Comparison of Effects of X-Ray and Thermal Neutrons on Dormant Seeds of Barley, Maize, Mustard, and Safflower

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COMPARISON OF EFFECTS OF X-RAYS AND THERMAL NEUTRONS ON DORMANT SEEDS OF BARLEY, MAIZE, MUSTARD, AND SAFFLOWER[1]

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COMPARISONS of the biological effects of X-rays and thermal neutrons on dormant seeds of barley have been made by seedling height studies, root tip cytological analyses, and cytological studies of pollen mother cells in the first generation following irradiation and by seedling mutation studies the second generation after irradiation. The results of these studies have been reported by CALDECOTT, FROLIK, and MORRIS (1952) and CALDECOTT, BEARD, and GARDNER (1954). The objective of the research reported in this paper was to extend the comparison of these two types of radiation by studying their effects on dormant seeds of four widely different crop species: barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), mustard (*Brassica juncea* Coss), and safflower (*Carthamus tinctorius* L.).

That survival or death of individual cells in an irradiated organism is dependent solely upon the nature and extent of the induced chromosomal changes has been postulated by various workers (SAX 1942; LEA 1947). The behavior of these individual cells is in turn thought to be reflected in the growth and survival to maturity of plants grown from irradiated seeds. In a study such as the one reported here, the use of four species which vary greatly in sensitivity to radiations as well as in many other characteristics should indicate whether or not the large differences in sensitivity are due to an inherent resistance of the chromosomes to radiation damage or to some other factor(s).

MATERIALS AND METHODS

Seeds of barley (var. Himalaya), maize (Single cross L289 × I205), mustard (var. Zellar's), and safflower (var. Nebraska 852) were irradiated with X-rays and thermal neutrons at the Brookhaven National Laboratory using the procedure described by CALDECOTT *et al.* (1954). Ten 500-seed samples of each species were subjected to irradiation and an eleventh 500-seed sample served as the non-treated control. The irradiation treatments consisted of five levels of

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X-rays and five of thermal neutrons for each species, with one 500-seed sample being used per treatment. As indicated in Tables 1, 2, 3, and 4, different dosage levels were used for the four species. The dose ranges were based upon preliminary experiments in which plant height was used as an indication of the relative sensitivities of the species studied.

Plant height and survival studies

For each species, 100 seeds from each of the 11 lots were planted in greenhouse flats. Seeds were spaced approximately one inch apart in rows spaced three inches apart. Determinations of plant height and survival were made at two or more times after planting for barley, maize, and mustard. For safflower, determinations of height and survival were recorded at only one age. All plant height measurements were made by holding the leaves erect and measuring the highest point of the highest leaf.

Pollen studies

Samples consisting of 250 seeds from each treatment of maize and mustard were planted in the field at Lincoln, Nebraska, in the spring of 1953. Samples consisting of 150 seeds from each treatment of barley and safflower were planted at each of two locations, Scottsbluff, Nebraska, and Fort Collins, Colorado (also in the spring of 1953). Pollen samples were collected at a stage when the pollen was stainable but before the anthers had dehisced, one sample being taken from each plant in each treatment. A 2- to 3-inch portion of the central spike of the tassel was collected from each maize plant, a single spike from each barley plant, a cluster of flowers from each mustard plant, and a single head (inflorescence) from each safflower plant. There was ample material in each individual sample for several separate determinations of pollen abnormality. Fresh samples were killed in Carnoy's solution (six parts 95 percent ethyl alcohol, three parts chloroform, and one part acetic acid) and stored at 0° F until analyzed.

The method used to determine percentage of normal pollen was similar for all species. Pollen was stripped from an anther in a drop of acetocarmine, debris was removed, and the drop was stirred to obtain an even distribution of normal and abnormal pollen grains. A cover slip was placed on the slide and counting was done with the aid of a microscope and a Micam camera with the ground glass screen in position. The 10× objective lens was used for all observations. The 25× ocular lens was used for observations of mustard and safflower pollen, and 10× and 15× oculars were used for corn and barley, respectively. Pollen grains which stained uniformly dark throughout were counted as normal, unless they were extremely small for the species concerned. All other grains, and the small pollen grains, were classified as abnormal.

A preliminary survey was made to determine the most efficient method of estimating the mean percentage of normal pollen from maize plants in the various treatments. The procedure was similar to that used by PITTENGER (1951). For each treatment in the study, ten plants, three flowers from each plant, and two anthers from each flower, all chosen at random, were used. After pollen from

each individual anther had been evenly distributed on a slide, counts of normal and abnormal pollen were made on three strips across the slide. Analysis of variance technique was then used to estimate the various components of variation contributing to "error" in estimating treatment means. The component of variation resulting from differences among plants was found to be the most important single factor in determining the variance of a treatment mean. The components of variance attributable to differences among flowers from the same plant, among anthers from the same flower, and among strips counted for the same anther were relatively small in most treatments. Hence the most efficient method of sampling to estimate the percentage of normal pollen for each treatment was to sample several plants per treatment using a single anther from each plant and counting a single strip on the slide. This also proved to be an efficient method of estimating the percentage of normal pollen for different treatments of barley, mustard, and safflower. Whenever possible, a sufficient number of plants was used to reduce the standard error of each estimate to approximately three percent.

RESULTS AND DISCUSSION

Results of the survival, height, and pollen studies are shown for barley, maize, mustard, and safflower in Tables 1, 2, 3, and 4, respectively. Correlation coefficients for the relationships between dose of X-rays or thermal neutrons and the various criteria of irradiation effect are presented in Table 5.

The preponderance of high correlation coefficients in Table 5 indicates that in the species tested, plant height, survival, and the frequency of normal pollen are, in most instances, reliable criteria of X-ray and thermal neutron effects. However, there are important exceptions. The need for care in interpreting plant height and

TABLE 1

Height, survival, and frequency of normal pollen in barley plants[1] from control and irradiated seeds

Treatment of seeds	Height (centimeters) Mean ± S.E.		Survival (plants/100 seeds)		Normal pollen (percentage) Mean ± S.E.
	11 days	32 days	11 days	32 days	
Control	18.5±0.18	65.1±0.67	98	98	78.3±1.5
X-rays					
8,000r	11.1±0.43	45.2±1.32	92	93	59.2±2.0
12,000r	7.4±0.61	38.0±2.00	69	58	62.0±2.1
16,000r	5.7±0.71	35.9±1.29	52	35	60.0±2.6
24,000r	3.1±0.63	30.9±3.41	28	14	57.3±4.3
32,000r	2.0±0.39	23.2±3.23	45	18	45.5±6.7
Thermal neutrons					
0.32×10^{13} N_{th}/cm^2	15.2±0.25	57.6±0.70	100	100	55.9±2.2
0.51×10^{13} N_{th}/cm^2	13.0±0.24	49.9±0.87	100	100	48.2±2.5
1.01×10^{13} N_{th}/cm^2	5.5±0.18	29.3±0.96	100	96	35.7±2.2
1.78×10^{13} N_{th}/cm^2	1.7±0.08	7.3±0.55	91	32	all plants died
1.80×10^{13} N_{th}/cm^2	0.9±0.08	6.5±3.91	70	3	all plants died

[1] Plants were grown in the greenhouse for height and survival studies and in the field for pollen studies.

survival data is clearly shown in the results with X-irradiated maize. In this case even plants of the highest dosage levels had relatively good survival at 12 days after planting, but at 32 days many of the plants from seed receiving higher doses had died and a striking increase in absolute magnitude of the correlation coefficient resulted. Conversely, at 12 days a close relationship existed between dose of X-rays and plant height, but at 32 days this relationship was much less distinct.

TABLE 2

Height, survival, and frequency of normal pollen in maize plants[1] from control and irradiated seeds

Treatment of seeds	Height (centimeters) Mean±S.E. 12 days	Height (centimeters) Mean±S.E. 32 days	Survival (plants/100 seeds) 12 days	Survival (plants/100 seeds) 32 days	Normal pollen (percentage) Mean±S.E.
Control	29.7±0.41	74.9±0.93	99	99	95.9±0.3
X-rays					
4,000r	25.2±0.38	65.7±1.04	94	94	86.5±3.4
8,000r	19.0±0.43	54.9±1.12	93	93	67.6±2.8
12,000r	10.5±0.53	36.5±1.48	92	73	58.0±3.1
16,000r	5.3±0.44	28.6±2.10	87	39	62.3±4.2
24,000r	2.9±0.47	51.5±6.73	91	7	60.8±11.2
Thermal neutrons					
1.01×10^{13} N_{th}/cm^2	22.8±0.30	61.9±0.77	95	95	70.9±3.2
1.21×10^{13} N_{th}/cm^2	18.1±0.34	52.2±0.81	94	92	58.0±2.8
2.00×10^{13} N_{th}/cm^2	12.7±0.34	40.1±0.77	93	92	41.1±2.9
3.25×10^{13} N_{th}/cm^2	3.7±0.13	11.8±1.29	95	16	all plants died
3.26×10^{13} N_{th}/cm^2	3.0±0.10	12.2±4.86	95	5	all plants died

[1] Plants were grown in the greenhouse for height and survival studies and in the field for pollen studies.

TABLE 3

Height, survival, and frequency of normal pollen in mustard plants[1] from control and irradiated seeds

Treatment of seeds	Height (centimeters) Mean±S.E. 15 days	Height (centimeters) Mean±S.E. 32 days	Survival (plants/100 seeds) 15 days	Survival (plants/100 seeds) 32 days	Normal pollen (percentage) Mean±S.E.
Control	6.3±0.30	23.0±1.16	41	41	93.4±1.0
X-rays					
48,000r	4.4±0.26	14.4±0.97	49	45	84.8±2.6
64,000r	3.8±0.25	13.5±0.90	46	40	63.1±3.0
80,000r	3.1±0.27	11.5±0.58	31	23	58.7±3.6
120,000r	1.6±0.08	2.8±0.58	42	9	35.8±5.2
160,000r	1.3±0.07	0.7	37	1	24.8±10.3
Thermal neutrons					
3.81×10^{13} N_{th}/cm^2	5.9±0.27	19.8±0.75	48	48	78.9±2.4
4.97×10^{13} N_{th}/cm^2	5.8±0.29	19.0±0.83	45	45	60.2±2.8
7.17×10^{13} N_{th}/cm^2	5.5±0.15	16.0±0.49	59	57	58.4±3.1
8.91×10^{13} N_{th}/cm^2	4.7±0.22	13.6±0.78	47	47	49.0±3.5
10.70×10^{13} N_{th}/cm^2	3.4±0.19	10.0±0.62	42	42	22.8±3.2

[1] Plants were grown in the greenhouse for height and survival studies and in the field for pollen studies.

TABLE 4

Height, survival, and frequency of normal pollen in safflower plants[1] from control and irradiated seeds

Treatment of seeds	Height (centimeters) Mean ± S.E.	Survival (plants/100 seeds)	Normal pollen (percentage) Mean ± S.E.
	19 days	19 days	
Control	13.9±0.56	57	95.6±0.8
X-rays			
8,000r	11.1±0.83	35	82.6±3.0
12,000r	11.4±0.62	43	80.6±2.8
16,000r	8.9±0.84	24	76.8±3.2
24,000r	7.5±0.47	22	61.0±4.3
32,000r	6.0±0.54	9	56.5±5.4
Thermal neutrons			
1.78×10^{13} N_{th}/cm^2	9.9±0.65	50	53.2±4.0
1.80×10^{13} N_{th}/cm^2	11.0±0.59	53	48.9±4.0
2.57×10^{13} N_{th}/cm^2	8.0±0.67	34	39.9±5.5
3.81×10^{13} N_{th}/cm^2	4.5±0.44	37	23.0±5.5
4.97×10^{13} N_{th}/cm^2	2.3±0.29	16	12.9 (1 plant only)

[1] Plants were grown in the greenhouse for height and survival studies and in the field for pollen studies.

TABLE 5

Correlation coefficients[1] for the relationships between various criteria of irradiation effect and dose of X-rays or thermal neutrons applied to seeds of barley, maize, mustard, and safflower

Species	Age[2]	Dose and plant height X-rays	Dose and plant height Thermal neutrons	Dose and survival X-rays	Dose and survival Thermal neutrons	Dose and normal pollen X-rays	Dose and normal pollen Thermal neutrons
Barley	11	—0.936†	—0.984†	—0.880*	—0.730	—0.907*	*—0.960**
	32	—0.943†	—0.997†	—0.923†	—0.891*		
Maize	12	—0.964†	—0.995†	—0.769	—0.526	—0.831*	*—0.991†*
	32	—0.660	—0.995†	—0.958†	—0.895*		
Mustard	15	—0.980†	—0.899*	—0.343	—0.212	—0.976†	—0.961†
	32	—0.986†	—0.983†	—0.911*	—0.217		
Safflower	19	—0.980†	—0.991†	—0.943†	—0.921†	—0.987†	—0.970†

[1] Degrees of freedom: underlined values—2, all other values—4.
[2] Days from planting to measurements of height and survival.
* Significant at the five percent level of probability.
† Significant at the one percent level of probability.

This change in relationship between dose and height was related to the fact that usually plants damaged most by the high doses were alive and measurable at 12 days, but many of these plants had died prior to the 32-day measurements. Thus the most appropriate time for recording survival data may differ appreciably from

the most suitable time for taking measurements of plant height. The data indicate that for many of the seedlings from X-irradiated seeds of barley, maize, and mustard and from neutron treated barley and maize seeds death occurred during the third and fourth weeks after planting. Therefore, for these species it appears desirable to record height measurements during the second week after planting and survival measurements about one month after planting.

The pollen data are subject to errors of interpretation similar to those just mentioned for plant height and survival data. Thus, in the higher treatment levels on all species, survival at the time of pollen shedding was considerably reduced, and it is probable that the plants remaining were not truly representative of the treatments. Despite this obvious weakness of pollen analysis as a measure of irradiation effect, statistically significant correlation coefficients with high predictive value were obtained for the relationship between dose and effect for all four species and both types of radiation.

To facilitate comparisons among the four species, plant height and normal pollen data for each species were expressed as percentages of their respective control values. Linear regression coefficients were calculated and lines showing the relationship between normal pollen and plant height were then plotted for each species for the two types of radiation. The regression coefficients and their standard errors are presented in Table 6 and the regression lines are shown in Figure 1 (X-rays) and Figure 2 (thermal neutrons). It is apparent that for each of the species tested, the slope of the regression line is greater for the X-ray treatments than for the neutron treatments. That is, per unit of reduction in normal pollen, plant height was reduced more in the X-ray series than in the thermal neutron series. MacKey (1951) and Caldecott *et al.* (1952, 1954) postulated an "extrachromosomal" or "physiological" effect of X-rays on seeds of barley. This suggestion was based on the observation that plant height and survival were reduced less, relative to the number of chromosomal aberrations, in plants grown from seeds exposed to fast or thermal neutron radiation than in plants grown from X-irradiated seeds. Later Caldecott (1956, 1958) and Caldecott *et al.*

Table 6

Regression coefficients indicating the regression of plant height on percent normal pollen in four plant species whose dormant seeds were treated with varying doses of either X-rays or thermal neutrons. Both measurements were expressed as percent of the control

Plant species	Age‡	Regression coefficients and their standard errors: X-ray treated	Thermal neutron treated
Barley (*Hordeum vulgare* L.)	11	2.20±0.54*	1.21±0.34
Maize (*Zea mays* L.)	12	2.06±0.47*	1.01±0.04†
Mustard (*Brassica juncea* Coss)	15	1.00±0.13†	0.61±0.11†
Safflower (*Carthamus tinctorius*)	19	1.33±0.15†	0.96±0.17†

* Significantly different from zero at the five percent level of probability.
† Significantly different from zero at the one percent level of probability.
‡ Days from planting to measurement of height.

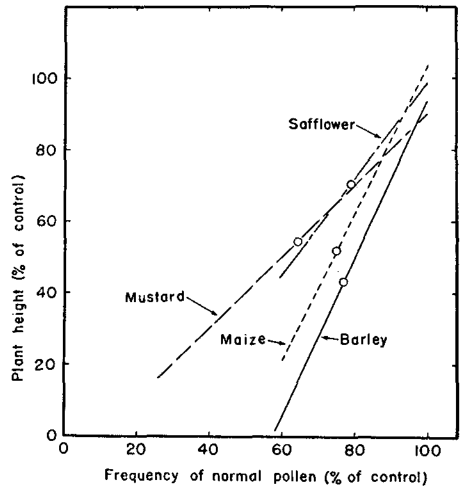

FIGURE 1.—Regression lines showing the relationship between plant height and percent normal pollen in four plant species whose dormant seeds were treated with varying doses of X-rays.

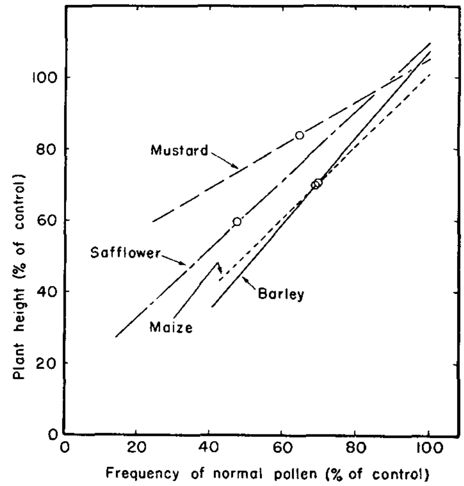

FIGURE 2.—Regression lines showing the relationship between plant height and percent normal pollen in four plant species whose dormant seeds were treated with varying doses of thermal neutrons.

(1957) suggested that the difference in linear energy transfer of the radiations was the direct cause of the differences in the biological effects of X-rays and fast or thermal neutrons. The variation between individuals within the X-rayed populations was shown to depend on a number of factors. Principal among them were moisture content of the embryo at the time of irradiation and environmental conditions during and after irradiation. Thus CALDECOTT (1958) has shown that it is possible to obtain a wide range in the frequency of mutations or meiotic chromosomal aberrations by controlling the water content of the dormant embryo before, during, and after irradiation. These later data appear to account, in a large measure, for the differences which were earlier attributed to an extrachromosomal or physiological effect of X-rays. Unpublished studies have shown that the X-ray induced injury to seeds of maize, oats, wheat, and tomatoes is subject to the same kinds of injury modification as barley. Thus it appears likely that the sensitivity of the seeds used in the present work may also have been modified by the environmental conditions preceding, during, or following X-ray treatment.

It is also apparent from the regression coefficients and lines that the four species differed somewhat in response to either kind of radiation, but that relative to one another they responded similarly to both kinds of radiation. For example, if the species are arranged in order of increasing regression coefficient, the same order is obtained for X-ray treatments as for thermal neutron treatments. However, the magnitude of the standard errors of the regression coefficients is such that one

must recognize the possibility that the responses of the four species within either kind of radiation were essentially alike.

The percentage of normal pollen shown in Tables 1, 2, 3, and 4 indicates that the dose of radiation required to produce a certain level of chromosomal abnormality differs widely for the four species. However, as may be seen from the regression lines in Figures 1 and 2, within either the X-ray or thermal neutron series, the four species are quite similar in the relationship between relative plant height and relative percentage of normal pollen. In other words, although the four species may differ widely in the sensitivity of the chromosomes to radiation, within each type of radiation the species appear remarkably alike with respect to influence on plant height per chromosomal abnormality.

SUMMARY

Seeds of barley, maize, mustard, and safflower were treated with various doses of X-rays and thermal neutrons, and observations of height, survival, and pollen abnormalities were made on the plants resulting from these seeds. In most cases a close relationship was found to exist between the three criteria of irradiation effect and dose of X-rays or thermal neutrons. However, it is necessary that care be used in selecting the age of plant at which height and survival data are taken.

The results suggest that although the chromosomes of the four species may differ widely in sensitivity to radiation damage, within each type of radiation the four species appear very similar with respect to influence on plant height per chromosomal abnormality.

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