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X-Ray Irradiation of Soybean Seed as a Technique for Production of Disease Resistant Plants

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X-Ray Irradiation of Soybean Seed as a Technique for Production of Disease-Resistant Plants[1]

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Abstract. Hawkeye soybean seeds were irradiated with X-rays at dosage levels of 2,500, 5,000, 7,500, 10,000 and 15,000 r. Irradiation had no effect on germination. Plant height 2 weeks after sowing was greatly reduced at 10,000 and 15,000 r., and morphological abnormalities were common. Fertility was greatly reduced at 15,000 r. When seeds were irradiated at dosage levels of 20,000 to 65,000 r., no plant matured and produced seed when dosage was above 35,000 r. The most obvious effect of high dosage X-ray irradiation of seed was the killing of primary leaves and apical meristems.

When tested for resistance to *Pseudomonas glycinea* in the first, second, third and seventh generations after irradiation of seed, progeny from immune plants became increasingly susceptible. When compared in the seventh generation, some plants were less susceptible than others. Resistance to *Diaporthe phaseolorum* var. *caulivora* evident in the first generation after irradiation was completely lost by the seventh generation.

The need for disease-resistant soybeans for use in improvement of the crop has been a problem for many years. The possibilities for production of disease-resistant varieties through use of various types of radiation has been pointed out by several authors (Frey, 1954, 1955; Konzak, 1954; Singleton, 1954). The first type of radiation to be studied in relation to production of plant mutations was X-rays. X-ray-induced mutations in plants were studied by Stadler as early as 1930.

Disease-resistant plants have been selected from progeny of irradiated seed. Field tolerance to crown rust was induced in oat plants grown from irradiated seed (Frey, 1954). Stem rust resistance in mutant oat plants rather than improved yielding ability may be responsible for increased yields (Frey, 1955). Disease resistance to race 7a of oat stem rust was induced by nuclear radiation (Konzak, 1954). Resistance to leaf spot of peanuts resulted from Gregory's work with irradiated peanuts (Singleton, 1954).

X-ray-induced mutations in plants have occupied Swedish workers for over 15 years. Mutations resulting in higher yields from bar-

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ley were reported by Gustaffsson (1941). X-ray resistance of seed of some argicultural plants was later reported by the same author (1944). X-ray susceptibility in cereals as influenced by seed size and hulls was studied by Froier and Gustaffsson (1944). X-ray-irradiated barley seed resulting from these studies produced economically utilizable mutant plants. In addition to producing higher yield, these plants had stiffer straw and larger kernels. Mutations that were agronomically desirable resulted from barley treated with X-rays by German workers (Freisleben and Lein, 1942). More recently, X-ray induced mutants in peanuts resulted in higher yields (Gregory, 1955).

The study reported here was initiated in 1953 to determine whether use of X-ray irradiation of soybean seed could be utilized as a method of producing disease-resistant plants.

Materials and Methods

Hawkeye breeder seed was used for this study. Moisture was adjusted to approximately 14 percent in all seed lots before exposing the seed to X-rays. For greenhouse studies the irradiated seed were planted in flats containing coarse sand. For field studies the irradiated seed were sown directly in field soil.

Irradiation of all seed lots was done with a General Electric Maxitron X-ray machine operated at 250 kvp. and 30 ma. A .25 mm. copper-aluminum filter was used.

All inoculations with the stem canker fungus (*Diaporthe phaseolorum* var. *caulivora*) were made in the greenhouse. Inoculum was prepared by growing the fungus on toothpick tips floating on potato-dextrose broth. Tops of plants to be inoculated were removed 1 inch above the third node from the tips of the plants. Toothpick tips were inserted in the cut ends of the plants and the ends sealed with petrolatum. Plants had bloomed prior to inoculation and in most cases pod development had begun at the upper-most node. The fungus growth in stems caused a brown discoloration. The length of the discolored areas was measured 4 weeks after inoculation, and the results expressed in mm. as "stem canker disease ratings".

All bacterial blight inoculations were made on field-grown plants. Inoculum was prepared by growing the bacterium (*Pseudomonas glycinea*) in 4-liter flasks containing 2 liters of potato-dextrose broth, for 48 hours prior to use. One liter of inoculum was diluted in 30 gallons of water before use. Plants were sprayed with inoculum delivered from a motor-driven sprayer at a pressure of 80 lbs/sq. in. They were inoculated when approximately 6 weeks of age and disease ratings were recorded 2 weeks later.

Plants were rated for bacterial blight on a scale ranging from 1 (immune) to 5 (completely susceptible). A rating of 2 indicated a plant was resistant; 3, slightly susceptible; and 4, moderately susceptible.

Progeny from irradiated seed were maintained through succeeding generations under field conditions in the following way. Individual plants from irradiated seed were harvested separately. Seeds from each plant were planted in a single row. Two seeds were harvested from each plant in a row and 40 of these were used for planting a row the following spring.

Results

The only information available on dosage levels was that of Gustaffsson (1944), who found a dosage of 7,500 r. optimum for production of mutations in soybeans when seed was irradiated. In a preliminary test, 5 levels of irradiation, 2,500, 5,000, 7,500, 10,000 and 15,000 r., were used. One hundred seeds were irradiated at each dosage. Three groups of 100 seeds each were nonirradiated and retained as controls. The seeds were sown after treatment, and percentage germination and height of plants were recorded 2 weeks after planting (Table 1). The reduced germination at 5,000 r. was due to damping-off. The dosage levels used had no effect on germination. Plant height was greatly reduced at 10,000 and 15,000 r. There were no visible malformations at 2,500 r. and few at 5,000 r., but at 7,500 r. most plants showed slight leaf malformations. At a dosage of 10,000 r., 25 percent of the plants showed severe stunting and malformations. At 15,000 r. stunting and malformations increased to 50 percent. Seed irradiated at the 10,000 and 15,000 r. dosage levels took 48-72 hours longer to germinate than did the control seed.

Table 1

Germination of 100 Hawkeye Soybean Seeds and Plants in Each of 3 Height Classes Resulting from Irradiation of Seed With X-rays at 5 Dosage Levels

Dosage (r.)	Germination (percent)	Plants in indicated height class[1]		
		0-50 mm.	50-100 mm.	100-500 mm.
0	94	0	0	94
0	94	0	0	94
0	94	0	0	94
2,500	93	0	4	89
5,000	83	5	14	64
7,500	'89	3	25	61
10,000	89	19	50	20
15,000	93	44	44	5

[1]Two weeks after planting.

Plants were selected at each dosage level and grown to maturity in the greenhouse. Only 3 out of 30 plants from seed receiving a dosage of 15,000 r. were fertile and produced seed. These plants produced few seed. They produced many pods from this dosage, but the pods usually contained no seed. Number of pods and seed yield increased as irradiation level to which seeds had been subjected decreased.

Seed harvested from all the first-generation plants from irradiated seed were sown in pots in the greenhouse. Two hundred thirty-one plants were grown to maturity. Only 15 of these were from the 15,000 r. lot and only 14 from the 10,000 r. lot.

Lots of 100 seeds were irradiated at 20,000, 35,000, 50,000 and 65,000 r. in order to determine whether X-ray dosages above 15,000 r. might be used and what effect such dosages might have on plant development. Seeds were again sown in flats containing sand, and percentage germination, plant height, and stage of growth were recorded after 2 weeks (Table 2).

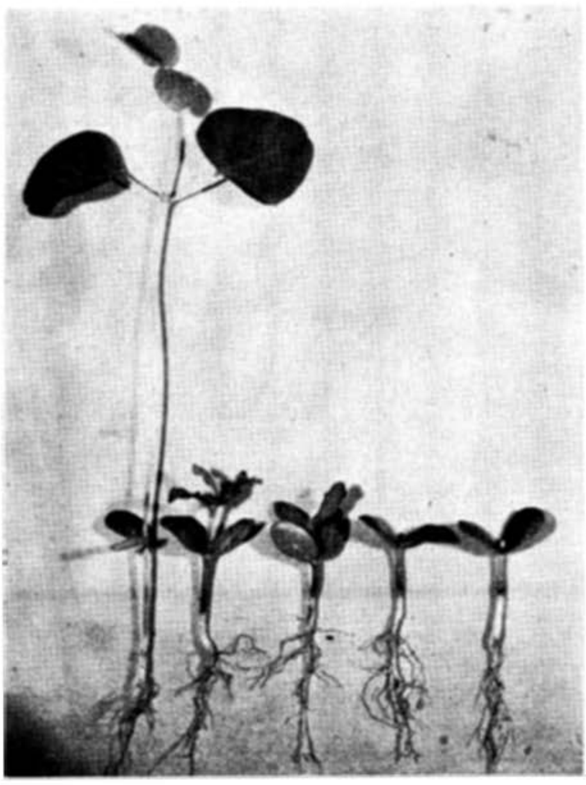

Figure 1. The soybean seedling at left was not irradiated. The four seedlings at right developed from X-ray-irradiated seed receiving dosages of (left to right) 20,000, 35,000, 50,000, and 60,000 r.

Table 2

Effect of 4 Levels of X-ray Irradiation of Seed on Development of Hawkeye Soybean Plants 2 Weeks After Sowing

Dosage (r.)	Germination (percent)	Height range (mm.)	Plants with primary leaves dead (percent)	Stage of growth	Plants developing after 60 days (percent)
0	100	180-210	0	first trifoliate leaf unfolded	100
0	100	175-215	0	first trifoliate leaf unfolded	100
20,000	96	30-130	47	37% of primary leaves unfolded; 93% of cotyledons expanded	32
35,000	96	30-50	86	3% of primary leaves unfolded; 86% of cotyledons expanded	2
50,000	96	25-40	100	83% of cotyledons expanded	0
65,000	97	20-35	100	68% of cotyledons expanded	0

Stunting was marked at the 20,000 r. dosage level and above (Figure 1), no plant being more than 50 mm. in height. One obvious effect of high levels of X-ray irradiation was the killing of the primary leaves and apical meristems of seedlings. Although germination was still very high at 50,000 and 65,000 r., all primary leaves were killed and no plant development occurred beyond expansion of the cotyledons. Two plants developed at the 35,000 r. level and one of these produced a single seed which germinated and produced a fertile plant. Only one plant at the 20,000 r. dosage produced seed. Ten seeds were harvested and sown but only 6 germinated. All these plants were fertile and produced seed.

Fifteen thousand roentgens is the practical X-ray dosage limit for Hawkeye soybeans. Beyond this point, death of primary leaves and apical meristems due to over-exposure to radiation is high, and sterility is very high in plants that develop beyond the seedling stage.

Plants in the second generation after irradiation of seed with X-rays at 15,000 r. were grown in the greenhouse and inoculated with the stem canker fungus. Five of the 15 plants inoculated were rated resistant (40 mm. or lower), whereas the control plants averaged 97 mm. (Table 3). The resistant plants were decidedly more stunted and abnormal than the susceptible plants, which were similar in appearance to the control plants but slightly shorter. The resistant plants were 8 to 18 inches shorter than the susceptible ones. The resistant plants had dark bluish-green foliage and stems, whereas the susceptible plants approximated the normal green color of the control plants. Leaves were puckered, sometimes asymetric, thick, stiff, and harsh to the touch.

Table 3

Stem Canker Disease Rating of Hawkeye Soybean Plants in the Second and Seventh Generation After Irradiation of Seed With X-rays at 15,000 r.

Plant group and number	Disease rating for second-generation plants (mm.)	Average[1] disease rating for seventh generation plants (mm.)
Plants from irradiated seed:		
1-1	40	85
1-5	10	87
1-7	10	96
3-4	10	95
5-1	30	108
Control plants	97[2]	90

[1]Average of 20 plants.
[2]Average of 4 plants.

Progeny from the resistant and control plants were maintained and retested for 7 generations after irradiation. The progeny from resistant plants lost their abnormal appearance and became similar to the control plants, except for slight shortness of 2 lines and smaller leaves of a different shape on another.

All plants tested in the seventh generation were as susceptible as the control plants. Resistance to the stem canker fungus was probably due to stunting caused by X-ray irradiation. After several generations the plants were restored to normal and lost resistance.

Seed was irradiated at 5,000, 7,500, and 10,000 r. for a field experiment. Each dosage level was replicated 4 times and 100 seeds were included in each replication. Seeds were sown at the rate of 2 per foot.

Germination and plant development were very similar to those in the greenhouse study; however, seed yield was greater. Plants from seed receiving 5,000 r. were slightly shorter and less vigorous than control plants (Figure 2). Plants from seed receiving 7,500 r. and 10,000 r. were decidedly stunted (Figure 3), and those receiving 10,000 r. were especially slow to develop.

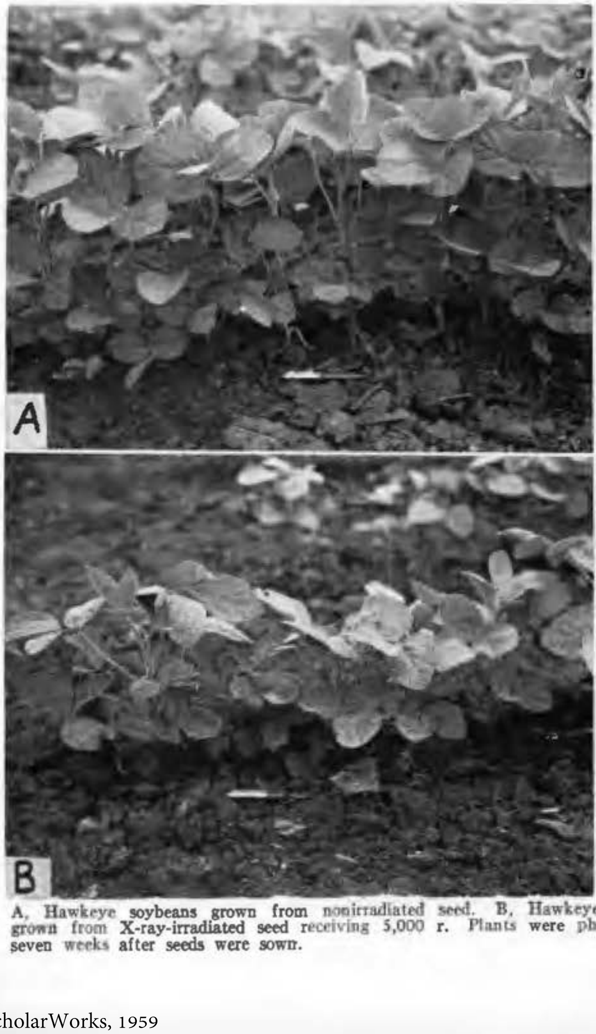

Figure 2. A, Hawkeye soybeans grown from nonirradiated seed. B, Hawkeye soybeans grown from X-ray-irradiated seed receiving 5,000 r. Plants were photographed seven weeks after seeds were sown.

Less than 1 percent of the plants from the 1,500 r. treatment were sterile, whereas 4 percent of those from the 10,000 r. treatment were sterile.

After the irradiation of seed, plants in the first, second, third, and seventh generations were inoculated with *Pseudomonas glycinea*, the organism causing bacterial blight of soybeans. The fourth through the sixth generations were grown in the greenhouse and were not tested.

In the first generation after irradiation, 2 plants were immune (Table 4). These plants (numbered 6 and 7) were of the abnormal type previously described in the stem canker study. In the second

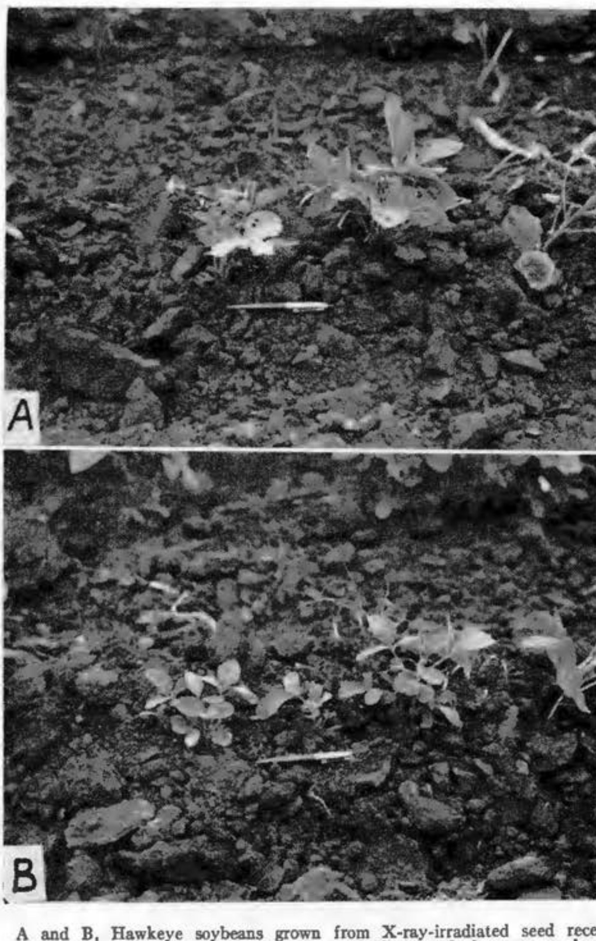

Figure 3. A and B, Hawkeye soybeans grown from X-ray-irradiated seed receiving 7,500 and 10,000 r., respectively. Plants were photographed seven weeks after seeds were sown.

generation, 1 of the progeny from plant 6 was resistant and the other was slightly resistant. Progeny from plant 7 ranged from resistant to slightly susceptible. All progeny were more normal appearing; and this was especially true of plants 6-3 and 7-1, except for the darker green color of their foliage. In the third generation, all plants ranged from slightly susceptible to susceptible. In the seventh generation, all plants were as susceptible as the controls except for plants 6-3 and 7-1, which were both only slightly susceptible. All progeny in each of these lines were uniformly less susceptible than the control plants.

Table 4

Bacterial Blight Disease Rating of Hawkeye Soybean Plants for Several Generations After Irradiation of Seed With X-rays at 10,000 r.

Plants from irradiated seed:	Disease rating in indicated generation		Average[1] disease rating[2] in indicated generation	
	First	Second	third	seventh
6-3	1	2.3	3.4	3.2
6-6		2.7	4.3	5.0
7-1	1	2.4	3.0	3.0
7-4		3.3	4.8	5.0
7-6		2.7	4.8	5.0
Control plants	5[3]	5.0	5.0	5.0

[1]Average of 20 plants.
[2]Plants rated from 1 (immune) to 5 (completely susceptible)
[3]Average of 4 plants.

The bacterial blight test demonstrated that it is possible to obtain a lower degree of susceptibility to disease through X-ray irradiation of soybean seeds. Although no resistant plants were found, use of a larger number of plants might have resulted in obtaining the desired mutation. Principal limitation of the technique is the production of abnormal plants that give resistant disease ratings but revert to susceptible plants in later generations.

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