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THE PRODUCTION OF MUTATIONS IN BARLEY
BY X-IRRADIATION

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U N I V E R S I T Y O F A L B E R T A

THE PRODUCTION OF MUTATIONS IN BARLEY
BY X-IRRADIATION

A DISSERTATION
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ABSTRACT

The effect of X-irradiation of barley seeds was studied at various levels of metabolic activity, which was experimentally controlled by varying periods of pre-soaking.

Increased metabolic activity resulted in increased susceptibility of seeds to irradiation effect. Optimum treatment levels appeared to be: 1. no pre-soaking, 15,000-20,000 r; 2. three hours pre-soaking, 12,000 r; 3. germinated seeds, 2000 r. A technique based on (2) seemed to be most satisfactory for practical work.

Some one hundred individual mutants, classified into twelve types, were induced and studied. All were attributed to recessive point mutations. All but one involved some form of chlorophyll deficiency, and all were deleterious. The ratio of lethal (mainly albino or near-albino) types to viable types was about 1:25 or 1:30.

Cytological observations at sporogenesis indicated little chromosomal breakage and rearrangement, but these phenomena were observed in root-tip preparations.

It is considered that induction of mutations is of doubtful value as a method of plant breeding.

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THE PRODUCTION OF MUTATIONS IN BARLEY

BY X-IRRADIATION

J. E. Fitzsimmons

INTRODUCTION

The type of penetrating radiations known as X-rays have long been known to have pronounced effects, presumably through their powers of ionization. But early experimenters were unable to reach definite conclusions because of variation in organic material and of ignorance of the properties of radiation. Results varied from one experiment to another when seemingly carried out under the same conditions. It was not until some twenty years ago that conclusive evidence was obtained on the biological effects of radiation.

The effects noted involved hereditary changes in various characters in different species. Such observation stimulated research in the application of radiations to determine:

1. the fundamental effects of radiation, and,
2. the effects of radiation on mutation rate.

Swedish workers, in particular, have been interested in the latter respect, and have made claims that they have been able to produce mutants which are superior to the original material in one or more respects.

THE PHYSICS OF X-RADIATION

X-radiation is an electro-magnetic radiation emitted in quanta from the target of an X-ray tube, with a wave length of from 0.05 to 10 Angstrom units. The electro-magnetic nature of the X-rays means that they have the same physical properties as light waves, but because of the shorter wave length the amount of energy involved is much greater.

These waves have the peculiar property of behaving as waves or as particles. This latter property is known as the quantum theory of radiation, and each quantum of energy is known as a photon. The amount of energy in a photon is inversely proportional to the wave length, and it can behave as though the photon were a particle with a definite mass. A theoretical mass can be calculated for a photon by using the famous Einstein formula, $E=mc^2$, where E is the energy in ergs, m is the mass in grams, and c is the velocity of light in centimeters per second.

The X-ray wave length in Angstrom units can be approximated roughly by the formula $12400/\text{voltage}$ applied. This gives the shortest wave length emitted, while the wave length of greatest intensity is about twice that. However, there is a spectrum of wave lengths emitted, ranging from those that are very short to long wave lengths that overlap into the ultra-violet range. Filters of aluminium or copper can be used to absorb some of the longer wave lengths so that a more monochromatic beam will be emitted.

X-rays are absorbed in matter by a process of ionization. Ionization takes place when a photon strikes an orbital electron and ejects the electron from an atom. The ejected electron then moves off with all the energy of the photon, less the energy required to eject it. This electron can strike other orbital electrons of different atoms and remove them. This process is repeated until all the energy is used up. The initial electron ejected is known as a photoelectron, and the process as the photoelectric effect.

The atom that loses an electron also loses one negative charge and thus becomes positively charged, or in other words, positive ions are formed. The ejected ions eventually tie up with other atoms when their energy is lost, and negative ions are formed. Thus ion pairs are formed. It is generally believed that only positive ions have any significant biological effect.

The energy from an X-ray wave of 1.5 Angstrom units is about 8 Kev, or 8000 electron volts. The energy required to eject an electron from an atom in tissue is about 0.5 Kev. Therefore the ejected electron has an energy of about 7.5 Kev which causes it to move about 1.5 microns in the tissue and cause about 230 further ionizations.*

Because of the penetrating power of X-rays, it is convenient to measure the energy absorbed in a given volume. The unit of dosage employed is the "roentgen" (usually designated by r) which is defined as:

"The quantity of X-irradiation which will produce one electrostatic unit of ions of either sign in one cubic centimeter of air under standard conditions of temperature and pressure."

* Lea (17, p10)

For convenience of making the physical measurement the roentgen is defined in terms of energy absorption in air. In tissue the energy absorption for the same incident intensity of radiation will be about one thousand times greater on account of the greater density of tissue, Lea (17, p7).

The biological effect of X-rays are due to the penetration and subsequent ionization that takes place. The ionization of an atom changes its chemical properties, and it also changes the chemical properties of the molecule of which the atom is a part. It is also possible that the chemical effect may be transmitted to adjacent molecules, i.e., those within chemical striking distance of the ionized molecule.

In a study of mutations the effectiveness of the radiation is limited to certain critical volumes in the cells, namely the chromosomes, and genes on the chromosomes. These become targets to be hit by the photons.

According to Lea (17, p78) each ionization in irradiated tissue has a certain probability of producing the effect, but the probability is uninfluenced since the last ionization occurred or before the next will occur. Therefore the effectiveness of the dosage depends on the number of roentgens applied and is independent of the intensity of the radiation and the manner in which it is fractionated.

Catcheside (4), however, believes that intensity and fractionation of the dose plays a significant part insofar as chromosomes are concerned. At low intensities breaks have a better chance to rejoin before another break is formed, thus reducing the chances of fragmentation. Fractionating the dose will have about the same effect.

Most workers, as reported by Lea (17), agree that gene changes are due to one ionization per gene, and so the greater the dose the more genes are affected. Lea states that the following results have been obtained:

1. Yield of recessive lethal mutations is proportional to dose.
2. For a given dose the yield is independent of the time over which the irradiation is extended.
3. The yield for a given ionization in the tissue diminishes with increase of ion density of the radiation.*

These are the principal tests by which one recognizes an action of radiation produced by a single ionization.

LITERATURE REVIEW

General

Muller (19) and Stadler (23, 24) first published results of the effects of X-rays on heredity. They worked independently, Muller with *Drosophila*, and Stadler with corn and barley. Both workers found similarity in the behavior of different organisms to the effect of X-rays as follows:

1. The rate of induced mutation was much higher than the rate of spontaneous mutation.
2. The mutations observed were like those of natural (spontaneous) occurrence.
3. Chromosomal aberrations occurred in all the organisms studied.
4. The rate of mutation was proportional to dosage.

* With different types of radiation, hard X-rays, soft X-rays, neutrons and alpha rays.

5. All mutations, with one or two exceptions, were recessive.

6. Many mutations observed were due to chromosomal deletions.

Stadler and Muller subsequently made rather extensive studies of the effects of X-rays on the induction of mutations. The mutations were almost invariably recessive and were found to segregate in a simple 3:1 ratio. This led to the hypothesis that mutations were caused by a single gene change. In other words, when a gene in a chromosome was hit and affected by the X-ray photon, only one of the two alleles was involved. Further substantiation was provided by the observation of reversible mutations and of mutations which were in multiple allelic series.

Stadler (25) defined a (point) mutation as any heritable variation which cannot be shown to be due to a change involving more than one gene. Mutations are identified by experimental tests, and these cannot establish conclusively that a change within a gene has occurred. Deficiencies may occur that do not involve the loci of two known genes, and which therefore cannot be distinguished from mutations. He, however, states that a "gene" or "point" mutation indicates that either a chemical change or a deletion may be involved. A hypothesis of chemical change cannot be tested directly but can only be inferred. Only the larger aberrations, such as translocations, fragmentation, inversions and large deletions can be detected by cytological examination, and therefore, it is highly probable that small undetectible deletions also occur. Such deletions can cause a phenotypic change which will show the same kind of segregation as a gene. Because of the greater prevalence of chromosome aberrations, Stadler stated that most artificially induced mutations are probably due to deletions rather than a

chemical change of the gene, and therefore, cannot safely be regarded as being the same as spontaneous mutations.

As indicated by Altenburg (1) mutations vary from very small, almost undetectable to very large, easily observable changes. In other words, there is a mutation spectrum. Some, such as the chlorophyll deficient mutants, are very easily identified; others, such as gross morphological changes, can usually be detected by careful observations; but in all probability many escape observation. Physiological changes, which Gustafson (13) has reported are very difficult to isolate. Consequently the mutation rates are only apparent.

Most mutations, whether spontaneous or artificially induced by radiation, are deleterious. A number of investigators (1, 6, 12, 19, 24) have reported that nearly all mutations are recessive, and the greater proportion are lethal. A few, however, are beneficial.

Applications to Plant Breeding

Stadler (24) stated that the use of artificially induced mutations in plant breeding would not be practical. Most of the mutations are deleterious and the chance of getting beneficial mutations is very low. Moreover, a positive trait tends to carry with it several negative ones. There tends to be a pleiotropic effect of the alleles, which tends to reduce vitality.

The plant breeder has at his disposal a large number of variations present in existing material and these variations are largely due to spontaneous mutations that have already been "selected" and adapted. The progress made by a plant breeder is certainly influenced by the extent to which such existent variability is used in his plant breeding program.

Lein (18) stated that the basis of plant breeding is, at present, limited to: 1. spontaneous mutations, 2. selection and 3. crossing. Of these three methods, the first is very limited in application because of rarity of occurrence, the second and third are almost exhausted. He feels that X-irradiation, by inducing large numbers of mutations, can produce variability in present day varieties which can be put to good use in a plant breeding program.

Gustafsson (13) stated that fifteen years of extensive work had not increased the yield of Scandinavian barleys. He reported that X-irradiation has produced a number of morphological and physiological mutants which are superior to the original material in several respects. Such mutants can be used to a good advantage in a plant breeding program. In

particular, he mentioned one mutant that produced an extra large head, and several "erectoid", or extremely strong strawed types. In another report (10, 12) he stated that he has isolated several other morphological and physiological types that are improvements over the mother line.

Techniques

All barley X-irradiation studies reviewed by the writer were conducted on the seeds. Freisleben, as reported by Lein (18), believed that pollen treatment would obviate many of the difficulties encountered in seed treatment, in that only one cell, the gamete, would be treated. But the practical difficulties involved in carrying out pollen irradiation are too great to make use of the method in a mutation study.

The difficulties encountered in seed treatment are due mainly to the fact that each seed contains many cells which are affected differently by the X-ray photons, some (according to Gelin, 9) being more severely damaged than others. In addition some of the cells may have undergone differentiation and, consequently, chimeral characteristics appear in the plants.

Caldecott (2) reported treatments in which the seed was placed one layer thick on a rotating plate, six to eight inches below the target of the X-ray tube. He did not state the type of filter, if any, that he used, but he said that many biologists question the use of filters

in this type of work, because any wave length that has sufficient energy for penetration, can cause ionization in the tissue and may result in an effect. Gustafsson (12) reported using an aluminium filter, but did not state why.

In a rather extensive survey, Gustafsson (12) reported the following dosages for a number of agricultural plants:

sunflower	5,000 r
barley	10,000-20,000 r
wheat	15,000-20,000 r
rye	12,500 r
flax	40,000-60,000 r
rape	90,000 r

Stadler (24) found that not all heads produced by a single seed showed mutations. There are several tiller primordia in the growing point of a developing embryo and if a cell or cells in one primordium are hit, only the tiller arising therefrom can produce a mutation. Therefore it is necessary to handle each head separately. He reported that three to four heads per plant can give a good indication of the apparent mutation rate in barley.

Researches on Barley

Researches on barley, as has been indicated, have been limited to mutation studies arising from irradiated seeds. A number of workers (3, 5, 9, 14, 15, 20, 26) have reported on the effects of different modifying factors such as heat treatments before and after irradiation, atmospheric changes, sonic energy, ultra violet rays and polyploidy used in conjunction with X-rays. There is a voluminous literature on the effects of X-rays on chromosomes of corn, Tradescantia and, in particular, Drosophila.

In mutation studies Frøer and Gustafsson (8) reported on the effect of seed size and hull on the efficiency of X-rays in producing biological effects. They concluded that the hull reduced the damage by absorbing some of the energy, and that large seeds were less susceptible than small, as measured by the rate of survival. They noted in a heavily irradiated sample containing seeds of various sizes, that the few plants which survived were larger, more vigorous and had more tillers than the check plants. They concluded that only the large seeds survived the treatment, and consequently the plants were larger. They stated that for best results only uniform seed samples should be used.

Several investigators (4, 6, 12, 19, 25) have reported on the effect of irradiation on chromosomes. These effects are usually translocations, inversions, deletions and fragmentation. In general, with increasing dosage, there is an increase in chromosomal aberration. As a result of such aberrations the course of mitosis becomes quite erratic. Gustafsson (12) reported that injured cells can enter prophase, and pass

through to telophase, but after a few divisions the nuclei disintegrate and disappear. The normal divisions of the undamaged cells pass the abnormal, and after a few divisions mostly normal divisions can be seen.

Gelin (9) reported on the effects of increasing the moisture content on susceptibility to X-ray damage. He found that there was an increase in chromosomal aberrations as moisture content increased. He used a dosage of 10,000 r and moisture contents of 10%, 15% and after soaking for 23 hours. The damage reported was 12.7% disturbed divisions for 10% moisture, 28% disturbances for 15% moisture and 53.8% after the soaking period. He found a marked parallelism between the percentage of disturbed cells, sterility and mutation rate. He stated that if the percentage of disturbed cells is doubled, the mutation rate will be doubled.

Gustafsson (11), and Freisleben and Lein (6) reported that irradiated seeds can start growth even when exposed to extremely heavy dosages, but not all seeds produce viable plants. They found that the proportion of plants surviving past the first leaf stage markedly decreased with increased severity of treatment. Gustafsson (11) reported that the treatment that produced the highest mutation rate is one that can cause a decrease of about 25% in the survival of treated seeds.

Gustafsson (13) has found that the mutation rate and partial sterility are closely related. The X_1 plants (i.e., those plants grown from irradiated seed) that have a high proportion of sterile florets tend to produce more mutations per X_2 plant than those having a higher proportion of fertile florets. He pointed out that highly fertile plants produce albinos, whereas highly sterile plants produce more of the rarer mutants. Gustafsson has concluded, from this and other evidence to be

presented later in the discussion, that there is a certain selectivity in the types of mutations found.

Gustafsson (10) has reported at great length and detail on the types of mutations, and the probabilities of certain kinds occurring. He has found it convenient to classify them as follows:

1. Chlorophyll mutants
2. Sterility or lethal mutants
3. Vital mutants:
 - (a) morphological
 - (b) physiological

He subdivided the chlorophyll mutations again as follows:

1. Albinos. These occur most frequently at the lower levels of dosage, and mostly from plants that are highly fertile. He found that with increasing dosage there was a decrease in the number of albino mutations produced. For this reason Gustafsson (10) refers to the proportionality between dosage and mutation rate to be a pseudophenomenon.

2. Xantha. This type appears at more severe treatments, usually in the progeny of highly sterile plants. Plastids of the xantha type are intermediate between the viridis and albino. These tend to appear in proportion to dosage.

3. Viridis. These mutations are most easily produced in highly sterile plants. The plants are normal to subnormal.

4. Tigrina. This is a viable striped mutant that appears only in the progeny of irradiated dry seeds.

5. Albioxanth⁹, which is intermediate between albinos and viridis in appearance and occur only in the progeny of highly hydrated seeds.

Sterility and lethal mutants are divided into two classes because 1. the lethals fail to develop to the flowering stage, and 2. the sterility class fails to set seed because of some change in the flower structure which prevents seed setting.

Vital mutants have been described under two subdivisions as follows:

1. Morphological mutants are those that are fully viable and quite fertile. The most commonly found are the "erectoid " types which arise from the same kind of treatments as the Xantha mutations. However, most of the morphological mutants show a pleiotropic genetic effect and are consequently inferior to the mother line in one or more respects, such as inferior yield and later maturity. Some, however, appear to be superior.

2. Physiological mutants differ from the mother line mainly in physiological characters, but some very slight morphological differences are usually apparent, such as 1000 kernel weight and width of leaf.

Gustafsson (13) has reported that fairly constant relative mutation rates exist between the different types of mutants. One induced morphological mutant, fertile or infertile, corresponds in frequency of occurrence to 22 chlorophyll aberrants, 15 mutants that prevent normal meiosis and gametic formation, or 30 cases of translocation sterility. In order to obtain one mutant distinctly better in one or more characteristics the barley breeder must induce about seven hundred to eight hundred worthless mutants.

MATERIAL AND METHODS

Material used in this study was largely confined to Montcalm barley. Barley, as previously indicated, is a good organism for the study of the effect of X-irradiation on mutation rate. Barley is also an important commercial crop, the variety Montcalm being one of the more valuable varieties. Irradiations of other crop plants such as peas, rape, flax and a selfed line of sunflowers were carried out on a small scale.

The X-ray apparatus used delivered 200 roentgens per minute at a distance of eight inches from the target. The machine was operated at 100,000 volts (100 Kv.) and 7 milliamperes (7ma.). Dosages used were based on those recommended by Gustafsson (13), usually 15,000 r for dormant seeds and smaller dosages for soaked or germinated seeds. No filter was used.

During the course of each irradiation treatment, the seeds were placed in a single layer in a petri dish, which was placed under the X-ray tube for the duration of the exposure. Because the X-ray beam is not homogenous it was deemed advisable to redistribute the seeds at regular intervals, so that each seed would have as good a chance as any other of getting hit by the same number of photons of similar energy value. The seeds were redistributed at least three times in each exposure, by pouring the seeds from one dish to another and thoroughly mixing them each time. About 250 to 300 seeds were treated each time.

Soaking was accomplished by placing the sample in a small sack which was immersed in a beaker of tap water. Moisture content was

estimated by weighing the samples before and after soaking and calculating the increase in moisture content. At the end of the soaking period the excess water was removed by rolling the seeds in paper towelling. The X-irradiation treatment followed immediately. Checks were treated in the same manner except that they were not X-rayed.

Germinated seeds were used in order to reduce the time needed for irradiation treatments. Germination was carried out in petri dishes, using moist blotting paper, and after 24 hours the seeds were ready for treatment. Treatment was carried out by leaving the seeds on a layer of moist paper during the period of exposure; but the seeds were redistributed at least twice during the treatment.

Treated and check samples were sown as soon as possible after treatment. In the field attempts were made to ensure uniform spacing of the seeds so that single plants could be harvested. In 1949 all seeding was done by hand, but in 1950 some was done using the Kemp V-belt seeder. Some treatments were sown in the greenhouse to get information on germination and survival rates of the different treatments.

X_1 plants were harvested in the field as single plants. Where possible, 4 heads were selected from each plant for use in studies of the mutation rates. Seeds from the four heads were sown in a separate row. Where one head produced a mutation, the progenitor X_1 plant was considered to have carried an induced mutation. Where all of the heads carried a mutation, the mutation was assumed to have arisen spontaneously prior to treatment. Where two heads each carried a mutation, two mutants were recorded. Where two mutations arose in one head,

two mutants were also recorded.

The mutation rate was calculated on the basis of the number of mutating plants per hundred plants tested, expressed as a percentage. This procedure was adopted because it has not been possible to sow and test all the X_1 plants, so calculations on the basis of number of seeds treated was not possible.

Most of the observations were made at the seedling stage in order to make use of the greenhouse facilities. The plants were allowed to grow for a period of 2 to 3 weeks.

In the field the same procedure was used, but viable mutations were grown to maturity. At maturity the X_2 lines that had shown mutations were harvested as single plants. Some of these lines were grown in the greenhouse to determine the mode of inheritance of the mutant types. Two heads of each plant of each line were grown, and the segregating ratios of the mutant character noted.

Cytological examinations were carried out on a limited amount of material. The acetocarmine smear technique, as outlined by Smith (22) was used throughout. Examinations were made on root tips of irradiated seed, and spore mother cells in the anthers at sporogenesis were examined in the X_1 and X_2 generations. Material was obtained from each treatment of the X_1 in the 1950 series and from the mutating lines of the X_2 .

In the 1950 series of treatments a fertility study was attempted. The number of fertile and infertile florets was estimated on each of the four heads of the harvested plants. To facilitate counting the awns were singed off by flaming with a bunsen burner. Germination

tests were carried out on singed and unsinged heads to determine the effects of the flame on the seeds.

OBJECTIVES OF THE STUDY

This study is preliminary in nature, designed primarily to evaluate the claims of the Swedish workers, who have reported getting mutations of economic importance. The present investigation has been mainly concerned with technique of seed treatment, classification of mutant types and determination of mutation rates.

EXPERIMENTAL RESULTS

The X_1 Generation

Plants other than barley

Flax, rape, peas and four selfed lines of sunflowers were treated at dosage levels recommended by Gustafsson (11). No apparent effect was noted in the X_1 of the flax or rape but the peas showed reduced growth. The peas were discarded because of the severe drought and lack of time to give them adequate attention.

Three of the sunflower lines received 5000 r each and the fourth line 4000 r. The germination of the first three lines was under 50%,

while the germination of the fourth was about 90%, which indicated that 5000 r dosage was approaching the upper limit. The surviving plants were selfed by bagging them, but a severe early frost killed most of the immature seed. The few available seeds of each X_1 treatment were sown but no mutations were apparent.

Barley

Effect of soaking on moisture content

To determine the water uptake of soaked seeds, weighings were made at twenty minute intervals, as indicated in Table 1. The rapid rise in the weight at the start is presumably due to intake of water by the process of imbibition. When the capacity to imbibe water is exhausted at the end of about two hours the weight becomes relatively constant for a period of one hour. The increased water content then appears to activate the metabolic process and enzymatic action changes starch to sugar. This conversion brings about an increase in osmotic pressure in the cells of the seed, and water intake is again initiated, this time by the process of osmosis. Equilibrium appears to be reached after about five hours of soaking. These data indicate that metabolic activity begins in about three hours of soaking.

Table 1

Effect of soaking period on water intake
by imbibition and osmotic pressure

Time	Average weight of three determinations	% increase
0:00	46.4 gms.	0
0:20	48.2	3.9
0:40	48.5	4.5
1:00	48.6	4.7
1:20	48.7	4.9
1:40	48.8	5.2
2:00	48.8	5.2
2:20	48.8	5.2
2:40	49.0	5.6
3:00	49.2	6.0
3:20	49.3	6.3
3:40	----	---
4:00	49.4	6.5
4:20	49.5	6.7
4:40	49.5	6.7
5:00	49.5	6.7
5:20	49.6	6.9

Effect of pre-soaking, pre-germination and radiation on germination,
survival and growth

Greenhouse observations: The effects of radiation were determined in
a preliminary survey in the greenhouse. The results are given in

Table 2.

Table 2

Effect of soaking and r dosage on seed germination,
and on survival and height of shoot

Dosage r	<u>Treatment</u> Period soaked hours	Germination %	Survival %	Height of shoot cms.
Check	0	100	100	11.6
5,000	0	92.5	87.5	11.0
10,000	0	95.0	92.5	10.2
15,000	0	97.5	75.0	6.1
20,000	0	95.0	72.5	6.0
Check	0		99.0	14
15,000	0		71.0	9
15,000	1.5		64.5	4.1
15,000	3		14.2	4

These results indicate that increased dosage above 10,000 r has a detrimental effect on survival and growth. Increased moisture content of the seed is directly associated with increased killing effect of irradiation.

Field observations: Observations of X_1 plants were limited to survival rates and fertility estimations. These results are shown in Tables 3 and 4. Inasmuch as the two years of the study were extremely dry the differences between treatments, other than regarding survival, were negligible. Differences were more apparent during the first three to four weeks of growth, but disappeared after that time. At maturity even the most heavily treated survivors appeared almost as vigorous as the checks.

Until July 16, 1949, practically no rain fell, but heavy rains occurred after that time. The result of such an increase in moisture supplies favored the development of second growth, which tended to mask any effect due to irradiation.

Before the crop matured heavy frost occurred, which prevented proper development of the seed, and consequently it could not be determined whether the observed effects were due to irradiation or to frost. Therefore it was not possible to obtain data on possible effects such as induced sterility.

In 1950 the season was again very dry. The dry weather retarded growth and heavy rains late in the season again caused excessive second growth. But as no frost occurred an attempt was made to collect data on induced sterility. These data are given in Table 3.

Table 3

Effect of soaking, germination and r dosage
on fertility of X_1 heads of barley

Treatment		Fertile florets %	Plants counted (4 heads/plant)
Check		93.7	18
4 hr. soak	15,000 r	83.4	20
Germinated	2,400 r	75.0	51
Germinated	3,000 r	57.5	48
Germinated	4,000 r	47.2	19

With increased dosage in the treatment of soaked or germinated seeds there are strong indications of decreased fertility.

It was also noted that a number of heads exhibited chimeral characteristics. Several heads showed high to complete sterility on one side. Unfortunately this condition was not observed until the counts were almost complete, so without doubt many such heads were missed.

Other chimeras noted were: a plant with striped leaves on one tiller; two plants having a branched rachis, resulting in a double head.

The X_2 Generation

Rate of induced mutation

Studies of X_2 plants were limited to determination of plant survival, rate of induced mutation, and relative frequency of mutant types.

Data on the effect of soaking on survival and mutation rates are given in Table 4. These data are compiled from both greenhouse and field-grown material. Results from X_2 seedlings grown in 1950 (greenhouse only) are given in Table 5. In this case both r dosage and period of soaking were varied.

Mutation rates shown are only apparent because:

1. Only four heads per plant were used, therefore some heads carrying mutated genes were, in all probability, not grown.
2. Viable mutants are difficult to pick out in the seedling stage and some were likely not classified.
3. A major difficulty encountered in determining mutations appeared when certain abnormalities occurred which might have been due to frost

Table 4

Effect of period of soaking, followed in each case by
15,000 r treatment, on survival and mutation rate
in the X_2 generation (1949)

Period of soaking	No. seeds sown	No. plants surviving	No. mutants	Mutation rate, %	
				No. seeds sown	No. plants
16	250	8	1	0.4	12.5
4.5	250	11	0	0	0
4	250	25	0	0	0
3.5	500	131	5	2.0	3.8
3	500	249	19	3.8	7.6
1	250	83	4	1.6	4.8
0	500	210	12	2.1	5.7

damage. In some cases abnormal leaves were diagnosed as symptoms of frost damage. In other cases very small white shoots which barely emerged occurred rather frequently. These were also attributed to frost damage, but a few may have been classified as mutations.

4. Germination was poor in the X_1 seed of 1949, and many seeds failed to grow. Consequently, in determining mutation rate, the assumption was made that the seeds carrying the mutated genes had the same probability of frost damage as the normal seed.

Table 5

Effect of varying r dosage and periods of soaking (carried in some cases to point of germination)
survival and mutation rate in the X₂ generation;
grown in the greenhouse (1950)

Treatment	No. seeds sown	No. plants surviving	No. plants tested	No. mutants	Mutation rate %	
					No. seeds sown	No. plants
Check	200	190	10	0	-	0
Dormant	20,000 r	50	11	1	-	10
4 hour soak	15,000 r	20	20	9	4.5	45
Germinated	3,000 r	40	36	5	1.2	14
Germinated	2,400 r	20	19	2	1	10
Germinated	2,000 r	210	23	8		34
Germinated	1,600 r	160	19	2		10

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Classification and relative frequency
of observed mutations

The types of mutations found, in order of relative frequency are:

1. Lethal mutations

A. Chlorophyll aberrants

i. Albinos

- a. pure white
- b. greenish midrib on leaf
- c. green tips

ii. Yellows

- a. pure yellow
- b. with a greenish tip

- iii. Dwarfs with small striped leaves. These mutants failed to develop seed.

2. Viable mutations

A. Chlorophyll aberrants

- i. Plants with striped leaves, later and smaller than the normals.

B. Non-chlorophyll mutants

- i. Plants with a lighter shade of green than the normals, with very short internodes, and ten to fourteen days later in maturing than the normal.
- ii. Notched leaf, found only in the seedling stage in the greenhouse.

The segregation ratio approached the 3:1 ratio, but as each individual row produced numbers of plants varying from three to thirty, observations on segregation ratios were deferred until the X_3 generation, which is discussed later.

Evaluation of r dosage as related to pre-soaking

An accurate evaluation is rather difficult because, among other limitations, the populations analyzed were rather small. A number of scheduled treatments had to be omitted when the X-ray apparatus failed at a critical point. This accounts, in part, for a number of treatments being missed.

In general, severity of treatment reduced germination and increased proportion of sterility in the heads. For dormant seeds, treatments approaching 20,000 r reduced survival to the point where too few plants are available in the X_1 for a study of mutation rate; while 15,000 r results in too large a population for convenient handling.

With pre-soaking and pre-germination, seeds become more susceptible to irradiation injury, survival rates are lowered and mutation rates per plant tend to increase.

The better treatments are considered to be: 1. 15,000 r with three to four hours soaking, or 2. about 2,000 r for germinated seeds. However, for practical purposes elimination of soaking and using dosages of 15,000 r or slightly greater can be expected to give fairly good results.

The X_3 Generation

Studies on this generation were confined, apart from a small amount of cytological work, to segregation among X_3 plants grown in the greenhouse. These plants were derived from X_2 head rows (field) that had shown mutations, i.e., heterozygous lines. In harvesting the X_2 rows each plant was kept separate.

Theoretically the X_2 rows showing mutations (presumably recessive) were mixtures of phenotypically normal homozygous and heterozygous types as well as the phenotypical mutant types. For example, a head row segregating in the field and producing albinos would be expected to include plants of genotypes GG, Gg and gg, the phenotypes being (homozygous) normal, (heterozygous) normal and albino respectively. The progeny of the heterozygote, Gg should segregate according to the 3:1 ratio. Theoretically the ratio of the homozygous to the heterozygous plants is 1:2.

As indicated in Table 6 the ratio of normal to mutants in the segregating lines was close to 3:1. Application of the chi-square test for goodness of fit gave X^2 equal to 0.30 indicating a very good fit to the assumed ratio. However, the proportion of homozygous to heterozygous approached a 1:1 ratio, rather than the expected 1:2. This discrepancy indicates that a deletion, rather than a point mutation may be involved.

The data, however, show that single recessive alleles at a single chromosome locus are operative in this case. Relating this fact to the concept of photon and target, the mutants are considered to be:

1. changes in single genes
2. small deficiencies.

This is supported by cytological evidence. Out of fifty heads examined at sporogenesis none showed any traces of chromosomal aberrations. This indicates that changes at single loci rather than gross chromosomal effects were produced by the X-irradiation treatment.

Attention should be drawn to instances of the occurrences of two mutations in a single plant. One of the heads from an X_1 plant grown

Table 6

Segregation in the X_3 generation, showing ratios of mutations, and homozygous to heterozygous plants, for 15,000 r dosage and varying periods of soaking

X_3 plot No.	Period of soaking (hrs.)	Type of mutant	No. normal plants	No. mutant plants	χ^2	No. homozygous plants	No. heterozygous plants
6004	0	yellow	279	105	0.20	7	6
6004	0	light green *	123	41	0.00	1	3
6005	0	albino	207	65	0.14	5	5
6006	0	light green*	539	199	1.51	12	11
6006	0	albino	238	64	2.33	16	4
6011	3	albino	152	67	3.65	10	6
6021	3	albino	214	64	0.38	8	10
6043	3	albino	28	10	0.03	0	2
6079	1	albino	315	109	0.61	13	9
6079	1	albino with green midrib	278	89	0.10	11	4
6081**	1	yellow	54	21	0.29	4	2
Totals			2447	834	0.30	77	62

* Viable mutants

** This plot also had one plant that produced 12 albinos, 3 albinos with green midrib and 35 normal plants.

in plot 6079 (Table 6) produced albinos, which segregated as 305 normal:109 albino and the other head segregated as 278 normal:89 albino with green midrib. Plot 6081 also provides an example of one X_2 plant that produced two mutations, the segregation being 12 albinos:3 albinos with a green midrib:35 normal. Such data show that more than one chromosome were involved in a few instances.

Cytological Studies

Root tip studies

Cytological studies were carried out on a limited amount of material in order to provide some indication of the observable chromosomal effect which might be produced by X-irradiation. Studies on root tips yielded very little information. They did show, however, the occurrence of fragmentation and bridges which were roughly proportional to dosage.

Considerable difficulty was encountered in getting root tips with dividing cells, particularly at the more severe dosages. A large number of root tips were examined and discarded because of the lack of dividing cells. In no case was it possible to get any pertinent data on X-ray damage to the plant as measured by chromosomal damage.

Sporogenesis studies

These studies were carried out on spore mother cells at sporogenesis. Examinations were made at metaphase I at a magnification of 440 diameters. Considerable difficulty was experienced in finding

material at the critical stage for examination. Inexperience in sampling was a contributing factor in this instance. Many of the spikes examined were too young but most of those discarded were too far advanced to obtain the proper stage of cell division.

With two exceptions all the X_1 heads examined appeared normal. The exceptions showed rings of 4 at diakinesis, indicating reciprocal translocations.

All the X_2 plants examined at the critical stage appeared normal, indicating that any mutations in the X_2 were likely due to a simple gene change or a small deletion, rather than to observable aberrations.

It is quite possible that a more experienced cytologist may have been able to detect small chromosomal aberrations that were not apparent to the writer. However, slides that were examined by more experienced persons were pronounced normal.

The writer feels that cytological examination can yield very little pertinent information in a study on mutations and mutation rates. A survey of the literature failed to reveal much evidence on the actual role of chromosomal aberrations, with the exceptions of reciprocal translocations and possibilities of deletions.

Aberrant root tip mitosis may be an indication of X-irradiation injury, but most of the injured cells are lost after a few divisions. If too many cells are too severely injured death of the plant results. There is some controversy in the literature regarding the effectiveness of any correlation between chromosomal injury and mutation rate (9, 10). As pointed out by Lein (18) most of the aberrations that survive in the mature plant fail to pass through meiosis, and sterility results.

However, it is difficult to correlate sterility with mutation rate because each spike comes from a different primordium of the seed. So that cytological examination of one spike cannot give any information on the sterility and/or mutations that may appear in the other spikes of the same plant.

The fact that mutations tend to arise more frequently in highly sterile plants indicates the greater probability of a primordium being hit at a critical point, and causing a chemical change in the gene or a deletion.

Experiments with simultaneous applications of sonic energy and X-rays indicate that more chromosomal injury becomes apparent when breaks are prevented from rejoining. Fragmentation and other chromosomal aberrations occur with greater frequency because the sonic energy prevents rejoining of the broken ends. Such information implies that actual breakage is greater than realized, because sonic energy by itself does not cause viable chromosomal injury. The full extent of the damage not being apparent indicated that a certain amount of rejoining has taken place. The rejoining, if fragmentation has taken place, may result in small deletions.

DISCUSSION

As indicated previously, several difficulties were encountered which detracted from the completeness of the study. Trouble with the X-ray apparatus forced abandonment of a number of scheduled treatments.

The extreme drought conditions of both 1949 and 1950 prevented normal development of the field grown material. Frost killing of immature seeds in 1949 was a further handicap. Lack of greenhouse facilities seriously limited the amount of X_2 and X_3 material grown.

An association of treatments with certain types of mutations was not apparent in this study, although most of the viable mutations produced appeared in the progenies of the irradiated dormant seeds.

There would appear to be inherent difficulties in applying X-irradiation techniques to plant breeding, as follows:

1. Difficulty in identifying small mutations, in particular, physiological types.
2. The large numbers of deleterious mutations that appear in proportion to the good ones. In this study about one viable mutant per twenty-five to thirty lethals was found.
3. Any viable mutant very likely carries with it several unfavorable traits along with the good. This pleiotropic effect may make the crossing and subsequent selection very difficult or impossible.

If, as Gustafsson (13) suggests, 700 to 800 mutations must be induced in order to get one good mutant, then the size of the nursery required will be excessive and require the services of a large number of trained workers. The production of a mutant of a desirable type would be a big step forward in plant breeding, but the cost of producing it may not be warranted.

Several modifications in technique could now be investigated since a new tube and new transformers have increased the output of the X-ray machine to 600 r per minute. This increased intensity may have a

greater effect per given dosage, in that the higher intensities may mean more chromosomal injury, (Catcheside, 4). For similar reasons fractionating the dosage may be an undesirable variable and experiments could be carried out to determine its effect.

Several criteria have been used to evaluate X-ray damage to seeds, such as chromosomal injury in root tips, survival and growth rates of X_1 plants, rate of induced sterility and of mutation in the X_2 . The criterion most easily used in large samples is the survival rate. A quarter value dosage, that is, one that can reduce the rate of survival to one-quarter of the number of treated seeds can be determined with relative ease. In such treatments the amount of treated seed can be fairly large, while the X_1 population can be kept rather small. According to Gustafsson (11) such treatments still leave enough surviving plants to give a good indication of the mutation rate, and a good probability of getting viable mutations.

Cytological examination of seed treated material is difficult to carry out on the large populations involved and data obtained cannot be correlated directly with mutations produced, since examination can be made only by killing the material involved. Sterility studies are also difficult to correlate directly because each head represents a different sector and partial sterility varies from head to head of the same plant.

All mutations noted were recessive and, as far as is known, deleterious. Most were lethal mutants, but some viable morphological types were found. It is felt that had the seasons been more favorable other mutations might have been found. According to Gustafsson (13)

the most common morphological mutant is the short erect "erectoid " type, but unfortunately, all of the barley studied was very short and very erect, so that the erectoid type, if it did appear, would not have been very obvious. However, as Gustafsson has pointed out, one morphological mutant for 20 albinos induced could be expected. In this study the proportion was less than he indicated.

The mutations found segregated in the 3:1 ratio, insofar as could be determined. It would appear, then, that a single gene was responsible for each mutant. However, in the case of the morphological ones found, one and perhaps more physiological characters were effected. The mutant type was much smaller, with shorter internodes and much later (about two weeks) in maturing than the normal plants. This indicates a pleiotropic effect of the gene.

CONCLUSION

Certain aspects of irradiation techniques show promise. Fairly severe treatments can be used to produce rather large numbers of mutations at a rate much above that of spontaneous mutations. The induction of mutations by artificial means does bring about a number of variations which can be used in genetic studies. The production of improved varieties through X-ray induced mutations shows some promise, but the cost of producing them may be too great to be of practical importance.

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1. The first of the two main parts of the book is devoted to a general survey of the history of the theory of the structure of the atom. It begins with the classical theory of the atom, which was based on the ideas of Rutherford and Bohr, and ends with the modern theory of the atom, which is based on the ideas of Heisenberg and Schrödinger.
2. The second part of the book is devoted to a detailed study of the structure of the atom. It begins with a study of the structure of the nucleus, which is based on the ideas of Rutherford and Bohr, and ends with a study of the structure of the electron cloud, which is based on the ideas of Heisenberg and Schrödinger.
3. The third part of the book is devoted to a study of the properties of the atom. It begins with a study of the properties of the nucleus, which is based on the ideas of Rutherford and Bohr, and ends with a study of the properties of the electron cloud, which is based on the ideas of Heisenberg and Schrödinger.
4. The fourth part of the book is devoted to a study of the applications of the theory of the structure of the atom. It begins with a study of the applications of the theory of the structure of the nucleus, which is based on the ideas of Rutherford and Bohr, and ends with a study of the applications of the theory of the structure of the electron cloud, which is based on the ideas of Heisenberg and Schrödinger.
5. The fifth part of the book is devoted to a study of the history of the theory of the structure of the atom. It begins with a study of the history of the theory of the structure of the nucleus, which is based on the ideas of Rutherford and Bohr, and ends with a study of the history of the theory of the structure of the electron cloud, which is based on the ideas of Heisenberg and Schrödinger.
6. The sixth part of the book is devoted to a study of the future of the theory of the structure of the atom. It begins with a study of the future of the theory of the structure of the nucleus, which is based on the ideas of Rutherford and Bohr, and ends with a study of the future of the theory of the structure of the electron cloud, which is based on the ideas of Heisenberg and Schrödinger.
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