THE EFFECT OF X-RAYS UPON DOMINANT MUTATION IN MAIZE

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No dominant mutations have been found in x-ray progenies of barley and maize, in the course of experiments in which many hundreds of recessive mutations have been detected. Many dominant alterations appear in the first generation progeny produced by the use of x-rayed pollen, but these alterations are lethal to the gametophyte and are therefore not reproduced in later generations. The recessive mutations detected are at least partially haplo-viable, since they must be transmitted through both male and female gametophytes before their effects may be observed in homozygous individuals. Since all of the induced alterations which are viable in haplo-phase are wholly recessive, these experiments provide no evidence of the production by x-rays of any mutant gene of positive action. The induced mutations observed may be merely gene losses tolerated by the gametophyte.²,³

The evidence against the occurrence of x-ray-induced dominant mutation is, however, inconclusive, for the following reasons:

1. The number of genes capable of showing the effects of dominant mutation may be much smaller than the number capable of showing recessive mutation, since many genes may be fixed by natural selection at a level maximal for phenotypic effect. The preponderance of recessive mutations may be only a reflection of the preponderance of such alleles in the genotype of the irradiated individual. The possibility of inducing dominant mutation therefore may be tested critically only by the effects upon known recessives, that is, genes with known dominant alleles.

2. Among known recessives many may be themselves deficiencies and therefore incapable of mutation. Critical evidence of failure to mutate to a dominant allele therefore may be obtained only from recessive genes which have previously been known to mutate to dominant alleles.

3. The only recessive alleles which meet this requirement are the so-
called variegation genes, which may be regarded as unstable recessives mutating frequently to a dominant allele. Each of these is characterized by a high spontaneous frequency of dominant mutation, so high that the effect of x-ray treatment in inducing additional dominant mutations probably would be inappreciable. Demerec's experiment with the unstable miniature-3 of Drosophila virilis is an example of this difficulty.

It is possible to avoid these difficulties in the case of one gene. The recessive gene a1 in maize has several known dominant alleles. Rhoades\(^5,6\) has shown that in the presence of another gene, Dt, mutations of a to various dominant alleles occur. With increasing dosage of Dt the frequency of dominant mutation of a is sharply increased, and in endosperms tripex for Dt and for a (a a a Dt Dt Dt) more than 100 mutations per seed are usually found. In the absence of Dt no dominant mutations of a have been reported, though a a a dt dt dt endosperms have been closely examined in large numbers. (Dr. Rhoades informs me that he has seen one instance of an apparent "dot" of anthocyanin colored tissue in the aleurone cells of an a a a dt dt dt seed.)

The gene a, then, is capable of mutation to a recognizable dominant allele, and can be tested for response to x-ray treatment in genotypes in which spontaneous dominant mutations of the gene rarely if ever occur. Since the effects of mutation are clearly recognizable in minute sectors of tissue, the treatment may be applied at a fairly advanced stage in endosperm development, so that many hundreds of cells may be tested for mutation by examination of a single endosperm. It is possible therefore to test for the occurrence of this mutation in practically unlimited populations.

The experiment was conducted as follows: The seed to be irradiated was produced by the cross a a \(\times\) A a, both parent stocks being homozygous for dt dt and for the complementary factors required for aleurone color development. The endosperms of half of the seeds produced are A a a (colored aleurone). These serve to indicate the size of the sectors resulting from genetic alterations induced by irradiation at the stage of development chosen, since induced deficiencies or recessive mutations of A in these endosperms result in sectors deficient in aleurone color. In the colorless seeds (a a a) induced dominant mutation of any one of the a genes would result in a corresponding sector of colored aleurone. The colorized seeds thus provide a basis for estimating the number of opportunities for the occurrence of detectable mutation in the colorless seeds, and for comparison of the relative frequency of induced dominant mutation and induced loss of A due to recessive mutation or deficiency.

X-ray treatment was applied to the ears 73–81 hours after pollination, following preliminary experiments which indicated that treatment at this stage results in sectors of suitable size. Treatments were made on field-grown plants by means of a mobile x-ray unit with self-rectifying tube,
operated at 88 K.V.P., 4.5 ma. tube current, and 15 inches target distance, with no filtration other than that of the overlying tissue. Under these conditions the time of treatment is 2.48 minutes per 100 r. The doses applied, uncorrected for filtration, were 800 r and 1600 r.

The lower dose was applied to 21 ears, which yielded a total of 8003 seeds. The effect of the treatment was clearly greater on the exposed side of the ear than on the opposite side, as indicated by the frequency of deficiency sectors and also by the occurrence of "scarred" endosperms. There was also appreciable variation in dosage along the long axis.

The average size and frequency of sectors showing "A-loss" (that is, loss of the A phenotype), was estimated by detailed examination of a small sample of seeds. From each of six representative ears given the lower dose of radiation, three seeds were taken from the treated side and three from the opposite side, with approximately even distribution along the long axis of the ear. Each of the 36 seeds was closely examined under magnification by the use of a dissecting microscope. This permits determination of the size of sectors by direct count of aleurone cells affected. In small sectors due to loss of A the aleurone cells are not wholly colorless, but the contrast in color is sharp enough to make the outline of the sector quite definite. In large sectors, produced by irradiation of the ear about 30 hours after pollination, the aleurone cells of sectors resulting from loss of A are colorless except for a rim of faintly colored cells about the margin of the sector. There are in addition occasional sectors of dilute aleurone color, probably resulting from similar changes affecting an unknown gene or genes. These are readily distinguishable from sectors involving A in the early-treated material, but are less distinct when the sectors are so small that marginal color may cover the entire sector. In order to minimize the error from this source, all sectors which seemed possibly due to loss of genes other than A were omitted. These made up nearly one-third of the aleurone sectors observed on the treated seeds. The ratio of dilute sectors to colorless sectors on early-treated seeds of the same cross is considerably less than 1:2, and it is therefore probable that the actual frequency of A-loss in the experimental material is somewhat higher than the data below would indicate. The observations are summarized in table 1.

The 18 seeds representing the exposed side of the treated ears (seeds Nos. 1 to 3 on each ear) yielded 618 sectors, or an average of 34 per seed; while those representing the opposite side yielded 248, or an average of 14 per seed. The sectors varied rather widely in size, the average number of aleurone cells included being 112. The frequency of A-loss sectors on untreated ears, determined by similar examination of 10 representative seeds from each of the 19 control ears, was 0.12 per seed. The 23 sectors found ranged in size from 9 to 750 cells.

The approximate number of aleurone cells in the entire endosperm was
determined similarly by counts of aleurone cells in representative areas. The number of aleurone cells in seeds of average size was found to be about 160,000. It may be roughly estimated, therefore, that at the time of treatment the number of cells per seed whose genetic alteration could be detected by sectors in the mature aleurone is about 1400 (160,000/112).

The colorless seeds of the same treated ears permit the detection of mutations of \( a \) to any one of the various \( A \) alleles producing aleurone color. Each endosperm cell contains three \( a \)'s, each of which is capable, under the influence of \( Dt \), of mutating to a colored aleurone allele. If the phenotypic effects of mutation and of deficiency are shown by the total cell progeny of the irradiated cell, or by the same fraction of this progeny, the result of mutation should be a colored sector equal in area to the color-deficient sectors produced by deficiency.

If either alteration requires for its realization a period of one or more cell generations in excess of the other, the resulting sectors may be of unequal size. It is not to be expected, however, that delay in realization will regularly reduce the size of sector by one-half for each cell generation. The sectors observed include only the cells of the aleurone layer which are included in the block of tissue affected; their reduction in size with advancing endosperm development depends upon the pattern of development of the tissues concerned. Actually, the average size of deficiency sector observed in ears treated at periods after pollination ranging from 73 to 81 hours was not changed enough to show any consistent relation among the 21 ears treated. Ears treated 96 hours after pollination yielded smaller but readily recognizable sectors. It is evident, therefore, that even if there were a delay in realization of gene mutation for several cell generations after irradiation, the resultant sectors would be detectable. Colored sectors as small as 10 cells in size are clearly recognizable on close examination of the seeds without magnification, and a single colored aleurone cell on a colorless seed is readily identified under magnification.

All of the colorless seeds were closely examined without magnification for sectors of colored aleurone. Occasional discolored areas of pericarp or endosperm were found, but under magnification these were readily distinguishable from colored aleurone sectors. No sectors of colored aleurone were found, among the 3832 seeds examined. On the basis of 1400 cells per seed, each containing 3 \( a \) genes capable of dominant mutation, this represents approximately 16 million chances for detectable dominant mutation (3832 \( \times \) 1400 \( \times \) 3). The treatment to which these cells were exposed produced in the adjoining colored seeds approximately 100,000 losses of \( A \). Since the number of \( A \)'s tested for frequency of \( A \)-loss was only one-third the number of \( a \)'s tested for frequency of dominant mutation, the experiment shows failure to induce dominant mutation in a trial on a scale sufficient to produce about 300,000 \( A \)-losses.
A similar examination was made of the ears irradiated at 1600 r. At this
dose the number of \( A \)-losses on seeds on the exposed side of the ear is too
great to permit an accurate count. On the opposite side of the ear the fre-
quency is very high also, but samples representative of the central portion
of this region (amounting to approximately one-sixth of the ear) were suit-
able for examination. In 8 of the ears treated a sample of 6 seeds per ear
was examined, the sample in each case being taken from the two rows least
exposed to the radiation. The number of \( A \)-losses from these samples
averaged 20.3 per seed. This is about three times the frequency of \( A \)-

<table>
<thead>
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<th>Table 1</th>
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**Frequency and Size of Sectors Due to \( A \)-Loss Following X-ray Treatment**

<table>
<thead>
<tr>
<th>Ear</th>
<th>No. 870</th>
<th>No. 876</th>
<th>No. 880</th>
<th>No. 881</th>
<th>No. 882</th>
<th>No. 884</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Interval, pollination to irradiation (hrs.)</td>
<td>75</td>
<td>75.5</td>
<td>80.5</td>
<td>81</td>
<td>81.5</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Number of sectors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed No. 1</td>
<td>22</td>
<td>32</td>
<td>26</td>
<td>36</td>
<td>49</td>
<td>40</td>
<td>205</td>
</tr>
<tr>
<td>No. 2</td>
<td>30</td>
<td>31</td>
<td>38</td>
<td>33</td>
<td>34</td>
<td>29</td>
<td>195</td>
</tr>
<tr>
<td>No. 3</td>
<td>49</td>
<td>43</td>
<td>34</td>
<td>23</td>
<td>37</td>
<td>32</td>
<td>218</td>
</tr>
<tr>
<td>No. 4</td>
<td>3</td>
<td>9</td>
<td>13</td>
<td>14</td>
<td>6</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>No. 5</td>
<td>20</td>
<td>12</td>
<td>17</td>
<td>12</td>
<td>14</td>
<td>9</td>
<td>84</td>
</tr>
<tr>
<td>No. 6</td>
<td>11</td>
<td>16</td>
<td>13</td>
<td>18</td>
<td>24</td>
<td>22</td>
<td>104</td>
</tr>
<tr>
<td>Number of aleurone cells per sector (frequency distribution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-10</td>
<td>12</td>
<td>12</td>
<td>16</td>
<td>9</td>
<td>23</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>11-50</td>
<td>38</td>
<td>43</td>
<td>48</td>
<td>42</td>
<td>47</td>
<td>41</td>
<td>259</td>
</tr>
<tr>
<td>51-200</td>
<td>71</td>
<td>64</td>
<td>46</td>
<td>63</td>
<td>82</td>
<td>69</td>
<td>395</td>
</tr>
<tr>
<td>201-500</td>
<td>14</td>
<td>23</td>
<td>27</td>
<td>22</td>
<td>11</td>
<td>19</td>
<td>116</td>
</tr>
<tr>
<td>500+</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

losses in seeds similarly located on the ears treated at 800 r, and inspection
of seeds in other regions of the ears indicates that this is a fair estimate of
the relative frequency of \( A \)-losses from the two doses used. The average
size of sectors was 113 cells.

The 15 ears treated at 1600 r produced a total of 2487 colorless seeds.
These were closely examined for sectors of colored aleurone, and in addition
a sample of 50 seeds from the most heavily treated region of each was
minutely examined under magnification. No sectors of colored aleurone
were found, with the exception of a single doubtful spot possibly including
3 aleurone cells, which could not be positively identified. The frequency of
\( A \)-losses, producible by the same dose in an equal population of \( A \)-genes,
estimated as before, is about 600,000.
The susceptibility to dominant mutation of the \( a \) genes used in this experiment was tested by pollinating each of the parental stocks by a homozygous stock designated \( a-dl \) \( Dt \). The gene \( a-dl \) is one of a number of "dotless" \( a \) alleles arising by mutation of standard \( a \) under the influence of \( Dt \). It is indistinguishable from \( a \) in phenotypic effect, but differs in showing almost no dots due to dominant mutation in the presence of \( Dt \). In endosperm triplex for \( a-dl \) and \( Dt \) an occasional small dot may be found, as in the case of the similar mutant \( a' \) described by Rhoades, but the rate is negligible in comparison to that of standard \( a \). In the endosperms of \( a a a-dl dt dt Dt \), produced by the crosses under discussion, the dots observed may be ascribed almost wholly to mutation of one of the two \( a' \)s under the influence of the single dose of \( Dt \). The mutation frequencies for x-ray action and \( Dt \) action are, of course, not directly comparable, since it is not possible to apply the two stimuli for comparable periods or to comparable populations of cells.

The number of dots per seed in endosperms of \( a a a-dl dt dt Dt \) produced by crosses on the female parental stock was 2.95, on the colorless seeds of the male parental stock 2.1. In each case the dots represent the number of mutations produced by two \( a \) genes in the presence of a single \( Dt \) gene. In the \( a a a \) seeds irradiated, therefore, the number of mutations to \( A \) which would be expected in the presence of one dose of \( Dt \) is about 4 per seed \((2.95 + 2.1/2)\). Rhoades\(^4\) has shown that the frequency of mutation of \( a \) to \( A \) is disproportionately increased by increased dosage of \( Dt \), and that about 17 times as many mutations are produced in \( Dt \) \( Dt \) \( Dt \) seeds as in \( Dt \) \( dt \) \( dt \) seeds. The population of 6319 seeds of \( a a a \), which failed to yield mutations to \( A \) under x-ray treatment, was therefore capable of yielding about 400,000 mutations to \( A \) under the influence of homozygous \( Dt \) \((6319 \times 4.0 \times 17)\).

*Summary.*—X-ray treatment failed to induce mutation of \( a \) to \( A \) (or to any other colored aleurone allele), in populations which, with the x-ray doses applied, were capable of yielding about 900,000 losses of \( A \) by deficiency or by mutation to a colorless allele.

The \( a \) genes used in the experiment, when combined with \( Dt \), yielded numerous mutations to \( A \) or other colored aleurone alleles. The population irradiated was large enough to have yielded, under the influence of homozygous \( Dt \), about 400,000 of such mutations.

\(^1\) Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Missouri Agricultural Experiment Station, University of Missouri. Missouri Agricultural Experiment Station Journal Series 940.


\(^4\) Demerec, M., these *PROCEEDINGS*, 20, 28–31 (1934).
