X-RAY STUDIES IN PHASEOLUS VULGARIS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

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1939

X-RAY STUDIES IN PHASEOLUS VULGARIS

by

Clarence Frederick Center

A THESIS
Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Farm Crops

1939

ACKNOWLEDGMENT

The writer is very grateful to Mr. H. M. Brown for invaluable advice and assistance throughout the course of this work.

Gratitude is also extended to Mr. E. E. Down and to Dr. C. R. Megee for their willing co-operation and helpful advice and criticism.

The writer is indebted to Dr. J. C. Clark of the Physics Department under whose direction all of the x-ray treatments were made. The writer is further indebted to Dr. Clark for technical advice in writing this paper, and for the formula for the calculation of the x-ray dosages used in these experiments.

CONTENTS

INTRODUCTION

Since the discovery of x-rays by Roentgen in 1895, many investigations have been carried on to determine their effects upon biological organisms, but there has been no reference in the literature concerning the genetic effects of x-rays on Phaseolus vulgaris. In 1938, an opportunity was presented to begin a study of this type, and the work herein reported was undertaken.

Before examining the data obtained in this study, the nature of the x-ray and the findings of the earlier workers will be discussed.

It is common knowledge that x-rays are produced when very fast moving electrons experience collisions with the orbital electrons of the stationary material of the x-ray tube. X-rays, which are vibrations similar in nature to visible light rays, vary greatly in length. The effective wave length of the x-rays produced is dependent upon the potential voltage difference between the source of electrons (cathode) and the target (anode), and decreases as the voltage increases. The long waves produced by low voltages are often designated in the literature as "soft" x-rays. Likewise, the short waves produced by high voltages are called "hard" x-rays.

The third International Congress of Radiology which met in Paris in 1931 adopted as a practical unit of x-ray quantity the "Roentgen" or "r" unit. This is the quantity

of x-ray energy which, when fully utilized in the production of ions, will produce one electrostatic unit of ions in one cubic centimeter of air at standard conditions of temperature and pressure.

For means of comparison, the dosage considered necessary to redden human skin is approximately 600 r units (27).

REVIEW OF LITERATURE

No attempt will be made to present an extensive review of literature. The material reviewed here will pertain chiefly to plants, and only those references that seem to bear directly on the discussion will be cited. A very complete review of the literature has been made by Goodspeed and Uber (7) and for further study the reader is referred to that comprehensive review and bibliography.

It should be added that for the purposes of this paper, the term "physiological effect" will be used to refer to the discernible effect of the x-rays on the treated generation, and the term "genetic effect" will be used to refer to the transmissible effect of the x-rays on the treated or on subsequent generations.

PHYSIOLOGICAL EFFECTS OF X-RAYS

Stimulative Effects

Although x-rays are now known to produce many genetic effects in irradiated organisms, the earliest investigations were entirely physiological in nature, and, if any significant cytological or genetic effects were produced, they were overlooked (7).

In 1927, Science (26) published the report that potted plants, grown from seeds that had been irradiated with "soft" x-rays, grew more vigorously, flowered and fruited from 1 to 3 weeks earlier and yielded from 15% to 170% more than the untreated material. Three lots of potatoes similarly treated were reported to have given yield increases of 35, 107, and 170 percent over non-irradiated controls.

Shull and Mitchell (27) claimed stimulation of wheat, corn, oats, and sunflower seedlings by treating with very low dosages of x-rays in the early stages of germination. Treatments of 30 to 120 r units were found to give best results in their experiments. They think that the optimum is probably specific for the various species and that the margin between optimum dosage and over-dosage is small.

By irradiating dry soy bean seeds with small dosages of soft x-rays, Long and Kersten (13) obtained slight stimulation of plant growth over non-irradiated controls, using the average green weight of the parts above ground as the basis of comparison. Working with large numbers of plants, they found that the average increase in weight of the irradiated plants exceeded three times the probable error. This suggests, but does not prove, a true stimulation as the result of x-ray treatment.

On the other hand, Johnson (9) found that the irradiation of sunflower seeds with a light dosage gave no increased rate

of germination, no increased percentage of germination, no increased growth during the seedling stage, and no increased height nor weight at maturity. Working later (10) with seedlings of tomato, sunberry, sunflower, and two species of vetch which had all been given light treatments, she found no stimulation of growth over controls as evidenced by measurements of height and green and dry weight.

Deleterious Effects

Whereas little has been definitely proven concerning possible stimulative effects of x-rays, much has been written to show conclusively that there may be very harmful effects of large doses on the irradiated organisms. Soon after irradiation experiments first began on living organisms, the fact that the effects of the treatments varied directly with the dosage in Roentgen units became evident. Many experiments have been conducted that quite conclusively prove this point (2, 11, 14, 21).

One of the most convincing works was that of Packard (22) in which he found that, if Drosophila eggs are exposed to x-rays for a definite length of time, the quantitative effects of the exposure, as measured by the number that fail to hatch, could be used as a measure of the dosage. He found that a carefully measured dose gave results which varied less than 5%.

Cattell (1) found in his study of irradiated wheat seedlings that, if the dosage remains the same, the physiological effects remain the same.

In a series of experiments with dry maize seeds, Collins and Maxwell (2) found that a range of x-ray treatments existed which caused all of the plants to die in the seedling stage without reducing the percentage of germination. However, as the dosages were increased, the rate of elongation of the roots and shoots was increasingly reduced, with complete "delayed killing" resulting from very high dosages (60,000 to 100,000 r units). They also found evidence that cell division took place after treatments of 60,000 r units, and that not all growth was due to elongation of the cells. They estimated that dosages of approximately 2,000,000 r units would be required to completely inhibit germination.

Johnson (9) found that the plants recovered from inhibitory effects of light doses in about three weeks and differed very little thereafter in any respect from the untreated controls.

Much variability of effect is shown by the plants that survive, the effects are not immediately apparent, and plants given lethal dosages have been found to live for several weeks though little or no growth is made (11).

In an attempt to find the reason for the apparent inhibition of growth in the treated plants, Cattell (1) found, by feeding x-rayed seedlings to mice, that the growth-promoting Vitamin B in the embryo was destroyed by heavy doses of x-rays.

Skoog (28) later found that the "growth substance" was inactivated by x-rays both in solution and in the intact plant, and by comparable dosages. A water solution of growth

substance, irradiated in an atmosphere of nitrogen, showed no immediate inactivation, thus indicating that the reaction was an oxidation. He also found that, if irradiated seedlings were given a continuous supply of growth substance immediately after irradiation, they were able to maintain a normal rate of growth compared to non-irradiated controls.

Cytological Effects

As far back as 1924, Komuro (12) made a rather thorough study of the grosser cytological effects resulting from very heavy doses of x-rays upon seedlings of Vicia faba. He found that in 1.5 hours after irradiation, vacuolization of the cytoplasm became apparent, chromatolysis was clearly evident, and all observed mitoses were abnormal. Six hours after irradiation, nuclear membranes were no longer visible due to degeneration. Abnormal, binucleate cells were present. Nine hours after irradiation, vacuolization of the cytoplasm was manifest. Escaped nuclei were very often seen in the cytoplasm. Many abnormal binucleate cells, many giant nuclei, and many multi-nucleolar cells were also found.

Stone (38) found that following x-ray treatment, mitosis was soon stopped for a period, after which growth was slowly resumed, with abnormal cell divisions generally occurring. Cells about to divide at the time of treatment were found to be prevented from further division, but those already in mitosis were allowed to complete the division, owing to the rapidity of the process.

GENETIC EFFECTS OF X-RAYS

In spite of profound physiological and cytological disturbances such as those already described, no genetic effects were discovered until Muller demonstrated in 1928 (15) that genetic variations could be induced in Drosophila by x-ray treatment. Since that time, the use of x-rays in genetic studies has been very extensive in both plants and animals.

The First Plant Mutations Induced by X-Rays

Stadler (29), by irradiating germinating seeds of barley, was the first to demonstrate that genetic effects, similar to those produced by Muller in Drosophila, could also be produced in plants. The cells from which the tillers will develop are already separated in the embryo of the dormant barley seed, and a mutation in one of these cells will affect only one tiller, unless an axillary bud subsequently develops above the mutation. To be certain that a variant type in the progeny was due to an induced mutation, the seed from each tiller was planted separately. If the same variation appeared in all of the head progenies of a single plant, the cause would probably be due to a hybrid condition of the seed from which the plant was grown. However, if a variation occurred in the progeny of one head while the progenies of the other heads of the same plant remained normal, the cause would probably be due to a genetic change that occurred during the development of the treated plant, presumably at the time of x-ray treatment. In the first successful experiment, three mutant types were

found in 70 head progenies from 26 plants grown from x-rayed seeds. One mutation was a white seedling that was colorless at the time of emergence and died in 2 or 3 weeks. One was a virescent mutation that was colorless at time of emergence, but became a pale green in a few days. One was green at emergence, but later became yellow and died. Each of the mutant types made up from one-fourth to one-eighth of a single head progeny from a plant of which other head progenies were entirely normal.

Rate of Induced Mutation in Barley

About 90% of the recognizable mutations induced by x-rays may be detected in the seedling stage (30). Using this assumption as an index upon which to base conclusions, several genetic reactions of barley seeds under various conditions of x-ray treatment have been noted.

The rate of mutation is not affected appreciably by changing the percent moisture in the seeds by soaking, within the limits of 15 and 40 percent moisture (33). The temperature of the seeds during irradiation had no pronounced effect when temperatures ranged from 10 to 50 degrees Centigrade (30). No significant difference was shown by equal intensities (r units) of irradiation when using 40,56,81,98 and 116 kilovolts as the potential difference between the anode and cathode of the x-ray tube (33).

The rate of mutation was found to be proportional to the total intensity of the irradiation applied, within the limits of the sampling error, for both dormant and germinating seeds (33).

The average rate of mutation was about four to eight times as high in germinating seeds as in dormant seeds in identical treatments near the limiting intensities for germinating seeds (33). However, dormant seeds were found to tolerate an x-ray dosage of 15 to 20 times as great as did germinating seeds, and the mutation rate possible with dormant seeds was about double that possible with germinating seeds (33, 35).

Mutation was induced in dormant seeds whether they were planted immediately after the treatment or not (30).

In some cases, two mutations occurred in the same head progeny (33).

Rate of Induced Mutation in Oats and Wheat

Each of the genera Avena, Hordeum and Triticum include species of 7, 14, and 21 pairs of chromosomes, but the cultivated barleys have only 7 pairs of chromosomes, whereas the cultivated oat and wheat varieties contain 21 pairs of chromosomes. The results of several experiments concerning the rates of mutations of oats and wheat have been presented by Stadler (32, 33, 35).

Avena sativa and A. byzantina with 21 pairs of chromosomes yielded no mutations with dosages sufficient to have produced 70 mutations in barley. Avena brevis and A. strigosa with 7 pairs of chromosomes gave 14 mutations at rates slightly lower than, but not statistically different from, barley (32).

Triticum vulgare with 21 pairs of chromosomes, yielded no mutations with dosages which would have yielded about 40

in barley. T. durum and T. dicoccum with 14 pairs of chromosomes mutated at a very low rate. T. monococcum with 7 pairs of chromosomes, in a small test, yielded mutations at a slightly higher, but not significantly different, rate than barley (32).

Stadler (32) suggested as a probable explanation of these results that in the formation of the polyploid species, a certain amount of gene duplication took place. A single recessive mutation induced in a cell containing two or three dominant factors for the determination of a single plant character would have no visible effect on the plant. Consequently, a much lower rate of mutation could be detected in the polyploid species. Stadler also noted that the species with the higher numbers of chromosomes were injured less by x-ray treatments.

MacArthur's Work with Tomatoes

In the progeny tests that MacArthur (14) made of tomato plants grown from x-rayed seeds, he apparently disregarded the possibility of there being chimeric tissue in the treated material. Single fruit progenies were grown from the irradiated material, and no attempts were made to determine whether the mutant tissue was sectorial or not. Out of 346 progenies thus grown, 43 mutations were found, all of which were recessive and monofactorial.

Experiments with Tobacco

Working with species of the genus Nicotiana, Goodspeed

and Olson (6) experimented to discover the effects of irradiation on sex cells. They x-rayed flower buds of all ages up to anthesis. More than 1,000 plants came to maturity, grown as seven populations from seven treated capsules. More than 20 percent of variants appeared, one capsule yielding 136 variants out of 168 plants. The majority of variants exhibited some reduction in fertility, but only rarely were they completely sterile, while a number were completely fertile.

In experiments in which pollen was x-rayed and used to fertilize untreated ovaries, variations appeared as readily as when entire buds were treated (5, 6).

Further observations on tobacco have been reported. No relation was found between the stage of maturity of the sex cells and the effectiveness of irradiation (4). Sex cells undergoing division yielded more mutations than those in the resting condition (5). Mutations and chromosomal aberrations may be produced with very low voltages by irradiation of the sex cells (7). Mature pollen, like dry, dormant seeds, is quite resistant to the effects of x-rays. (4, 7).

Time of Irradiation

Stadler (34) demonstrated that there was a great advantage in x-raying the embryo at an early stage of development. Corn treated after pollination showed affected areas varying from one-half of the endosperm in seeds treated 28 hours after pollination to barely visible sectors in seeds

treated 8 to 10 days after pollination. The entire embryo seemed to be affected by changes induced by treatment 28 to 48 hours after pollination, whereas the chimeras grew less and less in extent as more time elapsed between pollination and irradiation. Obviously, the amount of differentiation that had taken place in the embryo at the time a seed was x-rayed greatly affected the size of the sector that arose from any one disarranged cell.

Types of Mutations Found

Of the types of mutations that appear in the progenies of treated materials, chlorophyll deficiencies make up the vast majority of the mutants (14, 33). Numerous types of modification of both vegetative and floral parts have been reported (5, 6, 8, 14, 31, 33). Most of the mutant seedling characters are lethal and almost all are unfavorable to growth (33). No case of a dominant mutation has ever been reported from x-ray treated plant material, but in all cases, the few mutants that reach maturity breed true for their recessive characteristics (33).

IDEAS CONCERNING THE CAUSES OF INDUCED MUTATIONS

Stadler (35) defined a mutation as "a transmissible change in the gene." Workers in the field are still not agreed as to the real nature of mutations, nor are they sure that induced mutations result in the same manner as the so-called "spontaneous" mutations found occurring naturally (35).

Muller (16) argued against the idea that the x-rays produced a chemical within the cell and that the chemical then

caused the mutation to occur. He reasoned that if this were the case, other chemicals should accomplish the same effect. All attempts by Patterson and Muller (25) to produce genic mutations by the application of specific substances, without irradiation, failed. Neither was there any case of "delayed induction" as might be expected, if chemicals were the cause of the induced mutations (16).

The theory that induced mutations were the direct result of electronic hits which caused transmissible genic changes was next advanced and generally accepted (25). A second point was the fact that of two chemically identical allelomorphs present very near together in a treated cell, only one becomes altered (25). Third, the degree of phenotypic change (proportion of lethals to non-lethals) was obviously independent of the dosage (25). Another argument was the direct and simple proportionality that has been shown to exist between the frequency of the induced mutation and the amount of energy absorbed from the irradiation (7, 25). Also, the number but not the degree or nature of the individual mutations changed with the dosage (15).

IDEAS CONCERNING THE NATURE OF INDUCED MUTATIONS

Although breakage and rearrangement of the chromosomes was known to result from irradiation, the first mutations were thought to be caused entirely by chemical changes in the gene itself, apparently identical in gametic behavior and, in many cases, in phenotypic effect with well-known

mutations of spontaneous origin (7, 16, 17, 23, 24). In the few cases tested by crossing the mutant type onto untreated individuals, the mutant characters behaved as would be expected on the assumption that the mutation was a change of a dominant gene to a corresponding recessive in the somatic cell of a homozygous individual (33).

Cytological as well as genetic effects have been found to increase linearly with dosage, within the limits of their respective experimental errors (7, 36). Translocations between non-homologous chromosomes arose in irradiated Drosophila with nearly the frequency of detectable gene mutations (18). Occasionally entire chromosomes were lost from cells, but in the vast majority of cases, only a small deletion occurred (25). Working with Crepis, Navashin (20) concluded that there were no limitations on the size of dislocated chromosome fragments, nor any regulation as to the direction of the process.

Stadler (36) gives the types of chromosomal aberrations that have been found by cytological investigations. There may be the loss of either a terminal or internal segment of a chromosome, the removal of a segment to a new position in the same or in another chromosome, the inversion of a segment in its original position, and the interchange of segments between chromosomes. No case of simple translocation of a fragment to a whole chromosome has been found. Although many deficiencies are associated with translocation,

some deficiencies occur in cells which are otherwise normal. The importance of these alterations is still not fully appreciated and their presence and behavior is a subject open for much further discussion.

Like the induced gene mutations, translocations are commonly accompanied by lethal or deleterious character changes (18, 19). Partial sterility is also common as a result of chromosomal aberration because the alteration is often lethal in the haploid condition to the gametophyte generation (34).

Because the early mutations were all changes from the dominant to the recessive, some workers believed that they were due to destruction of the gene or parts of the gene by the x-rays (36). If such were the case, no forward steps could ever be taken. Considerable research was undertaken to attempt to induce dominant mutations. Muller (15), Patterson and Muller (25), and Timefeeff-Ressovsky (39), have claimed progressive mutations in Drosophila, produced by causing mutant types that originally resulted from irradiation to return to the original type by further irradiation.

The fact that mutations can be induced either of two different ways, and that a cycle of mutational change can be completed is considered by Patterson and Muller (25) as proof that not all mutational changes caused by x-rays consist of losses. They also believe that "progressive" mutations can probably be produced by irradiation in cases

where there is a possibility of their occurring at all. Muller (15) further believes that the production of mutations by x-rays in each of two ways proves that the x-rays have a reconstructive rather than a destructive action on the genes. The changes in the chemical composition of the genes is thought by Muller (16) to be "endless in their eventual possibilities." Muller also suggested that, if the "spontaneous" gene mutations serve as the basis of evolution, as is apparently the case, then the artificially produced mutations must also include amongst them changes as good as those which occurred naturally (16).

Stadler (37) questioned the assumption of Muller that the induced mutations are simple chemical changes of the genes. No one has proven the nature of change in the chromosome of a natural mutation, let alone of the induced ones. He says: "In plants it is only on the assumption that deficiencies are invariably lethal to the gametophyte that it is possible to justify the description of viable mendelian variations in general as gene mutations. This assumption is obviously invalid in the polyploid series, and is of doubtful validity in the other species." He believes (36) that mutations are not a single homogeneous class of germinal variations, but may include variations in the arrangement of the chromosomes as well as variations entirely within the gene itself. He stated that there are many induced mutations that are known by cytological investigation to be the result of chromosomal aberrations, and that it is very possible that the rest are

due to chromosomal aberrations that are too small to be detected cytologically. Although he grants that the reverse mutations are a convincing argument in favor of simple genic changes, he argues that cases are known in corn, in which mutant endosperm tissue, known to be due to a deficiency that had been induced by x-raying the pollen, occasionally grew small areas that showed the recovery of the lost dominant gene. He further believes that too little is yet known about the causes of natural mutations even to be able to prove that the types of mutations involved in gene evolution are affected by x-irradiation.

Muller and Altenburg (18) made the following statement regarding the evolutionary value of x-ray induced variations: "The lethal and other deleterious effects of most translocations, when homozygous, do not rule out translocations as factors in evolutionary change any more than the lethal or deleterious character of the vast majority of detectable gene mutations rules out gene mutations as the main building blocks of evolution. It is not to be expected that the majority of any changes occurring at random will have survival value."

EXPERIMENT AND RESULTS

OBJECT OF THE EXPERIMENT

The primary objects of the experiment with x-rays herein reported were to determine the types of mutations that might be produced, and the rates of these mutations in both dormant and germinating seeds of white pea beans. Of secondary importance was to be the study of any physiological and genetical variations that might present themselves.

MATERIALS FOR THE EXPERIMENT

For this study, the Michelite variety of Phaseolus vulgaris which was recently introduced by Michigan State College was used. The beans were a representative sample of an increase lot grown on the college farm in 1937.

Several factors entered into the choice of the white pea bean as the subject for this experiment. The treatments were to be given in the spring, and a second generation would necessarily have to be grown in order to allow any recessive mutations to segregate out. It was important, therefore, that the plants used be annuals. In order to have the segregation of recessive factors in the shortest possible time, it was necessary to self-fertilize the hybrid material, and a normally self-fertilized plant would be highly desirable. A plant that had not yet been studied for genetic effects of irradiation, and one that was of considerable importance in Michigan was further desired.

BASIS FOR THE CALCULATION OF DOSAGES GIVEN

Since intensity of electromagnetic energy, such as x-rays, is expressed in the units of energy per square centimeter per second, and since the inverse-square-law applies to x-rays as well as to visual rays, by making use of the work of Ulrey (40), on the spectrum of x-rays produced by a thick tungsten target bombarded by electrons of various energies, it may be shown that the intensity of the x-ray beam varies inversely as the square of the distance from the target, and directly as the first power of the tube current, and as the square of the tube voltage.

A Coolidge cathode x-ray tube with a thick tungsten target, such as was used in the experiments herein reported, when operating at 100 K.V. and 10 m.a. produces an x-ray beam of intensity equal to 0.34 r per second per square centimeter at a distance of one meter from the target (3).

In these experiments, the tube voltage and current was maintained at 60 K.V. and 10 m.a., respectively. The material for irradiation was placed as close to the x-ray tube target as was practical, both from the standpoint of electrical insulation and heating effect. This distance was 13 centimeters from the center of the target to the center of the irradiated material.

Remembering that the total energy given off from the target is proportional to the square of the tube voltage, and inversely proportional to the square of the distance from the target, and using the constant of 0.34 r per second

per square centimeter with the conditions of 100 K.V. and ten m.a. at 1 meter, the intensity of the x-ray beam 13 centimeters from the target of a tube operated at 60 K.V. and 10 m.a. may be computed by the equation

$$I = 0.34 \times \left(\frac{100}{13}\right)^2 \times \left(\frac{60}{100}\right)^2$$

$I = 7.2$ r per second per square centimeter

METHOD OF TREATMENT

In an attempt to reduce experimental error, the beans were graded over a size 13 round hole screen to remove the smaller sizes, and then all checked, weathered and distinctly odd-shaped beans were removed.

The beans were then divided into three groups for treatment. One group was left dormant, a second was germinated for 18 hours before treatment, and the third was germinated for 36 hours before treatment.

The seeds of the first two groups were irradiated for periods of 5, 15, 30 and 60 minutes. This is equivalent to dosages of approximately 2,160, 6,500, 13,000 and 26,000 r units respectively. The seeds of the third group were given a single dosage of twelve minutes which was equivalent to approximately 5,200 r units.

Care was exercised while the beans were being x-rayed to keep the seeds reasonably cool. A blast of air from an electric fan was kept on the apparatus to insure circulation of air over the beans.

The treatments are summarized in Table 1.

Table 1. Number of seeds treated and the length of treatment in minutes and dosage in r units.

Condition of beans at time of treatment	Number of seeds irradiated	Treatment in minutes	Dosage in r units
Dormant	300	0	0
	400	5	2,160
	400	15	6,500
	400	30	13,000
	400	60	26,000
Germinated 18 hours	200	0	0
	400	5	2,160
	400	15	6,500
	400	30	13,000
	400	60	26,000
Germinated 36 hours	200	0	0
	400	12 *	5,200

* The x-ray tube burned out and terminated this series of treatments at this point.

THE TREATED GENERATION

The seeds were all planted within an hour or two after the treatment was made. Plantings were made on the following dates in 1938:

All dormant seeds, treated and untreated----June 8.

All seeds germinated 18 hours----------June 9.

All seeds germinated 36 hours----------June 10.

Complications arose immediately after the beans were planted. Heavy rains fell either during the actual planting or within a very few hours afterwards in every case. The soil was fairly heavy and it baked more or less before the beans had germinated and broken through the surface of the ground. The fact that there was a crust on the ground undoubtedly affected the number of seedlings that broke the crust and survived.

Effects of the X-ray Treatments Shown by the Plants

Soon after the plants began to come up, it was noted that many of the seedlings had pushed through the ground, but had died without producing any leaves whatsoever. The stems would straighten up in most cases, and the cotyledons would open, but the shoot buds would produce no apparent growth. The seedlings might remain thus for a week or ten days and then die. The roots of these plants were found to be about one-half an inch long, thick and swollen. The root tips also failed to grow actively and finally rotted.

These dying seedlings, together with the fact that, due to the crust on the ground, beans still kept coming up after three weeks from the time of planting, made accurate germina-

tion and survival counts rather difficult. However, on June 27, the number of plants that were above ground and were apparently healthy were counted. The number that had come up and had died soon afterwards were also counted. The number that had failed to come up at all was obtained by subtracting the total number of plants that had come up from the number of seeds planted. This was done for each plot, and the results are given in Table 2.

When germinated seed was treated, the number that failed to come up increased with the length of the x-ray treatment. When dormant seed was treated, the number that failed to come up increased only in the 30 and 60 minute treatments, and only the 60 minute treatment showed a large increase in the number.

The germinating seeds were injured more than the dormant seeds by identical treatments except by the lightest treatment, in which little difference could be found between the two lots. The seeds that had been germinated for 36 hours before the treatment was given were severely injured by the 12 minute exposure, even more so than the seed that germinated 18 hours was injured by the 15 minute exposure. Apparently, the susceptibility to injury by x-rays increases very rapidly after the seeds begin to germinate.

It is not known how many of the beans that came up, after the first count was made on June 27, died without further growth.

Table 2. Germination and survival counts of treated beans.

Dose in min.	Number treated	Not up by June 27	Dead, June 27	Living, June 27	Living, July 8	Living, July 22	Total harvested
		Seeds dormant when treated					
0	300	7	23	260	265	264	258
5	400	28	35	337	346	344	340
15	400	22	45	323	330	330	322
30	400	39	95	266	256	242	168
60	400	170	118	112	82	73	62
	Seeds germinated 18 hours when treated						
0	200	9	14	177	177	176	0
5	400	28	31	341	352	350	336
15	400	61	52	287	271	260	223
30	400	183	70	147	136	121	95
60	400	282	54	64	54	52	49
	Seeds germinated 36 hours when treated						
0	200	9	20	171	173	173	160
12	400	130	50	220	220	212	200

The inhibitory effects of the x-rays on the plants that survived remained in evidence by the stunted growth of the treated material. The 5-minute treatments gave only a slight stunting effect, but the effects became progressively more severe as the length of the treatment increased. As in the seedling observations, the seeds that were germinated at the time of treatment continued to show more severe injury than the seeds treated in the dormant stage by identical treatments.

Many of the plants, especially in the more lightly treated plots, apparently recovered from the effects of the irradiation in three or four weeks and grew normally. However, even these never quite overtook the early lead gained by the controls. The more heavily treated plants were severely retarded and grew very slowly in the early part of the summer.

No great difference was noted in the shape or structure of the leaves or plants grown from the treated material. The only differences noted seemed to be due entirely to the slow growth of the plants.

Late in the summer when a period of wet weather set in, many of the heavily treated plants began to grow vigorously and many set a heavy crop of seed. Late in the fall, the treated plots were rather characteristically spotted by the dark green foliage of late-maturing plants that had been retarded earlier by the irradiation. The untreated controls matured much more evenly and a majority of the plants

matured much earlier than did the treated ones.

The Method of Harvest

Because there was so much variation in the time of maturity of the treated material, and since the most retarded plants seemed logically to be the most affected by the x-ray treatment, knowledge as to whether there was a correlation between the time of maturity and the percentage of mutations was desired. Accordingly, sacks were numbered consecutively, and as the plants matured, the pods were picked, placed in the proper sack, dried and stored. The results of this method of harvesting are given in Table 3.

It will be noted that in nearly every case, the heavier the treatment, the later was the date that the greatest number of plants were harvested. This seems to bear out very well the earlier conclusions made concerning the apparently retarding effects of the x-rays.

Production of Seed

During the winter months the seeds were threshed from the pods. Later, in getting the material ready for planting, the number of seeds from all of the treated material and from one dormant sample of untreated material was counted. These findings are given in Table 3.

With only one exception, the average number of seeds per plant dropped sharply on the final date of harvest. Because of this, the plants that matured late in the fall tended to reduce the average number of seeds produced within a given treatment. The only large differences in

Table 3. Dates of harvest, number of plants harvested (upper figure) and number of seeds per plant (lower figure).

Dose in min.	Number treated	Date harvested								Total
		9/12	9/15	9/19	9/23	9/30	10/6	10/13	Gr.*	
Seeds dormant when treated										
0	300	0 0	96 62.6	62 62.7	32 63.8	19 54.8	28 43.0	21 11.5	0 0	258 56.0
5	400	21 93.2	56 82.3	56 73.7	87 66.6	71 50.8	21 36.2	28 22.8	0 0	340 63.4
15	400	29 74.4	33 66.3	25 65.6	79 72.1	65 68.8	43 43.7	48 28.7	0 0	322 60.5
30	400	0 0	1 32.0	3 73.7	5 54.0	23 61.2	25 51.2	109 20.3	2 4.0	168 32.1
60	400	0 0	0 0	0 0	0 0	12 75.3	8 77.8	29 36.7	13 4.4	62 45.1
Seeds germinated 18 hours when treated										
5	400	26 64.1	43 78.9	39 70.6	78 57.3	55 48.7	9 31.2	85 35.4	1 6.0	336 54.5
15	400	14 84.9	17 82.8	20 92.0	39 71.0	24 67.5	41 55.4	68 26.3	0 0	223 57.0
30	400	1 45.0	7 27.4	6 80.0	15 60.0	12 49.1	8 39.3	41 29.3	5 4.0	95 40.1
60	400	0 0	1 91.0	2 99.0	0 0	10 80.9	3 90.0	22 36.5	6 7.0	49 54.5
Seeds germinated 36 hours when treated										
0	200	6	18	49	51	7	9	20	0	160
		No seed counts were made								
12	400	18 69.3	25 59.5	16 51.3	34 63.8	43 69.0	31 55.7	30 22.7	3 1.3	200 55.5

* The plants in this column were taken from the field Sept. 1 and matured in the greenhouse.

the average number of seeds produced per plant that were evident between the various treatments were in the 30-minute treatments of the dormant and the germinated seeds. In each of these treatments, a large percentage of the plants were harvested on the last harvest day.

About the middle of the flowering period a rather severe and prolonged drought occurred that may have influenced the seed set to quite an extent. The earlier plants would have set the most seed and the later ones would set under the handicap of dry weather. This drought, however, was not important to many of the more retarded plants because early in August a period of wet weather occurred and those plants, still growing steadily because they had not yet set any seed, flowered profusely and set a late crop. The fall was late and many of these late plants fully matured. Many, however, matured a very small number of seeds, the importance of which will be discussed.

THE SECOND GENERATION

In the spring of 1939, the material to be planted was sorted according to the number of seeds per plant in order that plots of the same length could be planted in a block. The seeds were planted about 3 inches apart in the row. Most of the material was planted June 7 just before a rain, but several of the longer plots were planted under rather unfavorable conditions two days later.

Several of the larger progenies of the more lightly treated material were not planted for lack of space.

Observations were made every few days after the beans first came up, and at longer intervals thereafter. Only well-defined variations were classed as mutant types.

Progenies showing mutant types were thinned to give the variants more favorable growing conditions.

Description of Mutant Types

A total of 152 mutants were found up to August 15, and a large variety of forms occurred. The mutations that were apparently the same in phenotypic appearance were grouped. The following classification of mutant types, with the number of progenies producing each type, resulted:

No. of progenies	Description of Mutant Type
29	Yellow seedlings that died soon after emergence.
13	White seedlings, entirely lacking in pigment, that died soon after emergence.
9	Light green seedlings that died soon after emergence.
8	Light green seedlings that lived and formed light green secondary leaves; some of these plants have died.
6	Green primary leaves; light green secondary leaves; some plants nearly normal in size.
6	Light green primary leaves; secondary leaves light green when first formed, but turn darker green with age.
4	Yellow primary leaves; secondary leaves light green.
3	Yellow primary leaves; secondary leaves dark green.

No. of progenies	Description of Mutant Type
2	Light green primary leaves; several very bright yellow secondary leaves produced; died in a few weeks.
2	Yellow primary leaves; produced a few secondary leaves; then died.
1	Light green seedling; many very small secondary leaves. Fig. 1.
15	Dark, glossy green; veins distinctly indented; chiefly dwarfs. Fig. 2.
1	Yellow edges and veins on secondary leaves; very small plants.
1	Secondary leaves light green along veins; plant viny; leaves somewhat smaller than normal. Fig. 3.
2	White chimeras; plants nearly normal in size. Fig. 4.
11	Leaves long and narrow, "willow leaf" type; plants rather small. Fig. 5.
3	Thick leathery leaves; slow growth. Figs. 6 and 7.
4	Small bushy plants with small, rough, irregular leaves. Fig. 8.
1	Narrow primary leaves, dwarf plant.
3	Appearance of leaves resembles mosaic; plants normal height, but slender. Fig. 9.
1	Mottled primary leaves; dwarf plant.
3	Dark green, small, slender vine; no branching.
1	Small plants with small narrow leaves.
2	Seedlings very erect and tall; very erect later growth.

No. of progenies	Description of Mutant Type
4	Long narrow leaves, but wider than "willow leaf" type.
7	Dwarf in size and structure; leaves proportional in size to the size of the plant; plants 4 to 7 inches high.
4	A great many very slender branches; plants of good size. Fig. 10.
1	Rounded leaves; number of leaflets varies from one to three. Fig. 11.
1	Leaves have 5 leaflets instead of 3; small plant. Fig. 12.
1	Center leaflet football shape; attachment to petiole flat. Fig. 13.
1	Dwarf, squat plants, rosette in form; died.
1	Erect, dense, cylindrical in form; dark glossy green color; plants 3 to 10 inches high. Fig. 14.
1	Stems appear very susceptible to rust; tips of vines green with the rust becoming progressively worse down the stem.

Approximately 67% of the mutations were chlorophyll abnormalities, almost all of which (i.e. of those that survived) were less than normal size despite the fact that the rows had been thinned to give the mutant types more favorable growing conditions.

Fig. 1. Small bushy plant with very small leaves. The primary leaves were light green.

Fig. 2. Dark, glossy green plant; veins distinctly indented; small in size.

Fig. 3. Plant showing light green areas along the veins of upper leaves; viny habit of growth.

Fig. 4. Plant showing a white chimera.

Fig. 5. Bean plant showing long, narrow, "willow leaf" type of leaves.

Fig. 6. Plant showing thick, leathery leaves; slow growth; clusters of very small leaves at the growing points.

Fig. 7. Glossy green plant showing thick lower leaves and clusters of very small leaves at the growing points. Normal plants on each side of the mutant in the center.

Fig. 8. Small plant in the center showing small, rough, irregular leaves. Normal plants surround the mutant.

Fig. 9. Plant in center showing small, dull, rolling leaves; Normal plant at the left.

Fig. 10.

Mutant in center showing a large number of very slender branches.

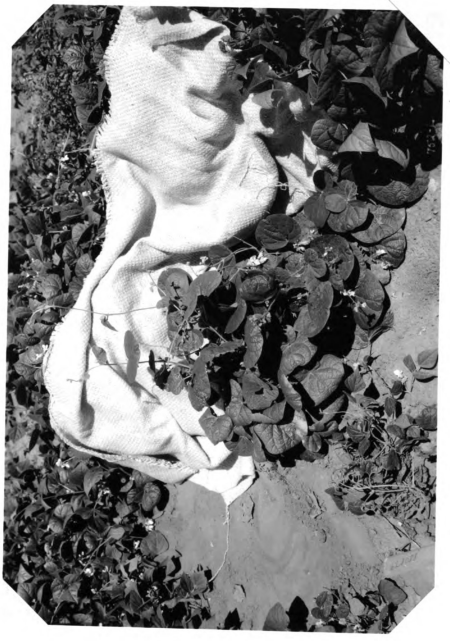

Fig. 11. Bean plant showing large rounded leaves and the number of leaflets varying from 1 to 3.

Fig. 12. Small plant showing the mutation from 3 leaflets to 5 leaflets per leaf.

Fig. 13. Small, glossy plant showing the football shaped center leaflet, and the flat attachment to the petiole of the leaf.

Fig. 14. Small center plants showing the mutant type with glossy green leaves, and erect cylindrical form.

In the plots segregating out the mutant types, the ratio of mutants to total progeny population varied greatly. At one extreme were segregations of 1:1 and 3:5 in the smallest plots, and 8:34, 7:30, and 13:42 in the larger plots. At the other extreme were ratios of 1:63, 2:105, 1:52, and 2:60. Ratios ranging from one extreme to the other were found.

Distribution of Mutations

Except for the large percentage of mutants in the 5-minute treatment, the percent of mutants appearing from the various treatments of dormant seeds was approximately proportional to the dosage given.

A similar relationship did not hold for the germinated seeds (Table 4). The percent of mutations that appeared as a result of the 15-minute treatment in this group was about three times that resulting from the 5-minute treatment, but the percent of mutations resulting from the heavier treatments was far below that expected on the assumption that the rate of mutation is proportional to the dosage.

The rate of mutation in the germinating seeds exceeded that in the dormant-treated seeds in identical treatments in only the 15-minute treatments.

Table 4 also shows that the rate of mutation tended to increase with the later dates of maturity.

Table 5 shows that the average percent of mutations appearing in the progenies increased with the size of the progeny in every case.

Table 4. Number of progenies planted in 1939 (upper figure) and number of mutations (lower figure) for each date of harvest in 1938, and percent of mutation.

Treatment in min.	Date harvested in 1938									% of mutations per treatment
	9/12	9/15	9/19	9/23	9/30	10/6	10/13	Gr.	Total	
Seeds dormant when treated										
5	7	42	27	52	27	15	26	0	196	
	1	2	1	3	0	2	1	0	10	5.1
15	17	24	16	48	43	38	47	0	233	
	0	2	4	8	2	0	4	0	20	8.6
30	0	1	3	5	23	25	109	2	168	
	0	0	2	1	8	7	12	0	30	17.9
60	0	0	0	0	12	8	29	13	62	
	0	0	0	0	6	5	7	1	19	30.7
Seeds germinated 18 hours when treated										
5	20	25	25	53	51	9	78	1	262	
	1	0	1	3	3	2	3	0	13	5.0
15	9	9	8	28	18	30	67	0	169	
	4	3	2	3	2	3	10	0	27	16.0
30	1	7	6	15	12	8	41	5	95	
	0	1	0	0	0	2	1	1	5	5.3
60	0	1	2	0	10	8	22	6	49	
	0	1	0	0	3	2	6	0	12	24.5
Seeds germinated 36 hours when treated										
12	18	25	16	34	43	31	30	3	200	
	0	1	0	1	5	2	7	0	16	8.0
Totals	72	134	103	235	239	172	449	30	1434	
	6	10	10	19	28	25	51	2	152	10.6
% of mutations per date of harvest	8.3	7.5	9.7	8.1	11.7	14.5	11.4	6.7	10.6	

Table 5. Number of progenies of the various sizes (upper figure), number of mutations appearing in each size (lower figure), and average percent of mutations in each size.

Treatment in minutes	Number of seeds per progeny						Not * planted	Total planted
	1-10	11-20	21-30	31-45	46-70	Over 71		
Seeds dormant when treated								
5	13	26	34	40	83	0	152	196
	0	0	0	2	8	0		10
15	12	32	29	54	86	20	89	233
	0	2	5	3	9	1		20
30	40	27	20	31	35	15		168
	0	2	3	5	12	8		30
60	19	2	8	9	11	13		62
	1	0	0	3	7	8		19
Seeds germinated 18 hours when treated								
5	22	23	47	65	91	14	74	262
	0	2	3	3	5	0		13
15	15	22	22	35	56	19	54	169
	1	3	5	6	7	5		27
30	11	16	16	25	12	15		95
	1	0	1	3	0	0		5
60	7	8	4	7	5	18		49
	0	0	1	4	0	7		12
Seeds germinated 36 hours when treated								
12	14	22	17	45	42	60		200
	0	3	1	5	5	2		16
Totals	153	178	197	311	421	174	369	1434
	3	12	19	34	53	31		152
Ave. % of mutations	1.7	6.7	9.6	10.9	12.6	17.8		10.6

* Not planted for lack of room. All progenies not planted contained more than 71 seeds.

Two types of mutations were found in each of 11 different progenies.

Only one segregating progeny was found among 160 controls, which tends to indicate that the material was rather uniform when the experiment began.

DISCUSSION

Several factors may have contributed in making the observed rate of mutation quite different from the actual mutation rate, especially in the germinated seeds. The crust that formed on the ground the first year and killed many of the weaker seedlings may have been a factor, because the data shows that the plants that were injured and retarded by the x-rays tended to produce the greatest percentage of mutations.

The large number that failed to mature may have been a factor also, because of the small progenies of many of the plants harvested late in the fall. The size of the progeny seems rather important to the observed results because the rate of mutation increases with the size of the progeny.

Several of the treated plants which were moved into the greenhouse September 1 either died without producing any seed whatsoever or matured a very small number of seed as shown in Table 3. These plants were the most retarded of those surviving the treatments, and they came chiefly from the heavily treated and the germinated plots.

Much more accurate results could undoubtedly be obtained, especially in the seeds most severely injured by the x-rays, if the treated material all fully matured, and if favorable

soil conditions were present to afford maximum survival of seedlings.

The regular increase in the rate of mutation as the size of the progeny increases is probably due to the fact that only a small sector of the plant may be affected by the induced mutation in the seedling. Consequently, the tissue is chimeric and the number of seeds that will be homozygous for the mutant character will depend upon the number of seeds produced and the chance that the character will be segregated out. If the mutant character is caused by a recessive factor, the expected ratio will be 3:1 for the number of seeds produced by the affected area. The larger the number of seeds produced, therefore, the greater the likelihood that the seeds produced by the affected sector will contain one or more seeds homozygous for the mutant factor.

The germinating seeds failed to exceed the dormant seeds in the rate of mutation in identical treatments except in the one treatment already mentioned (15-minute). This may have been due to the apparently high mutation rate in the 5-minute treatment of the dormant seeds, and to the unfavorable environmental factors to which the weakened heavily treated germinating seeds were subjected. By far the majority of the germinated seeds that were treated for 30 minutes matured plants very late in the fall and had a low average yield per plant. Many of the progenies of the heavily treated plants were grown under unfavorable conditions. It is also

apparent that the lethal dosage for germinating seeds is much smaller than for dormant seeds. There was a great difference in the effect of the x-rays on plants with a given treatment, and it is probable that only the least injured of the germinated seeds survived. More carefully controlled growing conditions would be necessary to accurately determine the true effects of the x-rays on the germinating seeds, but the environmental factors were so unfavorable to the injured plants that the data must have been greatly influenced.

No cytological studies were made to attempt to explain the causes of the mutations. However, the fact that certain types of mutations occurred several times, whereas many of the types occurred only in one progeny, promotes speculation as to probable cause. Are certain genes more easily changed than others or are there several complementary genes working together to produce the readily mutant characters? Surely translocations and inversions would not occur regularly in the specific manner necessary to affect any given gene. The field seems necessarily limited, therefore, to either deletions or simple chemical changes of the gene itself. And are the various chlorophyll abnormalities, leaf variations, etc., allelomorphic or is a different gene affected in each? The only means of determining the number of genes affected in the many progenies producing the same or similar mutant characters is by crossing plants, either of the type

or carrying the mutant factor, in one plot with affected plants from other plots.

Some question arose concerning the segregation for susceptibility for disease in one control plot. The ratio was 22:8 or nearly 3:1 for normal:disease. Since the ratio is so close to the expected ratio for a monohybrid, the author believes that it is due to a chance cross with a susceptible plant in 1937.

SUMMARY

Both dormant and germinated bean seeds were x-rayed to study the effects of the treatments on the treated generation, and to observe the types and rates of mutations that might show up in the progenies of the treated material.

The physiological effects of the x-rays varied with the intensity of the treatment. Germinated seeds were found to be more severely injured than dormant seeds by identical treatments.

The x-ray treatments tended to retard the growth and development of the plants of the treated generation.

Considerable differential effect was evidenced by the treated plants, but no great difference was noted in the shape or structure of the leaves or plants except that common to very slow growing bean plants.

Mutations were readily produced in the white pea bean and many different types of mutants were found.

The rate of mutation was roughly proportional to the intensity of the dosage for dormant seeds, but the propor-

tionality did not hold for germinated seeds.

The rate of mutation in the germinated seeds exceeded the rate in the dormant seeds in only the 15-minute treatments.

The rate of mutation tended to increase with the later harvest dates in the fall.

There was a steady increase in the rate of mutation as the size of the progeny increased.

A mutation produced in a treated plant is undoubtedly sectorial because of the wide ratios found in the progeny tests.

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