

After-Effects of Ionizing Radiation in Barley

III. Storage of Thermal Neutron Irradiated Seeds¹

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A PRONOUNCED INCREASE in damage has been observed in x-irradiated barley seeds following storage in oxygen at atmospheric pressure for six to eight weeks (Adams *et al.*, 1955; Adams and Nilan, 1958). These after-effects amounted to a twofold increase in frequencies of chromosome aberrations, and 70 to 75 per cent decrease in germination, growth, and survival to maturity. More recently it has been found that these after-effects were greatly enhanced when x-irradiated seeds were stored in oxygen at 100 lbs. pressure (Kronstad and Nilan, 1958; Sire and Nilan, 1957). The present study was conducted to determine if such a drastic postirradiation treatment would alter the chromosome-aberration frequency induced by thermal neutrons in resting barley seeds.

Several reports have shown that neutron effects are little influenced by oxygen concentration, moisture, or hydrogen sulfide when applied during the irradiation treatment (Giles *et al.*, 1952; Nybom *et al.*, 1952; Ehrenberg *et al.*, 1953; Caldecott, 1955). While this study was in progress, Ehrenberg (1954) and Curtis *et al.* (1957) reported that the growth of barley seeds treated with fast and thermal neutrons was not influenced by postirradiation seed storage under normal atmosphere.

Materials and Methods

Resting barley (*Hordeum vulgare* var. Himalaya $2n = 14$) seeds were treated with either X-rays or thermal neutrons at the Brookhaven National Laboratory. The X-rays were generated from a G. E. Maxitron Facility operated at 250 KVP and 30 ma and were filtered through 1 mm. of aluminum. The seeds were in a petri dish 35 cm. from the target and received 700r per minute at their surface as measured with a Victoreen Integron. A total dose of 5,000r was applied.

Thermal neutron radiations were conducted in the thermal column of the

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Brookhaven National Laboratory nuclear reactor. The seeds were given a total dose of $2.71 \times 10^{11} \text{ n}^{\text{th}}/\text{cm}^2$ while they were in a single layer in a lucite box. This dose approximated 5,000 *rep*. Gamma contamination in the Brookhaven thermal column was about 115*r* per hour. Additional details of the neutron treatment of resting barley seeds in the Brookhaven reactor have been published earlier (Curtis *et al.*, 1956).

Samples of seeds from each treatment were analyzed for irradiation damage immediately upon their arrival at Pullman. The analyses were made of the frequency of chromosome aberrations, including dicentric bridges and acentric (isodiametric and rod) fragments in shoot-tip cells of irradiated seeds. The details of the methods and techniques used in these analyses have been described earlier (Adams and Nilan, 1958).

The remaining irradiated and nonirradiated seeds were stored in specially constructed chambers in oxygen under 100 lbs. pressure for six weeks. The seeds were maintained at a moisture content of 7 per cent during the storage treatment by the addition of dry CaCl_2 in the pressure chamber. The oxygen was obtained from a commercial cylinder (Industrial Air Products) and assayed as follows: 99.6 per cent O_2 , .4 per cent N_2 . Samples of seed were removed at three and six weeks and analyzed for chromosome aberrations.

Results

Table 1 presents the frequencies of chromosome aberrations in x-irradiated and thermal neutron-treated resting barley seeds stored in oxygen under pressure. The data are the average of two replications from each of two experiments.

After three weeks storage, the X-ray treated seeds exhibited a marked increase in aberrations. After six weeks, there was no further increase in bridges but a slight increase in rod fragments and a greater increase in isodiametrics.

During the entire storage period the fragment frequency increased at a higher rate than the bridge frequency. Furthermore, this increase was greater for isodiametric fragments than for rod fragments. The proportion of isodiametric fragments to bridges increased with storage time at a much faster rate than did the proportion of rod fragments to bridges.

The data clearly show that the thermal neutron-treated seeds were unaffected by the postirradiation treatment. There was no real increase in bridge or fragment frequencies.

In the nonirradiated seeds there was an increase in isodiametric fragments as a result of the oxygen post-treatment.

TABLE 1. FREQUENCIES OF CHROMOSOME BRIDGES, ISODIAMETRIC FRAGMENTS AND ROD FRAGMENTS IN THERMAL NEUTRON-TREATED AND X-IRRADIATED BARLEY SEEDS STORED FOR SIX WEEKS IN OXYGEN AT 100 LBS. PRESSURE

(Summary of Two Experiments)

Treatment	Thermal Neutrons			$(2.71 \times 10^{11} \text{ n}^{\text{th}} / \text{cm}^2)$			X-ray (5,000r)			Control			
	Cells	Br./cell	Iso./cell	Cells	Br./cell	Iso./cell	Cells	Br./cell	Iso./cell	Cells	Br./cell	Iso./cell	Rod/cell
No storage*	800	.25	.99	.63	400	.29	1.45	.86	400	.002	.002	.002	.006
Oxygen 3 weeks	800	.27	1.08	.64	400	.74	5.04	2.32	-----	-----	-----	-----	-----
Oxygen 6 weeks	800	.22	.85	.59	400	.75	6.10	2.61	400	.004	.03	.005	.005

* Stored in air for 14 days prior to O_2 pressure storage.

Discussion

An oxygen post-treatment which drastically affected x-irradiated resting barley seeds had no effect on thermal neutron-treated seeds. This finding is similar to earlier reports (Giles *et al.*, 1952; Nybom *et al.*, 1952; Ehrenberg *et al.*, 1953; Caldecott, 1955) which have shown that various secondary factors applied before or during irradiation are much more effective in modifying the effects of X-rays than those of neutrons. It also supports more recent observations (Ehrenberg, 1954; Curtis *et al.*, 1957) that growth of barley seeds treated with fast and thermal neutrons was not influenced by postirradiation seed storage. The differences in the biological effects and degree of modification by secondary factors of the two kinds of radiations have been discussed earlier (Caldecott, 1955; Gray, 1953; Konzak, 1957) and have been related to the physical characteristics of the radiations.

It must be recognized that during the time between irradiation at Brookhaven and first analyses at Pullman (about two weeks) an appreciable increase in damage could have occurred in the x-irradiated seeds. Therefore, the X-ray induced chromosome-aberration frequency immediately following the irradiation would be somewhat less than that first recorded in the experiment and thus probably less than the frequency induced by the thermal neutrons. Nevertheless, after the storage treatment at Pullman the frequencies of bridges and particularly of fragments in x-irradiated seeds far exceeded those which were induced by the neutrons.

Marked "after-effects" on chromosomes induced by postirradiation treatments in x-irradiated seeds have been described earlier (Adams *et al.*, 1955; Sire and Nilan, 1957; Adams and Nilan, 1958). In these reports it was concluded that the postirradiation increase in chromosomal bridges and fragments in X-rayed seeds was due to (1) increased conversion of potential to primary breaks (cf. Swanson, 1954) and/or (2) decreased restitution of broken chromosomes (cf. Schwartz, 1952; Baker, 1956).

It is now appropriate to interpret the thermal neutron data in terms of similar hypotheses. It may be assumed (Caldecott, 1955; Caldecott *et al.*, 1954; Swanson, 1954) that the more densely ionizing neutrons will induce chiefly primary and few potential breaks in the chromosomes. Thus, during the postirradiation period few conversions of potential to primary breaks would occur; hence, little or no increase in chromosomal aberrations would result. The data may also indicate that the broken ends of neutron breaks are not affected by the postirradiation treatments so as to inhibit the restitution process. Most of the breaks probably reconstitute. The few that do not reconstitute give rise to

the frequencies of aberrations as recorded in the neutron-treated seeds. Thus, it can be concluded that most of the chromosome breaks induced by neutrons in resting seeds are not readily modified after irradiation to increase or decrease the frequencies of detectable chromosome aberrations.

The practical importance of altering the ratio of X-ray induced mutations to chromosomal aberrations in mutation plant breeding has been discussed earlier (Adams and Nilan, 1958). From the present study it would appear that neutrons may not be as satisfactory in some respects as X-rays for the plant breeder because the cytogenetic effects of neutrons cannot be experimentally modified. Substantiation of this conclusion must, however, await results from appropriate large-scale experiments involving induced beneficial mutations.

Results of these experiments have one other practical implication for plant breeders using ionizing radiation to induce beneficial variability in crop plants. If dry (7 per cent moisture) seeds are x-irradiated and several weeks or months elapse before the seeds are planted, the breeder must expect a considerable decrease in germination and survival. However, these changes should not occur when these seeds are thermal neutron treated. Evidence of this storage problem with x-irradiated seeds has been reported (Lawrence, 1955).

Summary

Resting barley seeds (*Hordeum vulgare* var. Himalaya) were x-irradiated or thermal neutron treated and then stored, along with nonirradiated seeds, in oxygen under 100 lbs. pressure for six weeks. The frequencies of dicentric bridges and acentric (isodiametric and rod) fragments were recorded at late anaphase of the first cycle of cell division in the shoot tips of the irradiated seeds.

The initial damage in the x-irradiated seeds was greatly increased by the postirradiation treatment. The neutron damage, however, was not modified by this treatment. The failure of induced aberrations to increase during the postirradiation period in neutron-treated seeds was attributed to the assumption that most of the breaks were induced at the time of irradiation and that the restitution of neutron breaks was not influenced by the postirradiation treatments.

Two practical implications of the results are discussed for the plant breeder who is employing ionizing radiation to induce genetic variability in crop plants.

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