MUTATIONS RESULTING FROM RADIATION
OF SORGHUM SEEDS BY GAMMA RAYS

By

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CHAPTER I

INTRODUCTION

Variability in the population of a material of agricultural significance is a necessary requirement for the successful application of breeding techniques to make desirable improvement in the material under study. Two methods of obtaining variability are from spontaneous mutations, which occur at a rather low frequency, and from mutations produced artificially by irradiation. The latter method has been found by many investigators to increase the mutation rate and provide variability although the variability may not be useful.

The purpose of the study reported herein, was to determine the number and types of mutations found after exposure of dormant seeds to three different dosages of gamma rays.

CHAPTER II

REVIEW OF LITERATURE

Since H. J. Muller demonstrated in 1927 that the exposure of germ cells to X-rays results in changes in hereditary material, the biological world has shown immense interest. (Purdom, 1963). Muller further stated that the explosion of the atomic bomb and the dispersion of radioactive debris gave further acceleration to this phenomenon. Singleton (1955) pointed out that X-rays were used to produce hereditary changes in a living organism, Drosophila melanogaster, a number of years before the researches of Muller. According to Singleton (1955), the research was conducted and the results published by James Watt Mavor in 1920, while working at Union College in Schenectady, New York.

Smith (1958) listed the four evolutionary forces that influence gene frequency. They are mutation, gene migration, selection, and chance. Since spontaneous mutations occur, mutation and selection are the two forces which influence variability in a population. By using irradiation, man has attempted to increase the mutation rate which occurs naturally at low frequencies. It is usually considered impractical to await the appearance of desirable mutations. "Radiation breeding" is used in an attempt to induce desirable hereditary changes. According to Smith (1958), once the variability has been induced by radiation the techniques of selection and testing are those of the conventional type.

Singleton (1955) pointed out that Dr. H. Nilsson-Ehle and Dr. A. Gaustafsson were the first to make practical applications of radiation induced mutants in plant breeding. Their work was conducted during the mid 1930's. They found and maintained many mutants of barley that had stiffer straw and some strains that were higher yielding than the untreated parental strain. Smith (1958) draws attention to work by Horlacher and Killough in 1933 in which they stated, "Mutations which are progressive have been produced in cotton by X-ray treatment of dry seeds." The mutations they had reference to were (1) a mutation from forked leaf shape to normal leaf shape and (2) a mutation from virescent yellow leaf and plant color to normal green leaf and plant color. The rate of these mutations was less than one percent in each case.

Most of the early work in "radiation breeding" employed either X-rays or neutrons. When a particle is ejected from a radioactive nucleus, it may not carry away all the excess energy which was the cause of the nuclear instability. (Purdom, 1963). The remaining energy is emitted at that time or later as electromagnetic radiation. This type of irradiation is known as gamma radiation. According to Purdom (1963), X-rays are the same kind of irradiation as gamma radiation. The only difference between the two is the source from which they are derived. Most mutations produced by radiation are of a deleterious nature, but the same can be said of spontaneous mutants. (Singleton, 1955). He pointed out that there is a vast difference between 100% harmful genes and 99.99%. Gustafsson, as quoted by Singleton (1955), estimated that only one out of one thousand mutations may be useful for plant breeding purposes. Nevertheless, the appearance of one desirable mutation among many deleterious mutations has paid very well in plant breeding.

The materials employed to incite reactions in the production of mutations may be mechanical forces, chemical processes, or combinations of the two extremes. (MacKey, 1956). Further, since the mutating in a limited material generally is concentrated to one type of mutation process, a more or less pronounced grouping will be observed in the frequency of visible mutations when compared to the other type. MacKey (1956) further stated that the mutation spectrum may be more restricted with spontaneous than with artificial mutagenesis, or the reverse may be true. There is no proof that either type should be more restricted. Point mutations, deficiencies, inversions, translocations, and duplications may occur as induced mutations.

Results obtained by Gustafsson and Nybom (1949) using five different colchicine solutions to initiate germination before treatment of the seeds with X-rays were those listed below. First, the mutation process was considerably altered by the application of colchicine previous to irradiation. Second, the xantha and alboveridis types were increased in number by the treatment. Third, viridis types became rarer, and fourth, the chlorophyll mutation types showed a negative correlation to the percent sterility of the R_1 plants, i. e., as sterility increased the number of mutations decreased.

Solutions of colchicine, potassium cyanide, hydrogen peroxide, butter yellow, uranyl nitrate, ferric sulphate, and distilled water plus two buffer solutions with a pH of 3 and a pH of 10 were used to germinate barley seeds prior to treatment with X-rays. (D'Amato and Gustafsson, 1948). They found that almost every chemical pre-treatment increased the mutation rate. An exception was a strong concentration of potassium cyanide which gave a lower mutation frequency than the control series. They also reported that colchicine in low concentrations did not increase the mutation rate, but it did widen the range of mutant types.

Ehrenberg (1955) irradiated barley seeds with X-rays and fast neutrons equilibrated with air to different relative humidities. He found that lethality, sterility, and chromosomal disturbances caused by X-ray irradiation of barley seeds decreased with increasing water content of the seeds, at the moment of irradiation, in the range of 8 to 18% moisture. When the mutation rate was studied, it was found to show a parallel dependence on moisture only at low contents. Fast neutrons were not dependent upon seed moisture for their effectiveness to produce mutations. When the X-ray irradiated seeds were germinated at a low temperature higher lethality was observed.

MacKey (1951) treated both dry and pre-soaked seeds of barley with X-rays and fast neutrons. The germination of dry, normal seeds was normal even after the application of the heaviest dose. However, initial seedling growth was retarded. The fraction of survival plants was found to be approximately equal in X_1 and N_1 doses. X_n and N_n are used to refer to the first or second generation after X-ray and neutron treatment, respectively. Pre-soaking of the seeds was found to increase the lethality of plants in the N_1 and X_1.

The types and frequencies of mutations were found by MacKey (1951) to be the same in the X_2 and N_2. He also stated that no correlation could be found between degrees of sterility in the X_1 and N_1 and different categories of chlorophyll mutation in the X_2 and N_2.

When germinated at $5^{o}C$ as compared to $20^{o}C$ resting seeds of barley, after irradiation by X-ray, were found to have a higher rate of mutation and greater sterility. (Ehrenberg and Lundqvist, 1957). They think that the results make it seem probable that the effect of temperature on X-ray-induced rate of point mutation involves a modification of primary injury. This should not be explained by the elimination of mutated cells at the higher temperature.

Some results obtained after treatment of sorghum seeds with X-ray and thermal neutron irradiation were: (1) height of seedlings from treated seeds was reduced with increasing dosage, (2) mature plants from treated seeds were depressed in stature, (3) plants from treated seeds were delayed in anthesis, (4) the average sterility of plants from treated seeds ranged from 12.5 to 75.8% from the lowest to the highest dosage, respectively, (5) the averages of the number of bridges per cell were 0.2 and 1.53, and (6) frequencies of seedling mutants ranged from 2.7 to 7.3 from the lowest to the highest dosage, respectively. (Kaukis and Webster, 1956).

Atomic bomb, X-ray-induced, and spontaneous seedling mutants in barley and durum wheat were studied by Moh and Smith (1951). The differences between mutants from the three sources were not large enough to be of any apparent biological importance. Six mutant types were described; all were chlorophyll characters. No distinct differences were found in the segregation ratios of the six types of mutants described. It was also found in the study that more than 20 percent of the mutants tested in the X_3 and X_4 segregated in ratios significantly different from the theoretical 3:1. Moh and Smith (1951) concluded that chromosomal aberrations associated with a portion of the mutations, or pleiotropic effects of the mutant genes, best accounted for the fact that over 20 percent of the mutants segregated in ratios that deviated significantly from the theoretical 3:1.

After irradiating barley with X-rays, Muntzing (1942) found several chlorophyll mutations: albino, xantha, alboviridis, viridis, and tigrina. Three doses, 5,000, 10,000, and 15,000r, were applied to the material. At 5,000r only albino mutations were found. With the increase in dosage to 10,000 and 15,000r, the entire range of chlorophyll mutations described above was found. The mutation rate was found to increase as dosage was increased from 5,000 to 10,000r, but not from 10,000 to 15,000r. The reason advanced for this was that the higher degree of sterility in the X_1 prevented the expression of all the mutations actually produced.

From irradiated seeds of the Huron variety of oats several agronomically desirable mutant strains were isolated by Frey (1955). Mutations that were desirable were ones for earliness, ones that were shorter in plant height, and those types having lower lodging percentages. Several of the mutant lines also yielded better than Huron, and were superior in test weight.

Thermal neutrons were applied to the Mohawk CI 4327 variety of oats. (Konzak, 1954). All except 74 plants in 49 progenies became heavily infected with oat stem rust, Puccinia graminis avenae race 7a, after artificially introducing it into the field. When grown in the greenhouse, progeny from these plants have shown that the stem rust resistance is also present in the seedling stage. Several other mutations were also observed which include virescent, virido-luteus, yellow green, and banded leaf. Mutations for short, tall, dwarf, fatuoids, awned, and black seeded types were observed.

Gregory (1956) reported on results obtained from selecting normal appearing plants in X-ray irradiated peanuts. Accomplishments he listed were: (1) improved yields of fruits in induced mutants, (2) stabilization of the yield character in later generations, (3) the occurrence of heterosis among hybrids of mutant sibs of the same inbreeding variety, (4) the grade variability of the same mutant from different families, and (5) the reconstruction of normal or near normal phenotype by re-irradiation of extreme mutants. Gregory (1955) found that the total genetic variance among randomly chosen irradiated normals was four times that measured in control progenies.

When different lots of dormant seeds of sorghum were treated with X-radiation, Quinby and Karper (1942) found nine mature plant characters which had not been reported previously. The characters, dwarf, freckled leaf, zebra stripe, midget, yellow leaf tip, fired leaf, mottled leaf, and two red leaf, were found to be inherited as simple recessives. No desirable mutations were reported.

Tomato seeds from two varieties were X-ray irradiated and a screening system employed to detect mutations which increased earliness and plant size. (Mertens and Burdick, 1957). They produced two lines that were earlier than the control lines from which they were taken. The R_1 plants were backcrossed to controls and earliness traits were evaluated. The results indicated that similar mutations might be induced and recovered if the methods which were outlined in the report were followed.

Seven mutants selected from Maja barley were analyzed with regard to yield production under different year conditions and with varying amounts of manure. (Gustafsson and Nybom, 1950). The morphological types of the mutants were erectoides 12, 13, 16, and bright green 2. The physiological types were "strawstiff early", "seeds differently colored", and "broad-leafed, late". It was found that when the environmental conditions favored the mother strain the yield of the mutants was low, and when the reverse conditions were encountered the mutants produced greater yields than did the mother strain. The mutants were not as responsive to increases in manure as was the mother strain.

Hundreds of recessive mutations have been detected in various crops. Stadler (1944) used X-ray treatment to try to induce a mutation of a to A in maize endosperm cells. The symbol A is an aleurone character that allows the expression of color in the grain of corn; the allele inhibits the expression of seed color. The treatment to which these cells were exposed produced in the adjoining colored seeds approximately 100,000 losses of A. Since the number of A's tested for frequency of A-loss was only one third the number of a's tested for frequency of dominant mutations, the experiment showed a failure of induced dominant mutation in a trial on a scale sufficient to produce about 300,000 A-losses. The populations used were capable of yielding about 900,000 losses of A by deficiency or by mutation to a colorless allele. No change of a to A was found.

Kaukis and Reitz (1955) treated air-dry dormant seeds of sorghum with thermal neutrons and X-rays. Unthreshed X_1 heads were then planted in greenhouse sand benches, and the location of mutants on the panicles segregating for mutant characters was carefully noted. The purpose of the study was to determine whether a single or several cells of the plumular apical meristem of the dormant embryo could be identified as panicle primordia. From the results obtained it was decided that the tissues associated with sorghum panicles commonly are derived from more than one cell in the dormant embryo.

Work by Mertens and Burdick (1957), lends support to the above finding. In their work, they noticed a high frequency of chlorophyll sectoring in the first true leaf of R_1 tomato seedlings, less in the second leaf, and none in the other leaves. Also they found recessive mutants in one inflorescence on a plant, but not from others on the same plant. The conclusion that Mertens and Burdick (1957) reached is that there is competition among the progenies of individual irradiated cells. The cells forming flower tissue survive a selection process that favors a more rapid growth rate. The mutations that have a slightly beneficial effect on growth rate in the heterozygous condition must stand a good chance of being included in gametes of the R_1 plant.

Smith (1958) stated that since it is possible to produce desirable mutations, that is, variability by irradiation, the question of using the method in agriculture becomes largely one of economics.

Harlan (1956) stated that certain crops grown in the United States are all introductions from some other part of the world and that these crops are composed of such an enormous array of variants that it is impossible to introduce more than a sample of the array. He further stated that plant exploration is a very inexpensive method for obtaining useful and desirable variants. As an example of this, he cited work done in melon and cotton improvement by predetermining the desirable character needed and then searching through the world collection until the desired character was found.

CHAPTER III

MATERIALS AND METHODS

Irradiation Procedure:

Two varieties of *Sorghum vulgare* Pers. were chosen for irradiation treatment during the spring of 1963. Four lots of each of the two varieties, Wheatland and Combine Kafir-60, were sent to the Agricultural Research Laboratory, Oak Ridge, Tennessee. Prior to treatment with gamma rays the seeds were equilibrated in a relative humidity of 70%. This generally takes two weeks. The seeds were then packaged in polyethylene, sealed and irradiated on June 4, 1963. The source of gamma rays was cobalt-60. Each variety was given three different doses; 15,000r, 30,000r, and 45,000r. The remaining lot from each variety was used as the control in the experiment.

Field Procedure of R_1 and C_1

Immediately after receiving the seeds from the Agricultural Research Laboratory, Oak Ridge, Tennessee, samples from each treatment were germinated to determine whether or not allowances for field planting should be made for irradiation damage. The three irradiation treatments and the untreated control of each variety were replicated six times making a total of twenty-four rows of each variety. The planting was made on June 13, 1963, at the Oklahoma Agricultural Experiment Station, Perkins, Oklahoma. The plots were given the same cultural treatments as other sorghum material. Supplemental irrigation was provided as needed to help insure maximum plant growth and seed set. At the time of flowering the heads of each plant were bagged to insure selfing. Observations were made from the time the seeds emerged until the plants were mature to detect any visible dominant mutations. Each R_1 and C_1 head was harvested at maturity and threshed.

Germinator Procedure:

The decision was made to germinate 300 seeds from each head in every treatment including the control in a Stults Day and Night Water Curtain Seed Germinator. One replicate for a variety was brought into the laboratory and a sample from every head was germinated until all replicates, except those from which samples were drawn for the field planting, were observed. The purpose of this part of the study was to seek the kinds and frequencies of mutations as revealed in R_2 seedlings. Germinator studies were begun February 4, 1964. Samples, after being treated with captan, N-trichloromethylmercapto-4-cycolhexene-1,2-dicarboximide, were germinated at alternating conditions of light and temperature. For twelve hours the lights were off and the temperature was 68^{o}F. During the remaining twelve hours, the lights were on and the temperature was 86^{o}F. These temperatures were the same as those recommended by the Agriculture Handbook No. 30 (Washington, D. C., 1952). The practice of germinating 300 seeds from each head was employed until November 19, 1964, at which time it was decided to decrease the number of seeds tested per R_1 head to 100 seeds. This was done because a rather large amount of R_1 material was yet to be tested for mutations and time was becoming limited. No heads were tested in the germinator from which samples were taken to be planted in the field the summer of 1964.

Field Procedure:

To detect and observe mutations affecting either/or both seedlings and mature plants, 300 seeds were drawn from thirty heads of each treatment per variety. Two replications were planted at the Oklahoma Agricultural Experiment Station, Perkins, Oklahoma; 150 seeds from each head were planted in each replicate. The planting was made June 10, 1964. As soon as the plants were emerged, the types and frequencies of seedling mutations were recorded. Through-out the growing season the plants were observed for the expression of mutant characters. As these appeared, the number found and a description for each mutant type was recorded. Mature plants expressing mutations were selfed for further studies to determine the number of genes involved and the type of gene action. Plants from rows segregating for seedling mutations were selfed for the same purpose.

CHAPTER IV

RESULTS AND DISCUSSION

Plants classified as mutant types were those plants that were visibly different from the normal or control plants. Mutant types so classified and reported included those expressing chlorophyll characters, differences in plant height, colorless coleoptile, sterility, late maturity, and leaf characteristics. R_1 and R_2 are used to denote the first and second generations after gamma ray treatment respectively. Similarly C_1 and C_2 are used to denote the first and second generation plants of the controls used during the course of study.

Results of R_1

The data shown in Table I indicated that increasing the dosage of gamma rays did not have a deleterious effect upon seed germination. This is in agreement with data reported by MacKey (1951). The conclusion drawn from these data was that dosage did not affect the percent germination. Therefore, the same number of seeds from each treatment per variety was planted June 13, 1963.

From the time the R_1 and C_1 plants emerged until harvest, they were observed for possible dominant mutations. One plant was found that appeared to express a dominant mutation. Upon further examination, both in the germinator and in the field the following year, it was found to breed true. The mutant occurred in the Wheatland material that received 15,000r of gamma rays. It was yellow green in plant color and while in the milk and dough stages, the seed color was yellow. At maturity the seeds were of the same color, salmon, as the control treatment.

TABLE I

PER CENT GERMINATION OF SORGHUM SEEDS NEWLY IRRADIATED WITH GAMMA RAYS

Variety and Treatment	Total Seeds Tested	Total Seeds Germinated	Per Cent Germination
Wheatland			
Control	100	94	94.0
15,000r	100	91	91.0
30,000r	101	92	91.1
45,000r	94	88	93.6
Combine Kafir-60			
Control	100	95	95.0
15,000r	101	98	97.0
30,000r	101	94	93.0
45,000r	99	94	94.9

Germinator Study

The per cent germination and diseased seeds of R_1 and C_1 heads as observed in the germinator study are shown in Table II. Also included in this table are the total number of seeds germinated for each treatment. These data present evidence that the decrease in germination per cent was not due to irradiation. Rather the decrease in germination per cent of Wheatland at 45,000r and 15,000r was caused by the incidence of mold upon the seeds. Although the seeds were treated with a fungicide,

TABLE II

COMPARISON OF GERMINATED AND DISEASED TO THE

TOTAL NUMBER OF SEEDS TESTED IN THE GERMINATOR*

Treatment	Total Seeds Tested	Total Seeds Germinated	Total Seeds Diseased	Per Cent Germination	Per Cent Diseased Seeds
Wheatland					
Control	16,400	13,954	4,722	85.1	28.8
15,000r	16,850	14,043	5,840	83.3	34.6
30,000r	15,300	13,149	4,145	85.9	27.1
45,000r	6,500	5,243	2,331	80.6	35.8
Wheatland Total	55,050	46,389	17,038	84.3	31.0
Combine Kafir-60					
Control	6,950	6,028	1,231	86.7	17.7
15,000r	10,600	9,042	2,458	85.3	23.2
30,000r	8,500	7,174	1,945	84.4	22.9
45,000r	5,600	4,759	1,322	85.0	23.6
Combine Kafir-60 Total	31,650	27,003	6,956	85.3	22.0
Grand Total	86,700	73,382	23,994	84.6	27.6

*Represents 592 first generation plants; or 461 R_1 plants and 131 C_1 plants.

the material was not 100% effective.

Table III shows the number of seedling mutations found for each treatment of R_1 and C_1 plants for each variety in the germinator. These data do not indicate that increasing the dosage of gamma rays increased the mutation rate. However, when the data from the entire study was examined, it was found that for every increase in dosage of gamma rays there was also an increase in the number of mutations found.

TABLE III

NUMBER OF SEEDLING MUTATIONS IN R_2 AND C_2 PLANTS FROM GAMMA RAY IRRADIATED SEEDS OF SORGHUM AS OBSERVED IN THE GERMINATOR*

	Treatment			
Variety	Control	15,000r	30,000r	45,000r
Wheatland	0	3	7	3
Combine Kafir-60	0	5	3	5
Total	0	8	10	8

*Total Mutations found in 461 R_1 and 131 C_1 heads.

The mutation rate as determined from observation of R_2 material in the germinator was found to be 5.64%. The different mutant types found in each variety as observed in the germinator are shown in Table IV. The mutations were divided into the four classes for convenience. Albino, yellow green, and yellow mutations accounted for 19 of the 26 mutations found in the germinator study. The class "other" includes such mutations as dwarfs, white coleoptile, banded leaves, and one for short seedlings.

Table V shows the types of mutants found in each treatment. These data show that the different mutant types may be expected in any gamma irradiation treatment from 15,000r to 45,000r.

TABLE IV

NUMBER OF DIFFERENT MUTANT TYPES PER VARIETY

FOUND IN R_1 AND C_1 PLANTS AS OBSERVED IN THE GERMINATOR*

	Mutant Types			
Variety	Albino	Yellow Green	Yellow	Others
Wheatland	3	3	3	4
Combine Kafir-60	7	1	2	3
Total	10	4	5	7

*Total Mutations found in 461 R_1 and 131 C_1 heads.

TABLE V

NUMBER OF MUTANT TYPES PER

TREATMENT FOUND IN THE GERMINATOR*

	Treatment			
Mutant Type	Control	15,000r	30,000r	45,000r
Albino	0	3	5	2
Yellow Green	0	1	2	2
Yellows	0	1	1	2
Others	0	3	2	2

*Total Mutations found in 461 R_1 and 131 C_1 heads.

Field Results

The purpose for this phase of the study was two fold: (1) to observe detectable seedling mutations, and (2) to observe detectable mature plant mutations. The number of mutations found in the R_2 plants is found in Table VII. Both seedling and mature plant mutations are included.

In Table VI is presented the field germination per cent by variety and treatment. These data indicate that an increase in irradiation dosage decreased germination in the Combine Kafir-60 variety. In the case of Wheatland there appeared to be no affect of dosage upon germination.

These data support the theory that an increase in irradiation dosage results in an increase in mutation rate. This was true for both varieties. The mutation rate found for the field material was 6.66% for the untreated control and 17.22% for the treated material. The mutations observed in the control were unexpected.

The types and distribution of mutations observed under field conditions by variety and treatment are presented in Table VIII. As observed in the data from the germinator study, the majority of mutations were those involving chlorophyll characters.

Table IX gives the overall number and frequency of mutations per treatment. R_1 progeny of 510 gamma irradiated seeds expressed 57 mutations for an irradiation induced frequency of 11.17%. The chlorophyll mutations, albino, yellow green, and yellow, represented the majority of the mutations produced, i. e. 76.68% of the total number. Of the 57 mutations observed in the treated material 15 or 26.32% of

TABLE VI

PER CENT GERMINATION OF FIELD MATERIAL

BY VARIETY AND TREATMENT

Variety and Treatment	Total Seeds Planted	Total Seeds Germinated	Germination Per Cent
Wheatland			
Control	9,000	6,975	77.5
15,000r	9,000	6,838	76.0
30,000r	9,000	6,785	75.4
45,000r	9,000	7,115	79.1
Total	36,000	27,717	77.0
Combine Kafir-60			
Control	9,000	7,803	86.7
15,000r	9,000	7,425	82.5
30,000r	9,000	7,062	78.4
45,000r	9,000	6,879	76.4
Total	36,000	29,169	81.0
Overall Total	72,000	56,886	79.0

TABLE VII

NUMBER OF SEEDLING AND MATURE PLANT MUTATIONS

OBSERVED IN R_2 AND C_2 PLANTS UNDER FIELD CONDITIONS*

	Treatment			
Variety	Control	15,000r	30,000r	45,000r
Wheatland	4	2	6	9
Combine Kafir-60	0	2	5	7
Total	4	4	11	16

*Includes progeny of 180 R_1 and 60 C_1 plants.

TABLE VIII

DISTRIBUTION OF MUTANT TYPES AS OBSERVED

UNDER FIELD CONDITIONS*

Variety and Treatment	MUTANT TYPES			
	Albino	Yellow Green	Yellow	Other
Wheatland				
Control	1	2	0	1
15,000r	1	1	0	0
30,000r	2	3	1	0
45,000r	2	4	2	1
Combine Kafir-60				
Control	0	0	0	0
15,000r	0	1	0	1
30,000r	1	1	1	2
45,000r	3	0	0	4

*Includes 180 R_2 and 60 C_2 plants.

TABLE IX

OVERALL NUMBER AND FREQUENCY OF MUTATIONS

PER TREATMENT

Treatment	Number of R_1 Seeds	Number of Mutations	Mutation Rate %
Control	191	4	2.09
15,000r	211	12	5.68
30,000r	183	21	11.47
45,000r	116	24	20.69
Total	701	61	

the total number of mutations from treated seeds were classified as "other" mutant types.

Table X presents the number of R_1 and C_1 heads which had an insufficient number of seeds to provide samples either for the germinator or field study. Heads of R_1 and C_1 plants not having a sufficient number of seeds to provide a 300 seed germinator sample were omitted. These data definitely indicate that increased dosages of gamma rays decreased the fertility of the R_1 plants. This was especially true for the Wheatland variety. The sterility was probably due to an increase in the number of translocations, deletions, etc. which are known to affect fertility.

Discussion

One plant was observed in the R_1 generation to express what was thought to be a dominant mutation. During the summer of 1964 a

TABLE X

R_1 AND C_1 HEADS WITH TOO FEW SEEDS
TO TAKE SAMPLES

	Treatment			
Variety	Control	15,000r	30,000r	45,000r
Wheatland	3	6	14	38
Combine Kafir-60	0	0	7	23

Wheatland control was found to be segregating for the same type of mutation as described above. This presents evidence that the mutation is a recessive one. Two explanations for the appearance of the mutant character in the R_1 generation are suggested. First, the plant from which seeds were drawn for treatment was heterozygous for the character. Therefore, in the next generation both homozygous recessives and heterozygous individuals would be expected to appear. The plant could be a mutant homozygous for a spontaneous mutation. Second, instead of being a homozygous recessive spontaneous mutation, the seed which produced this plant may have been heterozygous for the character. At the time of treatment with gamma rays the dominant allele could have mutated to the recessive, thus allowing the expression of a mutation the first generation.

Chlorophyll mutations represented the majority of mutations found both in the germinator and the field. The chlorophyll mutations were divided into three types, albino, yellow green, and yellow mutations. These types need no description. A description of the seedling mutations classified as "other" are as follows: (1) three for colorless coleoptile, (2) one which had numerous light green spots on the first true leaves, and (3) three causing shorter plant height. Plants of one of the mutations in the last group were transplanted to the greenhouse for further observation. It appears to be a dwarf with a prostrate habit of growth.

No evidence was found in the germinator study to indicate that the different dosages had any effect on the germination of the R_2 and C_2 seeds. However, there was evidence that an increase in the incidence of mold on the seeds decreased the per cent of germination. Mold on the seedlings was due to the favorable environmental conditions which resulted when the heads were bagged. The environmental conditions would include high humidity, darkness, and an adequate food source. The degree of damage due to mold could not be determined in the field germination study. Evidence from the field germination data indicated that increasing dosage of gamma rays in the Wheatland variety did not result in decreased germination as it did in Combine Kafir-60.

In the field study, as in the germinator study, the majority of the mutations found were those involving chlorophyll characters. Two of the mutations classified as "other" were actually ones involving chlorophyll and were expressed only in the seedling stage. The seedlings of both were gray green. And both of these mutations were found in Combine Kafir-60 treated with 45,000r. One of the mutations was lethal while the other was viable.

The remaining types of mutations classified as "other" are presented below. Two mutations were found involving plant height one of which was taller than the normal plants and the other shorter than the normal plants; one for sterility; two which delayed flowering by about one month; and two which involved both plant height and leaf characteristics. One of the mutations involving height was observed in a Wheatland control. The mutant plants were about one-third normal height. The other mutation involving plant height was found in Combine Kafir-60 material that received 45,000r. The mutant plants were on the average 49 centimeters taller than the average of the pure line Combine Kafir-60 for the same year. Both mutations which delayed flowering were found in Combine Kafir-60 material. One was found in material that received 15,000r and the other from material that received 45,000r.

The mutations that involved both plant height and leaf characteristics were found expressed in the progenies of two Combine Kafir-60 R_1 seeds. The mutants had stiff, erect leaves. All plants exhibiting this type of leaves, were reduced in height, but some never got over 6 inches high while the others were about 2/3 normal height. Since these plants were found in the R_2 generation, the first segregating generation after gamma ray treatment, the short ones may be homozygous recessives and the taller ones heterozygous for the mutant character. However, it must be kept in mind that only a portion of the inflorescence may have been segregating for the mutant character, which would explain the relatively low ratios obtained.

The mutation rate for 510 treated seeds, R_1, was found to be 11.17%. An increase in dosage of gamma rays resulted in an increase in per cent of mutations. The four mutations found in the field in the C_2 generation were not expected. They were found among the progeny of 191 control seeds of the C_1 generation and the resulting spontaneous mutation rate for this experiment was 2.09%. This is certainly higher than the present accepted spontaneous mutation rate of 1 out of 1,000,000. The only explanation is that this ratio was the result of chance.

For a more complete understanding of the results of gamma rays and the increase of irradiation dosage, more study of this material is needed. This is especially needed to determine the type of damage caused by the gamma rays and to determine segregation ratios of the various mutant types.

CHAPTER V

SUMMARY AND CONCLUSIONS

Seeds of the two varieties of Sorghum vulgare Pers., Wheatland and Combine Kafir-60, were irradiated at the Agricultural Research Laboratory, Oak Ridge, Tennessee. The treatments each variety received were 15,000r, 30,000r, and 45,000r of gamma rays and an untreated control. Observations of the R_1 material revealed one plant that expressed a mutant character, yellow green plant color. Further examination of the progeny of the plant proved that the character was fixed and the plants bred true. Two explanations have been advanced earlier to account for the occurrence of a mutation the first generation after gamma ray treatment.

Germinator and field experiments of the R_1 progeny revealed that the most frequent mutation types were those involving chlorophyll characters, albino, yellow green, and yellows. Mutation types classified as others include (1) three involving plant height, (2) one for plant sterility, (3) two for maturity, and (4) two involving plant height and leaf characteristics. Of the three mutations involving plant height, one resulted in plants about two-thirds the normal height. One of the others, involving Combine Kafir-60 that received 45,000r caused the mutants to be 49 centimeters taller than the average of the pure line Combine Kafir-60 for the same year. The other mutation affecting plant height was found in the germinator in Combine Kafir-60 that received 15,000r gamma rays. The mutants appeared to be dwarfs. Observations of these plants in the greenhouse revealed that they have a prostrate habit of growth.

Both mutations affecting plant maturity were found in Combine Kafir-60 lines that received 45,000r. The mutant plants were approximately one month later to flower than the normal plants. Mutations involving plant height and plant characteristics were found in Combine Kafir-60 lines that received 30,000r of gamma rays. The leaves were stiff, thicker than normal, and erect. Some of the mutants were only six inches high at maturity, while others were about two-thirds normal height.

Frequencies of mutations found in gamma ray irradiated sorghum were (1) 5.68% for the 15,000r treatment, (2) 11.47% for the 30,000r treatment, and (3) 20.69% for the 45,000r gamma rays. The study involved 510 irradiated seeds. Studies of the R_2 generation revealed an overall mutation rate of 11.17% in irradiated sorghum. Four mutants were found in the progenies of 191 control lines. This resulted in a 2.09% mutation frequency for untreated sorghum in the study.

Data presented definitely indicated that as irradiation dosage was increased the mutation frequency is also increased. Further study is needed with this material to determine the kind of chromosomal change that results as a result of gamma ray irradiation.

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