QUANTITATIVE VARIATION INDUCED BY GAMMA RAYS AND FAST NEUTRONS IN ARABIDOPSIS THALIANA*

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Daly K. Quantitative variation induced by gamma rays and fast neutrons in Arabidopsis thaliana. Radiation Botany 13, 149-154, 1973.—To test the effectiveness of radiations which differ in linear energy transfer (LET) for the induction of quantitative variation, dry seeds of Arabidopsis thaliana (L.) Heynh. (Cruciferae) were exposed to varying doses of cobalt-60 gamma rays and fast neutrons. Flowering time was delayed for all neutron treatments in the M₄ generation, but only at the highest gamma-ray dose level, 150 kR. Tests for increased variance in the M₄ generation due to irradiation were significant at each dose level of gamma-ray and fast-neutron treatment.

Fast neutrons were found to be more efficient than gamma rays for induction of genetic variance. This induced variance increased linearly with dose in the case of fast neutrons, implying that these quantitatively inherited variations arose primarily from chromosomal alterations, rather than ‘point’ mutations. The absence of a similar dose-response relationship for gamma rays is attributed in part to lack of enhancement of this radiation’s effect due to reduced availability of oxygen immediately following irradiation in these experiments.

INTRODUCTION

In recent years, a number of workers have attempted to determine the effectiveness of ionizing radiation for the induction of variation which reflects the mutability of polygenic systems controlling quantitative traits. This has been especially true for self-fertilizing crop species in which such variation could be particularly desirable if incorporated in a breeding program. The investigations which first tested the effectiveness of mutagenic treatment on quantitative traits included the work of Czarnecki,(14) Mertens and Burdick,(15) Oka et al.,(16) Rawlings et al.(20) and others on self-fertilizing plants. From these results, it was concluded that irradiation increased the genetic variability available to selection in quantitative traits, which has led to further studies on the nature and magnitude of this variation.

The purpose of the investigation described in this report is a test of the effectiveness of radiations which differ in linear energy transfer (LET) for the induction of quantitative variation. One of the most prominent features of radiobiological damage is that as the LET increases so also does the relative biological effectiveness (RBE) up to a certain optimal density. The inference is that if the target is inactivated only when several ionizations occur within it, then densely ionizing radiation will be more effective than sparsely ionizing radiation. Cobalt-60 gamma rays were chosen as representative of sparsely ionizing radiation; fast neutrons served as an example of the densely ionizing radiations.

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The biological material was a self-fertilizing diploid species, *Arabidopsis thaliana* (L.) Heynh. This plant is a rapidly reproducing member of the family Cruciferae which can be cultured on a defined medium. Flowering time was chosen as the quantitatively inherited trait which would serve as an index of the response to radiation. This characteristic has been utilized previously in testing for increased genetic variance following seed irradiation in various plant species, including *A. thaliana*.*

**EXPERIMENTAL PROCEDURE**

Seeds of the race 'Estland' of *A. thaliana* were derived from a single plant in a stock supplied by Dr. John Langridge of Canberra. This race, collected originally by Laibach, is rapid flowering with vigorous, uniform growth. Prior to irradiation, seeds were allowed to equilibrate over dry calcium chloride at room temperature.

**Seed irradiation**

Dry seeds were exposed to gamma rays from a cobalt-60 source at Brookhaven National Laboratory. Total exposures were 0, 50, 100 and 150 kR at a dose rate of 410 kR/hr. Exposures of dry seeds to fast neutrons were made using the thermal column of the Brookhaven Graphite Reactor, which has been described previously. The neutron spectrum was essentially a fission spectrum and gamma-ray contamination was estimated to be less than 10 per cent of the absorbed dose. The average linear energy transfer of the neutron spectrum was calculated to be 49 keV/μ. Neutron dosimetry was performed using a tissue-equivalent ionization chamber, measuring in rads. Exposure times in the thermal column ranged from 2 to 8 hr at dose rates of either 1880 or 2000 rads/hr.

**Post-irradiation treatment**

Following radiation exposures, seeds were immediately hydrated in distilled water which had previously been boiled and then chilled to 2–4°C. Seeds were allowed to remain in distilled water for a period of one hour, while helium was continuously bubbled through the chilled water. Post-irradiation treatment of one hour in chilled water was designed to reduce the availability of oxygen for enhancement of biological effects. Thus, any differential response observed following exposure to radiation would not be attributable to an oxygen effect.

After hydration, seeds were transferred aseptically to individual culture tubes (16 x 150 mm) containing nutrient agar. All cultures were first placed at 5°C for 24 hr, followed by four days at 20°C, before being grown to maturity at 25°C. Individual plants of the *M*₁ generation were allowed to reproduce by self-fertilization; the seeds produced were bulked and sampled at random to obtain the *M*₂ generation.

**RESULTS**

The results presented here consist of observations on time of flowering which served as the index of radiation-induced variability. Data on the number of days to flowering in the first (*M*₁) and second (*M*₂) generations following seed irradiation were used for the estimation of means and variances.

**M*₁ generation**

Germination percentages were high in all treated groups in the *M*₁ generation. The number of plants which survived to flowering was greater than 50 per cent in each treatment (Table 1). No artificial selection was practiced on this generation on the basis of *M*₁ fertility.

**M*₂ generation**

A description of the flowering-time distributions in the *M*₂ generation is given in Table 2. Mean flowering time is delayed only at the highest dose level, 150 kR, in the case of gamma rays. In each of the fast-neutron treatments, however, the mean flowering time is significantly greater than the non-irradiated control.

The estimates of within-plot variance for irradiated and control groups in the *M*₂ generation are listed in Table 2. In the non-irradiated control, the within-plot variance is assumed to be entirely of environmental origin. Hence, it is possible to estimate the induced genetic variance in each of the irradiated groups by computing the difference between their respective within-plot mean squares and the within-plot mean square of the control. This variance component, *σ*², estimates the transmissible variation induced by radiation, which is assumed to be
Table 1. Survival and flowering in the M₁ generation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exposure*</th>
<th>Seed no.</th>
<th>Survival to flowering, %</th>
<th>Flowering time, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma rays</td>
<td>0 kR</td>
<td>300</td>
<td>87.3</td>
<td>23.1±0.15</td>
</tr>
<tr>
<td></td>
<td>50 kR</td>
<td>180</td>
<td>66.1</td>
<td>21.4±0.16</td>
</tr>
<tr>
<td></td>
<td>100 kR</td>
<td>180</td>
<td>78.3</td>
<td>22.3±0.19</td>
</tr>
<tr>
<td></td>
<td>150 kR</td>
<td>180</td>
<td>58.9</td>
<td>25.2±0.23</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>0 krad</td>
<td>150</td>
<td>91.3</td>
<td>22.7±0.27</td>
</tr>
<tr>
<td></td>
<td>~3.6 krad</td>
<td>150</td>
<td>83.3</td>
<td>22.7±0.32</td>
</tr>
<tr>
<td></td>
<td>~9.4 krad</td>
<td>150</td>
<td>83.3</td>
<td>26.0±0.20</td>
</tr>
<tr>
<td></td>
<td>~12.0 krad</td>
<td>150</td>
<td>73.8</td>
<td>26.1±0.24</td>
</tr>
<tr>
<td></td>
<td>~16.0 krad</td>
<td>150</td>
<td>60.7</td>
<td>29.2±0.97</td>
</tr>
</tbody>
</table>

*1 kR = 0.95 krad.

Table 3. Regression of induced genetic variance (Y) on dose (X)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proportion of Y sum of squares due to X</th>
<th>Proportion of Y sum of squares due to X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma rays</td>
<td>( Y = 4.205 - 0.005X )</td>
<td>0.02</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>( Y = -0.719 + 0.426X )</td>
<td>0.94*</td>
</tr>
</tbody>
</table>

*\( P < 0.05 \).

Whether the predicted responses to selection can be realized will obviously depend upon breeding tests, particularly since the heritability values may over-estimate the proportion of the total variance which can be utilized by selection.

DISCUSSION

The characteristic dose-response curves of high LET radiations are linear, while those of low LET are curvilinear. The expected relationship holds for the fast-neutron treatments, which are in agreement with a simple linear regression model (Table 3). However, the increase in variance due to gamma rays is not proportional to dose. Lack of a consistent dose-response relationship for gamma rays may be due to additional uncontrolled environmental variation, for the response to sparsely ionizing radiation is notably modified by environmental variables, such as seed moisture and oxygen availability.\(^7\)

The failure to obtain a linear dose-response is unexpected in view of an earlier report on radiation-induced variability in A. thaliana.\(^9\) In that case, the magnitude of the genetic variance for flowering time induced by gamma-ray treatment was closely correlated with dose (Fig. 1); the least-squares regression of induced variance did not deviate significantly from linearity \((r = 0.99)\). Similarly, Lawrence\(^17\) concluded that the amount of induced variation for flowering time was linearly related to dose from examination of the selection response in advanced lines of A. thaliana.

There is one important difference, however, in post-radiation treatment conditions, which may account for the difference in response to gamma rays. In the prior experiment,\(^9\) seeds were allowed to hydrate after irradiation without...
Table 2. Analysis of flowering time distribution in the M₁ generation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exposure</th>
<th>Plant no.</th>
<th>Flowering time, days</th>
<th>Within-plot mean square</th>
<th>Genetic variance, s²</th>
<th>Heritability, h</th>
<th>Confidence limits on h, 90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma rays</td>
<td>0 kR</td>
<td>250</td>
<td>23.18 ± 0.16</td>
<td>2.2968</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50 kR</td>
<td>243</td>
<td>22.95 ± 0.21</td>
<td>7.1890†</td>
<td>1.8422</td>
<td>0.68</td>
<td>0.60 &lt; h &lt; 0.71</td>
</tr>
<tr>
<td></td>
<td>100 kR</td>
<td>262</td>
<td>22.85 ± 0.17</td>
<td>4.2322†</td>
<td>1.9334</td>
<td>0.46</td>
<td>0.32 &lt; h &lt; 0.57</td>
</tr>
<tr>
<td></td>
<td>150 kR</td>
<td>232</td>
<td>24.56 ± 0.25*</td>
<td>6.6413†</td>
<td>4.3445</td>
<td>0.65</td>
<td>0.56 &lt; h &lt; 0.73</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>0 krad</td>
<td>234</td>
<td>20.56 ± 0.10</td>
<td>3.5550</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3-8 krad</td>
<td>213</td>
<td>21.82 ± 0.16*</td>
<td>4.8688†</td>
<td>1.3138</td>
<td>0.27</td>
<td>0.08 &lt; h &lt; 0.42</td>
</tr>
<tr>
<td></td>
<td>9-4 krad</td>
<td>215</td>
<td>21.85 ± 0.17*</td>
<td>6.2833†</td>
<td>2.7283</td>
<td>0.43</td>
<td>0.29 &lt; h &lt; 0.55</td>
</tr>
<tr>
<td></td>
<td>12-0 krad</td>
<td>193</td>
<td>21.73 ± 0.20*</td>
<td>7.5657†</td>
<td>4.0107</td>
<td>0.53</td>
<td>0.41 &lt; h &lt; 0.63</td>
</tr>
<tr>
<td></td>
<td>16-0 krad</td>
<td>207</td>
<td>21.72 ± 0.22*</td>
<td>10.1794†</td>
<td>6.6244</td>
<td>0.65</td>
<td>0.56 &lt; h &lt; 0.72</td>
</tr>
</tbody>
</table>

*Significantly different from control mean (P < 0.05).
†Significantly greater than control mean square (P < 0.05).
attempting to exclude oxygen. The present results are based on seeds which were also hydrated immediately after irradiation, but in a manner designed to minimize the availability of oxygen in the post-radiation period. In other respects, the experimental conditions are considered comparable. These results suggest that the genetic alterations induced by gamma rays which are reflected by increased variance in quantitative traits are oxygen dependent. In this way, they resemble a variety of other biological effects of sparsely ionizing radiations which also exhibit a marked oxygen sensitivity.

Although high LET radiations, such as neutrons, have been shown to be more efficient for inducing a variety of genetic changes, a review of the results for quantitative variation is inconclusive. This outcome may be accounted for in part by problems of dosimetry, since most of the investigations have utilized thermal neutrons. However, by making determinations of the elemental composition of seeds of A. thaliana, Brock was able to compare the variation induced by thermal neutrons and cobalt-60 gamma rays on the basis of absorbed dose. The RBE values estimated in this manner ranged from 8.0 to 18.2 for flowering time and plant weight and are similar in magnitude to those that have been reported for other neutron effects in plants.

It is apparent that the fast-neutron exposures described here are more efficient than gamma rays for the induction of variation in flowering time (Fig. 1). The data are in contrast with previous comparisons of fast neutrons to sparsely ionizing radiations which have not demonstrated a clear difference in effectiveness. The present results imply that the sensitive target for genetic alterations which yield increased quantitative variability requires more than a single ionization for such an alteration to occur. This conclusion is consistent with the hypothesis that quantitatively inherited variations arise primarily from chromosomal alterations rather than 'point' mutations.

Indications of the relative frequency of mutations increasing ('plus') and decreasing ('minus') the manifestation of a character are usually obtained from a comparison between means of M₄ and control lines. On this basis, the present results would indicate a preponderance of mutations in the 'plus' direction. However, Lawrence has concluded from analysis of flowering time in irradiated populations that mutations delaying flowering ('plus') are relatively less frequent but produce a larger average...
effect. Hence, conclusions as to the relative frequency of 'plus' and 'minus' mutations may not be meaningful, since the assumption of equality of average effect of these mutations is in doubt.

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REFERENCES

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