Effects of Co 60 Gamma Irradiation on Corn Pollen

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INTRODUCTION

Both sparsely and densely ionizing radiations have been useful tools for researchers working in the field of biology. To the plant breeder, ionizing radiations have come to represent a powerful source to increase the frequency of mutations and thus enhance the chances of obtaining some that seem to be useful.

Application of radiation to seeds and pollen grains of several plant species has been made by many investigators. Criteria used to measure the effects of radiations have been based mainly on inhibition of pollen germination, pollen-tube growth, and pollen-tube mitosis (*2*).[2] F_1 and F_2 generations from treated pollen grains and seeds have also been studied. Germination ability of seeds from irradiated pollen or seeds themselves, as well as seedling height and survival, have also been used to measure the effects of radiations (*2*).

For the experiment to be reported here mature corn pollen was irradiated with Co 60 gamma rays, and then applied to silks of the female plants 24 and 48 hours after treatment. The purpose of the experiment was to determine whether there were any differences in the number of "good" kernels per ear, germination ability, and seedling height when pollen was used 24 and 48 hours after irradiation, as well as to obtain the dose response of these characters. No cytological observations of the pollen tube or pollen-tube germination were made in this experiment. Corn pollen, because of its trinucleate condition at maturity, presents many difficulties for germination studies *in vitro* (*1*).

REVIEW OF LITERATURE

Responses of pollen to treatment with ionizing radiations are different, depending upon the characteristics under study. Mericle and Mericle (*7*) reported that pollen germination and pollen-tube growth were greatly inhibited with doses of radiation (LD50 ranging up to 55,000 r. Tube elongation needed median lethal doses of 25,000 r, while for pollen-tube cytology studies a median dosage of 250 r was necessary. For endosperm and F_1 mutation studies 1,000 r were needed, and for mean lethal seed treatment, from 1,000 r to 2,000 r were used (*7*).

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 16–17.

A very detailed review of literature on radiosensitivity of pollen from different plant species has been made by Brewbaker and Emery (*2*). Comparisons of X-rays and ultraviolet light (*8,11,13*) and X-rays and neutrons (*3,4,5,6,12*) have also been made, showing some differences among treatments. Radiosensitivity of pollen grains is influenced and modified by well-known factors such as oxygen, storage, and freezing (*2*). Desiccation of pollen, stage of maturity, size of pollen grain, and content of N and B are also factors that affect radiosensitivity of the pollen (*2*).

Data on pollen radiosensitivity are quite complete for many plant species. Because of its trinucleate condition at maturity, data are far from satisfactory for corn pollen, except for cytological studies, as stated by Brewbaker and Emery (*2*). Some studies on seedling height and survival to maturity have been made by Frolik and Morris (*5*) after irradiating corn tassels in a nuclear reactor.

Treatments with X-rays and neutrons (fast and thermal) of barley seeds have been performed by Caldecott (*3*). Subsequent seedling growth and F_1 abnormalities from treated pollen have been made by Schmidt and Frolik (*12*), after irradiation in a nuclear reactor. According to Brewbaker (*1*), results are not completely reliable because gamma contamination was not taken into account

MATERIALS AND METHODS

Pollen from R^{mb} (marbled aleurone) and R^r (purple aleurone) corn was irradiated in a Co 60 gamma source with doses from 250 to 20,000 r, at intervals of 250 up to 1,500 r, and intervals of 500 up to 20,000 r. Irradiation of the excised corn tassels was accomplished by putting them in a polyethylene bottle with circulating compressed air. The bottle was lowered into the Puerto Rico Nuclear Center Co 60 gamma pool where a 2,400-c. source is housed.

After irradiation, tassels were bagged individually and kept in tapwater at room conditions for 24 hours. At this time the first pollination was made. After the pollination the used tassels were bagged again and placed in the flasks containing tapwater and kept for another 24 hours, when the second pollination was made. Ten ears were pollinated as five tassels were irradiated at each dose level.

Gross pollen observations under a stereoscope revealed that a very high percentage of pollen appeared normal when used even at the highest doses which went to 20,000 r. However, no seed was obtained in ears from tassel doses above 4,000 r. Approximately 1 month after pollination the ears were harvested and the number of well-developed kernels per ear was determined. These seeds were planted in flats containing about 50-percent soil and 50-percent *cachaza* (a sugarcane end-product) and put in growth chambers for scoring germination percentage 1 week after planting. The seedling height was measured 1 and 2 weeks after planting. Temperature in the growth chamber was 70° F. at night and 85° F. during the day cycle. Both temperature and humidity were checked with a Brown recorder.

EXPERIMENTAL RESULTS

The average number of good kernels per ear is presented in table 1. Results are quite similar for both groups, even though consistency in the pattern followed is different, being more uniform for the group from R^r pollen.

TABLE 1.—*Mean number of good kernels per ear from R^{mb} (marbled aleurone) and R^r (purple aleurone) corn pollen treated with different gamma-ray doses at 2 pollination intervals*

Gamma-ray dose (r)	R^r Pollen		R^{mb} Pollen		Average
	24 hr.[1]	48 hr.[1]	24 hr.[1]	48 hr.[1]	
0	187.8	70.5	143.4	136.0	134.3
250	203.6	92.3	110.0	143.8	137.4
500	168.2	64.0	156.8	185.2	143.6
750	180.5	125.6	83.2	136.8	131.5
1,000	198.0	106.6	187.0	84.5	144.0
1,250	191.5	107.3	159.2	98.2	139.0
1,500	119.5	92.3	108.4	103.2	105.8
2,000	98.4	92.5	95.4	113.0	99.8
2,500	52.2	33.2	28.6	58.4	43.1
3,000	40.0	42.0	13.4	28.4	31.0
3,500	19.4	43.7	10.8	22.0	24.0
4,000	29.2	12.5	7.6	4.6	13.5
Above 4,000[2]					

[1] Time after irradiation when pollen was used.

[2] No seed.

When available, 25 seeds per ear were planted in flats and the germination percentage was scored at 7 days after planting. In some of the higher dosages, especially from 2,500 up to 4,000 r, it was quite difficult to get sufficient seeds for testing germination. Table 2 shows the results of germination-ability.

As can be observed, there is not a consistent pattern between pollen used 24 hours after treatment and pollen used 48 hours after treatment. At some points the 48-hour pollen has apparently produced kernels with better germination ability. There is a dose response in both groups and with both ages of pollen within groups. The germination percentage decreased with increased dose.

Seedling height was measured 1 and 2 weeks after planting. Table 3 shows the average seedling height for each sample of seeds tested for every elapsing time and each dose.

In the range from 0 to 2,500 r sufficient seedlings were obtained to produce a reliable mean height for each dose treatment. No statistically significant differences were observed for seedlings coming from pollen used at 24 hours from that used at 48 hours after irradiation.

TABLE 2.—*Mean germination percentages of seed from R^{mb} (marbled aleurone) and R^r (purple aleurone) corn pollen treated with different doses of gamma rays at 2 pollination intervals*

Gamma ray dose (r)	R^r Pollen		R^{mb} Pollen		Average
	24 hr.[1]	48 hr.[1]	24 hr.[1]	48 hr.[1]	
0	75.2	80.0	69.3	74.5	74.8
250	75.0	60.0	71.8	75.4	70.6
500	65.6	72.0	56.8	15.7	52.5
750	29.3	22.7	34.0	58.1	36.0
1,000	25.2	53.0	71.2	41.3	47.7
1,250	63.0	23.0	19.6	66.3	50.5
1,500	49.3	30.0	37.2	68.8	46.3
2,000	43.7	50.5	33.6	33.6	40.4
2,500	21.6	6.6	15.5	12.6	14.1
3,000	9.3	10.3	0	0	4.9
3,500	6.7	4.0	0	1.8	3.1
4,000	8.0	0	0	20.0	7.0
Above 4,000[2]					

[1] Time after irradiation when pollen was used.

[2] No seed.

Figures corresponding to doses from 3,000 r to 4,000 r are not reliable since the number of seedlings obtained at these levels were one or two per treatment, but they are presented as they were measured.

DISCUSSION AND CONCLUSIONS

The effects of different kinds of radiations as measured in terms of pollen-tube germination, pollen-tube elongation, production of viable seeds, and studies on seedling height and survival have been studied by many workers in several plant species (*3,4,5,7,8,11,12*). In the experiment reported here the number of "good" kernels per ear, the germination percentage, and seedling height were studied.

"Good" kernels (at time of classification it was not known whether they were viable or not) could have been produced by genetically damaged pollen nuclei. Pollen irradiation may lead to impairment of viable-seed production, primarily via its effects on: 1, Pollen germination; 2, zygote formation and embryo growth; 3, endosperm development; 4, viability of the mature seeds; and 5, "death" of seedling (*2*). Since our material is corn (trinucleate pollen), both generative nuclei can be affected independently by irradiation and the effects may be manifested separately. Usually, as stated by Brewbaker and Emery (*2*) and others for seed abortion—many were observed from 1,500 r up in this experiment, and inviability—very low germination at higher doses are caused mostly by embryo lethality rather than endosperm damage. Seed abortion and endosperm underdevelopment were not systematically scored in this experiment, even though incidental observation showed an increase of such abnormal kernels associated with increasing doses.

TABLE 3.—*Mean plant heights (cm.) of seedlings grown in growth chambers from seed of ears pollinated with R^{mb} (marbled aleurone) and R^{r} (purple aleurone) corn pollen treated with different gamma ray doses at 2 pollination intervals*

Dose (r)	7 days					14 days				
	R^r Pollen		R^{mb} Pollen		Average	R^r Pollen		R^{mb} Pollen		Average
	24 hr.[1]	48 hr.[1]	24 hr.[1]	48 hr.[1]		24 hr.[1]	48 hr.[1]	24 hr.[1]	48 hr.[1]	
0	9.3	7.9	7.9	9.1	8.6	33.98	32.5	34.9	37.01	34.6
250	7.8	6.1	8.1	7.2	7.3	38.5	36.4	38.0	36.8	37.4
500	5.1	5.6	5.5	3.3	4.9	33.2	35.9	33.3	29.0	32.8
750	4.7	6.8	5.2	5.6	5.6	28.8	31.3	34.7	34.6	32.4
1,000	5.0	8.9	9.3	5.0	7.0	27.7	37.6	39.4	29.2	33.5
1,250	5.8	8.8	7.2	9.7	7.9	34.2	37.8	38.4	39.2	37.4
1,500	7.8	7.3	5.3	5.8	6.6	36.0	39.6	27.9	31.7	33.8
2,000	8.6	9.0	4.2	4.7	6.6	36.0	37.3	20.7	23.5	29.4
2,500	7.8	6.8	4.3	6.0	6.2	35.9	32.8	26.2	25.1	30.0
3,000	6.3	5.8	0	0	6.0	25.2	25.5	0	0	25.4
3,500	6.2	6.5	0	6.2	6.3	27.1	31.5	0	27.5	28.7
4,000	4.5	0	0	2.0	3.2	23.6	0	0	15.1	19.4
Above 4,000[2]										

[1] Time after irradiation when pollen was used.

[2] No seed.

As shown in table 1, the number of good kernels per ear was lower for high doses and longer time elapsing between treatment and use of the pollen, except for R^{mb} pollen which showed an increase at some doses. Within the limits and conditions of this experiment the pollen used 24 hours after irradiation produced, on the average, more seeds per ear than the pollen used 48 hours after treatment. Doses above 2,000 r increasingly reduced numbers of good kernels produced, although the amount of seed does not bear a strong relation to the dose received below 1,500 r.

The results for germination ability are not as consistent in this experiment, as shown in table 2. It should be noted that from 2,000 up to 4,000 r the quality of the seed itself was not at the same level as for lower doses, but the best were selected among those available at all levels. This partly accounts for higher dose treatments having a marked decrease in the germination percentage. It is expected that damage to the embryo will occur more often as the dose increases. This damage will be reflected in inability of the seeds to germinate. In general, it can be stated that the decreasing germination percentage is a reflection of induced lethality to embryo by radiation. There does not seem to be any difference attributable to the storage period after irradiation of pollen.

Data on this kind of work are not so extensive for seedlings being produced by irradiated pollen, even though cytological observations of radiation-treated pollen have been made for Tradescantia, Datura, and Petunia (*2*). Schmidt and Frolik, (*12*) compared the effects of thermal neutrons and X-rays on pollen and seeds of corn. They obtained a decrease in seedling height when seeds received about 7,500 r and pollen received about 1,200 r.

The average height of the seedlings did not show a strong dose response. If the seedling lived, it grew about as well, regardless of the dose received by the pollen grain giving rise to the seed. There might be some reduction of height at the highest doses, but the number of plants surviving to provide measurements was less than five for any group above 2,500 r, and many of the groups had no survivors, thus recording a zero for seedling height. All survivors at 7 days continued to grow to 14 days when they were all discarded. The rate of growth from 7 to 14 days showed no influence of the dose. Thus, if a plant survived the initial growth, it continued through the seedling stage. The radiation damage was manifest as lack of seed produced by the irradiated pollen, or by lack of germination of seed that were produced, but was not a factor in seedling height.

SUMMARY

Corn tassels were irradiated using Co 60 gamma rays at dose levels from 250 to 20,000 r. Twenty-four and forty-eight hours after irradiation the pollen was collected from the tassels and used to fertilize ears. The quantity of good seeds per ear, the germination percentage of these good seeds, and the seedling height 7 and 14 days later of the plants from these germinated seeds were recorded. No seeds were obtained beyond 4,000 r. There was little effect from pollen used 24 hours after treatment compared with pollen used 48 hours after treatment for any of the characters measured. There was a general dose response for number of good seeds per ear, and also in the percentage of germination of those seeds classified as good. The higher doses reduced both numbers of good seeds and their germination. There was no evident dose response of seedling height. If the seed did germinate it grew about as well as any at the dose levels measured.

RESUMEN

Se irradiaron inflorescencias de maíz con rayos gamma de cobalto-60, exponiéndolas a una dosis que fluctuó entre 250 y 20,000 roentgenios. Veinticuatro y 48 horas después de irradiadas, se recobró el polen para fertilizar las mazorcas. Se obtuvieron los siguientes datos: número de semillas buenas, o sea, semillas bien desarrolladas; porcentaje de germinación de la semilla buena, y altura de las plantas a los 7 y 14 días después de haber germinado la semilla. No se obtuvo semilla de la que se irradió con una dosis en exceso de 4,000 roentgenios. El polen que se trató por 24 horas afectó poco los caracteres que se midieron, al compararse con el que se trató por 48 horas. La reacción fue general en cuanto a la cantidad de buena semilla producida por mazorca y a su porcentaje de germinación. Las dosis altas disminuyeron tanto la cantidad de semilla buena como el porcentaje de germinación. No hubo evidencia en cuanto a la reacción de las plantas irradiadas con referencia a su altura. Si la semilla germina, su crecimiento es igual sin importar la dosis aplicada.

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