $2-2\frac{1}{2}$ cm. in diameter, contained 20-30 seeds of normal size.

Offspring from 43-c were all similar to the mutant. The progenies of the offspring plants consisted exclusively of mutants. Crosses of 43-c x normal Chatham and 43-c x daclry were made. The results are described in Table 15. The F1 progenies were composed entirely of normal plants. This result indicates that this mutation was probably determined by one or more recessive genes.

From the cross of 43-c x daclry, an F2 generation was

Table 15. Results of progeny tests made in the study of slender-stem

parentage	generation of progeny	slender-stem plants	normal plants
43-c	R3	all	0
43-c x daclry	F1	0	24
daclry x 43-c	F1	0	22
Chatham x 43-c	F1	0	20
43-c x Chatham	F1	0	20
43-c x daclry	F2	9	38
43-c x (43-c x daclry)	BC1	14	21

obtained with 38 normal plants and 9 slender-stem, small plants. The proportion of mutants was 19.15 percent. This deviation from the expected 25 percent is significant statistically. The deviation may perhaps be due to lower viability of the homozygous recessive seeds or to the small numbers.

Seventy seeds were produced from the backcross of 43-c x (43-c x daclyry) and planted; 45 seeds germinated; and of these 10 seedlings died of damping-off disease. Among the 35 surviving plants 21 were normal plants and 14 mutant-phenotype plants. The deficiency of mutants from the expected 1:1 ratio is highly significant. Here again the deficiency of recessives may be due to the lower viability of homozygous recessive seeds.

In spite of the deficiency of mutants in the F2 generation and backcross generation, this slender-stem character induced by radiation would appear to be the result of one recessive gene. The symbol sm is proposed. Further studies would be desirable to confirm this hypothesis and to determine its linkage relationship.



Fig. 19. Slender-stem mutant

9. Grey-necrotic. (Figs. 20 and 21) Four R1 abnormal plants produced 4 progenies which had grey-necrotic segregation. The results are shown in Table 16.

The grey-necrotic plants were all weak and small. There were numerous minute, greyish, necrotic spots on the young leaves which spread and covered the whole leaves, causing the early death of the leaves and resulting in early death of the plant before it reached maturity. Microscopic examination revealed no evidence of the presence of a pathogen.

Because all the necrotic plants seemed to be identical, only B18 was used for the study of inheritance. Crosses were made between B18-1 and normal Chatham. The F1 generation appeared to be similar to Chatham, indicating that the necrotic character is completely recessive to normal (non-necrotic). The F2 generation gave a segregation of 75 normal plants and 22 grey-necrotic plants, which is approximately a 3:1 ratio. Twenty F2 normal plants were saved at random, selfed, and backcrossed to B18-1. Among these twenty plants, five proved to be homozygous, because their selfed seeds and backcrossed seeds produced only non-necrotic normal plants; fifteen proved to be heterozygous, because their selfed seeds and backcrossed seeds produced both normal and necrotic plants.

It appears that this radiation-induced grey-necrotic character is controlled by one simple recessive gene. From the tomato linkage map assembled by Butler (4), the description of gene ne is similar to this radiation-induced mutation. Since there is no tester plant for ne available at the present

Table 16. Results of 4 progeny tests which had grey-necrotic segregation

plant number	treatment thermal neutrons	description of plant which produced grey-necrotic seedlings	grey-necrotic plants	normal plants
38	7.54×10^{13}	mottled-leaf branch	4	10
736	4.58×10^{13}	curly, wrinkled-leaf branch	2	3
250	6.56×10^{13}	abnormal shape, mottled-leaf branch	2	4
B18	7.54×10^{13}	small-leaf branch	3	5

time. The symbol ne-1 is proposed temporarily for this radiation-induced gene mutation to distinguish it from ne.

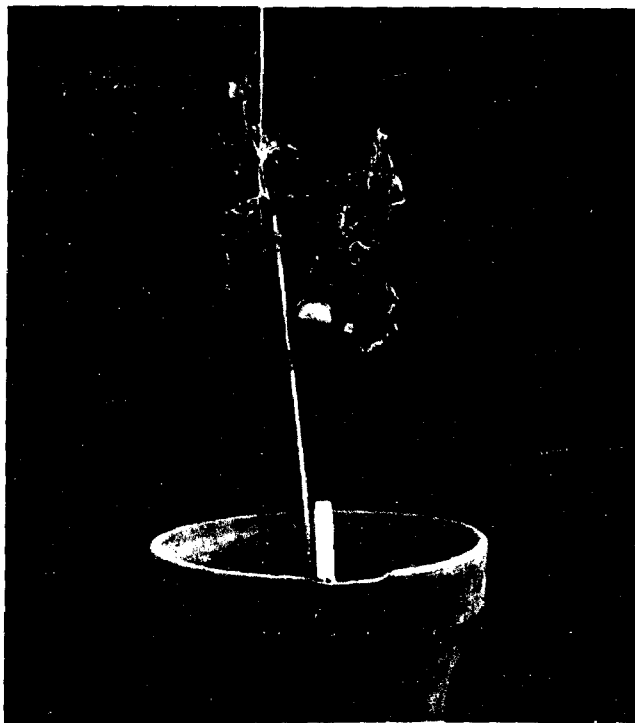


Fig. 20. Grey-necrotic mutant



Fig. 21. A comparison of a grey-necrotic mutant and a normal plant

Left: grey-necrotic mutant
Right: normal plant

10. Wiry-leaves. (Figs. 22, 23, and 24) Wiry-leaf mutants are dwarf and their internodes are very short. The leaves are variable in shape and size, having a tendency toward reduction of the lamina. Some leaves, from the lower nodes, are nearly normal in shape. Some leaves have a very wide range of shape and size, from shoestring-like leaves to leaves with normal leaflets at the tip. The flowers are very irregular. Sepals and petals are separate, numerous, and linear. The stamens, which are linear in shape, are free from each other. The styles are long and curled. An aggregate fruit is produced consisting of several distinct but partially-fused carpels. Pollen is scarce. These plants are completely sterile but a few parthenocarpic fruits can be obtained (Fig. 26).

It was thought that this character might be due to infection by tobacco mosaic. This hypothesis was tested by grafting and proved not to be true.

There were three progenies which produced wiry-leaf segregates. The results are shown in Table 17.

Since crosses could not be made and seeds could not be obtained, the inheritance of this mutation could not be determined. Lesley and Lesley (14) in 1928 reported a similar "wiry" tomato mutant which was obtained by spontaneous mutation, from which a few seeds were obtained. They found that the "wiry" character was inherited and controlled by one simple recessive gene. Again in 1956 Lesley and Lesley (15) found a "wiry" which was induced by P³² treatment.

Table 17. Results of 3 progeny tests which had wiry-leaves segregates

plant number	treatment thermal neutrons	description of plant which produced wiry-leaf seedlings	progeny	
			wiry-leaf plants	normal plants
115	6.56×10^{13}	leaf chimera	1	4
177	6.56×10^{13}	wrinkled-leaf branch	2	8
1001	6.56×10^{13}	abnormal fruits	2	6

We believe there is sufficient similarity between the thermal neutron induced wiry-leaf mutant in this experiment and those of Lesley and Lesley to assume that they are identical.



Fig. 22. A comparison of a wiry-leaves mutant and a normal plant

Right: wiry-leaves mutant
Left: normal plant



Fig. 23. Wiry-leaves mutant at a later stage

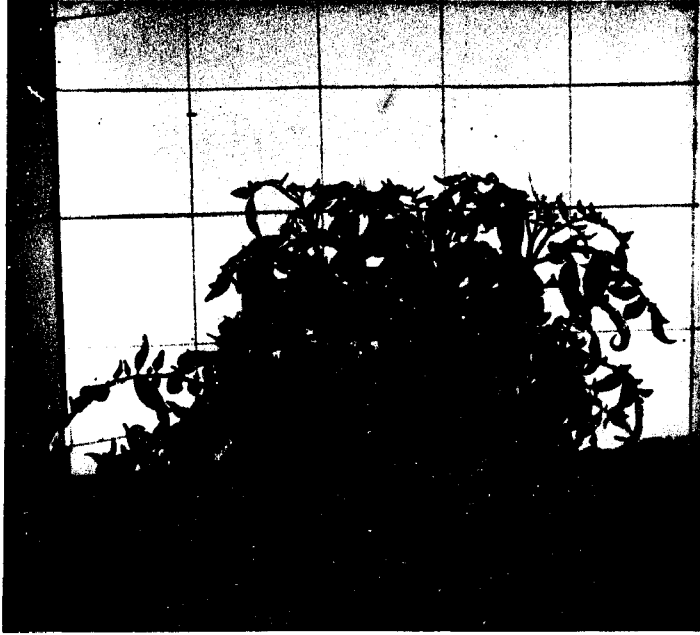


Fig. 24. Wiry-leaves mutant in the field

Non-inheritable mutations. The progeny tests in the greenhouse indicated that several mutations, mostly affecting leaf color and texture, were probably not inheritable. Of these, only one, glossy leaf surface, was selected for further study. (Fig. 25)

Abnormal plant No. 505a, from the 4.85×10^{13} thermal neutron treatment, showed no evidence of glossy leaf surface when young. In the middle of July, two shoots showed the glossy-leaf character. Two, normal medium-sized fruits were obtained, which also had a glossy surface. A microscopic examination of the leaf revealed that the palisade tissue consisted of three layers of cells, and that the cells were smaller and crowded than normal palisade cells. Offspring from the seeds of the mutated branches gave only normal plants which did not have the glossy leaf surface. The only method of maintaining this mutation is asexual propagation by means of cuttings.

There were 52 R1 plants which had glossy-leaf branches, although some of them were less glossy than 505a.

The progeny tests of these plants gave the same results, producing normal, non-glossy-leaf plants. This indicated that the glossy-leaf character induced by irradiation in this experiment is not inherited. Perhaps this mutation affected only the outside layers, and the inside layers which give rise to gametes were not affected.



Fig. 25. Glossy-leaf mutant

Possible mutations. Thirteen selections of plants resistant to early blight were made in the fall of 1957, on the basis of fruit production and relative freedom from defoliation. Progenies were planted in the greenhouse on March 1, 1958. Inoculations were made by Dr. A. E. Rich, Pathologist of the Department of Botany, when the seedlings reached about three inches in height. The first inoculation was made on March 28, 1958, and the second inoculation on April 5. Observations were made on April 14, and results were recorded on the basis of infected leaf area and the number of infected spots. It was found that the control was the most susceptible line, with an average rating of 5, and lines Rs1, Rs3, Rs5, Rs7, Rs9, Rs10, Rs11, Rs12, and Rs13 were severely infected, with average rating ranging from 3-5. Lines Rs2, Rs6, and Rs8 were slightly infected, and line Rs4 was the most resistant line with a rating of 1-1.5. These four lines were planted in the field in the summer of 1958 for further examination. Leaf mold (Cladosporium sp.) disease was prevalent and most of the plants were severely infected with it before early blight appeared. Thus it was impossible to determine the disease-resistant qualities of the selected lines. However, Rs6, although susceptible to leaf mold, was selected because the progeny exhibited uniform earliness (about 7-10 days earlier than the controls). Rs4 was selected because it was less infected by leaf mold. A further investigation for the early blight resistance of these two selected lines is necessary.

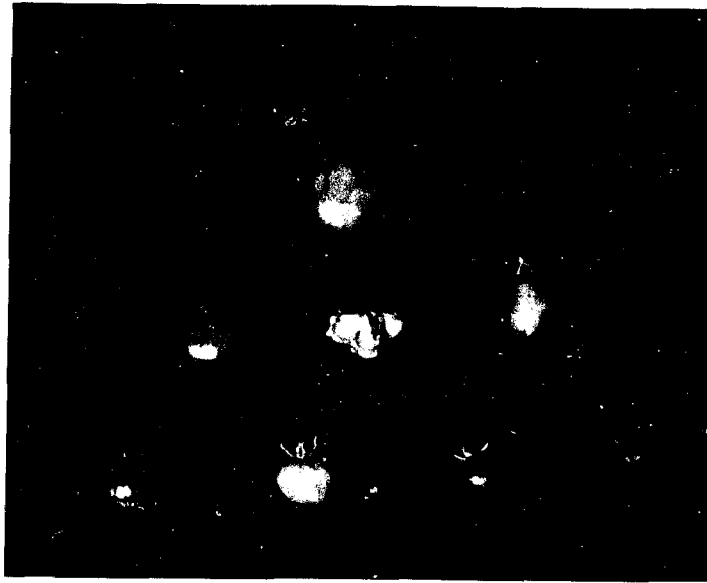


Fig. 26. Abnormal fruits

1. normal fruit;
2. fruit with loose, wrinkled epidermis; this plant had no other abnormalities and is not discussed further.
3. aggregate fruit associated with wiry-leaves mutant.
4. nipple fruit from normal branch, inheritable but not discussed further.
5. small, furrowed fruit associated with curly mutant.
6. small, firm fruit from drooping-leaves mutant.
7. small, brownish, oblate fruit associated with green-stem mutant.
8. parthenocarpic fruit from sterile plant.

CONCLUSIONS AND SUMMARY

This project was initiated in an attempt to induce by seed irradiation a mutation that could be used in tomato breeding for early-blight (*Alternaria solani* (E. & M.) Jones & Grout) resistance. This furnished an opportunity to study the effect of various kinds and amounts of irradiation on tomato seeds, including the frequency of mutations, their nature, and their inheritability.

The Chatham variety was selected because of its genetic purity, its earliness, and its desirable characters. The seeds were irradiated at the Brookhaven National Laboratory at Upton, L. I., New York. The X-radiation was made with the G. E. Maxitron operated at 250 KVP and 30 ma. The machine delivered approximately 800r per minute. The thermal neutron radiations were made in the thermal neutron column of the nuclear reactor. 30,000r and 50,000r X-ray dosages and 20, 26, 30 and 40 hours at a flux of approximately 7.0×10^8 thermal neutrons / cm² / sec. were employed.

Measurements of the effects of the treatments on germination, emergence and survival were made. The appearance and growth of the seedlings were also observed.

The percentage of germination of the irradiated seeds was normal, but increasing amounts of irradiation tended to delay the emergence of seedlings in soil. There was also a reduction in the percentage of survival as the dosage increased. The 50,000r X-ray treatment was lethal to all

seedlings and with the 1.0×10^{14} thermal neutron treatment very few plants survived from the treated seeds. The thermal neutron irradiated seeds were much more uniformly affected than the X-rayed seeds. The 6.56×10^{13} thermal neutron treatment resulted in the largest percentage of abnormal plants in relation to the number of seeds, but the 7.54×10^{13} thermal neutron treatment produced the highest percentage of abnormal plants among those that survived. The same type of abnormalities were induced by both X-rays and thermal neutrons. The morphological abnormalities included curly leaves, irregular leaves, narrow leaves, large leaves, leaf chimeras, entire-margined leaflets, brown-necrotic, grey-necrotic, small leaves, glossy leaf surface, wilted leaves, mottled leaves, wrinkled leaves, weak plants, super-vigorous plants, dwarf plants and depressed plants. Chlorophyll changes included light yellow, yellow, very light green, light green, grey-blue-green, and dark green.

In general, surviving plants were delayed in blooming and fruiting as compared with the controls. Of the 5703 surviving plants from irradiated seeds with both X-rays and thermal neutrons, 12.56 percent produced sterile branches, and 0.47 percent of the plants were completely sterile. Among the 969 affected plants, 73.37 percent had sterile branches and 2.64 percent were completely sterile.

Progeny tests of fertile abnormal plants from irradiated seeds were made in the greenhouse. Among 186 progeny tests there were 47 progenies which showed abnormalities in

the following or R2 generation; of these only 8 progenies had abnormalities similar to those of their mother plants.

Ten inheritable mutations, of which nine were recessive and one was dominant, were discovered.

Two of these inheritable mutations occurred in the R1 generation:

1. chlorophyll deficiency

2. curly-leaves, which was found to be controlled by one recessive gene. When tested with d a c l r y, no linkage was revealed. The symbol cu₂ is proposed.

Eight appeared in the R2 generation:

3. suppressed-root, which is controlled by one dominant gene. The symbol Sr for this is proposed.

4. green-stem, which differs from the previously reported a, and is controlled by one recessive gene, for which the symbol a₂ is proposed.

5. round-leaflets, which appears to be controlled by two recessive genes.

6. tripinnate-leaves, which is controlled by one recessive gene.

7. drooping-leaves, controlled by one recessive gene. No linkage was indicated when tested with d a c.

8. slender-stem, controlled by one recessive gene. For this the symbol sm is proposed.

9. grey-necrotic, which is controlled by one recessive gene.

10. wiry-leaves, from which plants no seed could be

obtained. The wiry-leaves mutant appeared to be similar to those reported by Lesley and Lesley.

Other mutations include glossy leaf surface, dark green and light green color which were somatic and hence not inheritable, and grey-blue-green leaf color (absence of palisade cells), which occurred only on sterile branches.

Other possible mutations include resistance to early blight and earlier ripening. These deserve more study.

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