SPECIAL ARTICLES

MUTATIONS IN BARLEY INDUCED BY X-RAYS AND RADIUM

AT the Nashville meeting of the American Association last December I reported the occurrence of mutations in barley following X-ray treatment.[1] The experiments, which were independent of and coincident with those of Muller,[2] though by no means so comprehensive and thorough, confirm Muller's discovery of the power of X-rays to induce mutation and show its application to plants. They show also that mutations may be induced similarly by radium treatment.

The treatments were applied to germinating seeds, and the induced mutations recorded were in all cases somatic mutations affecting the progeny of only part of the plant. The experiments were set up in this way in order to exclude the possibility that new characters appearing after treatment might be ascribable to some irregular segregation from hybrid ancestry. The barley plant produces several tillers from axillary buds, each tiller terminating in an inflorescence of about thirty self-fertilizing flowers. In the dormant embryo the first three or four leaves are already differentiated, and the cells from which the tillers will be developed are separated. A mutation occurring in one of these cells, therefore, will affect only one tiller, and, whether dominant or recessive, will segregate in the progeny of only one head.[3] Its absence in the progeny of other heads of the same plant shows that the genetic change occurred during the development of the plant treated, for any ancestral character, however inherited, would affect all head progenies of the individual similarly.

The X-ray treatments were applied intermittently in twelve equal exposures at one-hour intervals, while the seeds were germinating under optimum conditions. A 30 m.a. "radiator-type" self-rectifying Coolidge tube was used, operated at a tube current of 5 m. a. Treatments were made at seventy-eight kilovolts (peak), two and three quarter minutes per exposure, and at fifty-four kilovolts, five and one half minutes per exposure, in an attempt to apply approximately equal quantities of radiation differing somewhat in quality. At each voltage a "heavy" and a "light" treatment were applied simultaneously, at target distances of 22.7 and 45.4 cm, respectively. The radiation passed through two samples of seed at shorter distances, and the filtering effect of the wet blotters and seeds must be considered in computing dosage. Ionization measurements made later showed that this reduced the intensity of the radiation at the higher voltage by about 52 per cent., and of that at the lower voltage by about 65 per cent. The relative ionizing intensity of the heavy and light treatments at the higher voltage and the heavy and light treatments at the lower voltage was in the ratio 100: 21: 50: 9. The so-called heavy doses were not heavy enough to reduce viability appreciably, but a dose of approximately three times this intensity, with the higher voltage, was found to be partially lethal. I am indebted to the department of physics of this university for the use of the X-ray equipment, and particularly to Mr. R. T. Dufford, of that department, for much advice and assistance and for the construction of an ionization chamber of the Duane type,[4] with which the dosage measurements above were made.

The radium treatments were applied under similar conditions, using as a source 50 mg of radium in the form of radium sulfate, sealed in a thin glass tube within a tube of silver 1 mm thick. Dr. Dudley A. Robnett, of Columbia University, generously lent his personal supply of radium for the treatments. The seeds, germinating in stacked watch glasses, were exposed continuously for twelve or twenty-four hours at distances ranging from one and a half to eleven cm. The maximum dose (applied to seeds in the dishes immediately above and below that containing the radium tube) was well below the limit of tolerance.

The mutations previously reported were three seedling chlorophyll defects, "white," "virescent," and "yellowing." White seedlings are colorless from emergence, and die in two to three weeks. Virescent seedlings are colorless at emergence, but gradually develop a pale green color. With care they may be kept alive for a long period, but they grow very slowly. Both white and virescent seedling types have previously been reported as Mendelian characters in barley. Yellowing seedlings are green and apparently normal at emergence, but about a week later they pass through intermediate shades to full yellow and die soon after.

These three mutations were found among seventy-seven head progenies, representing twenty-six X-rayed plants. Each mutant type made up one fourth to one eighth of a single head progeny from a plant of which other head progenies were entirely normal. If the segregation is due to a mutation affecting one head

[1] Experiments on the effects of X-rays on crossing over and chromosome distribution, reported in the same communication, have been published separately. (*Proc.* Nat. Acad. Sci., 14: 69–75, 1928.)

[2] SCIENCE, 66: 84–87, 1927.

[3] Under favorable conditions tillers are sometimes developed from the axillary buds of earlier tillers. In such cases more than one head may be affected by the same mutation.

[4] *Am. Jour. Roent. and Rad. Therapy,* 10: 935–943, 1923.

or part of a head, some of the normal plants from this head should segregate the same mutant character in the following generation, while none of the plants from the unaffected heads should do so. This expectation was realized in the next generation, in which eight of the sixteen normal plants tested from the head which segregated white seedlings were found to be heterozygous for white, seven of the twelve plants tested from the head which segregated virescent were heterozygous for virescent, and three of the seven plants tested from the head which segregated yellowing were heterozygous for yellowing. In each case about fifteen plants from unaffected heads of the mutating plant were tested, and all gave entirely normal progeny.

The remaining progenies have since been planted and seedling segregations noted. The total frequency of mutations resulting in definite and conspicuous seedling characters is shown below:

	Total number of head progenies examined	Number segregating mutant seedling characters
X-ray treated:		
Higher voltage		
Heavy dose	210	6
Light dose	259	1
Lower voltage:		
Heavy dose	494	6
Light dose	280	1
Total X-rayed	1,243	14
Radium treated:		
Total for all doses	1,039	3
Untreated	1,341	0

The majority of the mutations listed were white seedlings. Tests of genetic identity have not yet been made, but it is probable that many of these represent mutations at different loci. At least three genetically distinct white seedlings have previously been reported in barley, and in maize, in which genetic analysis has been more intensive, dozens of genes for white seedling are known.

The mutations following radium treatment included two whites and one virescent. Since the radiation passed through 1 mm of silver and at least 5 mm of glass before reaching the seeds, it consisted largely of gamma rays. The fact that mutation frequency was lower in the radium series than in the X-ray series does not imply that radium is less effective than X-rays in inducing mutation, since the intensity of the radium treatment was probably much lower. All three mutations occurred in the plants receiving the heavier doses.

A preliminary trial was made also of the possibility of increasing the effectiveness of X-ray treatment by impregnating the seeds with salts of heavy elements. Since in general X-ray absorption increases approximately in proportion to the fourth power of the atomic number of the absorbing element, it may be increased materially by the presence of even a small amount of some heavy element within the cell. The seeds were soaked for seven hours in M/5 solutions of the salts listed below. The X-ray treatment, which began fifteen hours later, was similar to that described above as heavy dosage at the higher voltage, but the intensity was about 40 per cent. higher. The frequency of mutation for distinct seedling characters is shown below:

Treatment		Number of head progenies	Number of mutations
Radiation	Chemical		
X-rayed	$Ba(NO_3)_2$	136	9
X-rayed	$Pb(NO_3)_2$	133	9
X-rayed	$UO_2(NO_3)_2$	194	11
X-rayed	none	72	2
Not X-rayed	$UO_2(NO_3)_2$	53	0
Not X-rayed	none	76	0

Although the numbers are too small to justify a final conclusion, the results suggest that the chemical treatments increased the effectiveness of irradiation. The mutations included, in addition to types already noted, the following seedling characters: "yellow," "pale yellow," "yellow-green," "banded" (transverse white bands), "striped" (two distinct patterns) and "tapering" (a morphological peculiarity). Several possible mutations for less conspicuous seedling characters are omitted from the summary pending further investigation.

In all, forty-eight mutations causing distinct seedling characters have been found following irradiation. These include almost all the seedling characters of barley previously reported and several not previously described. No mutations have yet been found in untreated plants, of which about fifteen hundred head progenies have been examined.

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THE NUCLEAR CONDITIONS IN THE SPERMATOCYTES OF DROSOPHILA MELANOGASTER

IT is a surprising fact that very little has been known until recently concerning the nuclear condi-