Somatic mutation frequency in the stamen hairs of
*Tradescantia* KU 7 and KU 9 clones exposed
to low-level gamma rays

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ABSTRACT

Two triploid clones (KU 7 and KU 9) of *Tradescantia* heterozygous for
flower color were exposed to 1 to 42.3 R of gamma rays or the scattering
radiation in the gamma field of the Institute of Radiation Breeding. Oc-
currence of somatic pink mutations in the stamen hairs was investigated 10
to 16 (or 14) days after irradiation. The mutation frequency was found to
increase linearly with increasing gamma-ray exposure in the both clones, and
the frequencies of 0.437 and 0.468 pink mutant events per $10^3$ hairs per R
were determined for KU 7 and KU 9, respectively. When the data collected
in the present study were analyzed together with those obtained in earlier
experiments in the gamma field, linear relationships of the somatic mutation
frequency with gamma-ray (2.1 to 201.6 R) and scattering radiation (0.72 to
57.6 R) exposures were confirmed. Scattering radiation was found to have a
genetical efficiency more than two times higher than that of gamma rays.
Variation of spontaneous mutation frequency observed in the present study
and in earlier studies was inversely correlated to temperature variation.

1. INTRODUCTION

While the risk estimate of low-level ionizing radiations has been regarded
as an urgent problem to be solved (see BEIR Reports 1972, 1980), the genetic
effect at low-dose levels has been relatively well studied with higher plants
rather than with animals and microorganisms. This is because two botanical
systems, *Tradescantia* stamen hairs heterozygous for flower color (see Under-
brink et al. 1973; Ichikawa 1974) and cereal pollen grains possessing suitable

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2) Visiting Researcher to the Laboratory of Genetics, Faculty of Agriculture, Kyoto Univer-
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starch characters (see de Nettancourt et al. 1977), have been proved to be most excellent test systems for such studies. In particular, the characteristics of *Tradescantia* stamen-hair system, that is, the capability of detecting all pink mutant cells easily without being concealed by other cells as well as the relative easiness of handling a great number of samples, have proved to be especially suitable for studying the genetic effect of low-level radiations (Underbrink et al. 1973; Ichikawa 1974, 1976, 1981b).

In fact, Ichikawa (1971, 1972b) demonstrated that the somatic pink mutation frequency in the stamen hairs of *Tradescantia* KU 7 clone kept a linear relationship with chronic gamma-ray exposure down to 8.0 R, after repeating experiments in the gamma field of the Institute of Radiation Breeding, Ohmiya, Ibaraki. He also reported a higher genetic efficiency of scattering radiation in the gamma field at low-exposure levels such as down to 0.96 R. Sparrow et al. (1972) demonstrated further that the somatic pink mutation frequency in *Tradescantia* clone 02 stamen hairs increased linearly with increasing acute X-ray dose in the extremely small-dose range of 0.25 to 6 rad and that the mutation frequency with 0.43-MeV neutrons was linear down to 0.01 rad. Ichikawa and Takahashi (1977) also confirmed such linear relationships between acute gamma-ray exposure (3.1 to 50.8 R) and somatic mutation frequency in the stamen hairs of KU 9 and KU 20 clones of *Tradescantia*, and Ichikawa et al. (1978) reported that the somatic mutation frequency in clone 02 stamen hairs was linear when exposed to gamma rays at lower exposure rates such as 0.026 to 0.52 R/min.

Furthermore, the genetic effects of relatively high natural background radiation levels have been detected with the stamen-hair system of *Tradescantia* (Mericle and Mericle 1965; Nayar et al. 1970), as well as the significance of internal exposures from ³H (Nauman et al. 1979) and ¹³¹I (Tano and Yamaguchi 1979) at low levels. Increased somatic mutation frequencies in the stamen hairs have been also reported by growing *Tradescantia* in soil samples from the Bikini Island (Ichikawa and Nagashima 1979) or near nuclear facilities (Ichikawa 1981a). Comparable findings obtained from other organisms are only those of the cytological effects of higher natural radiation levels detected in the root-tip cells of some plant species (Gopal-Ayengar et al. 1970) and in the spermatocytes of scorpions (Takahashi 1976).

The present paper describes the results of further experiments with *Tradescantia* performed in the gamma field of the Institute of Radiation Breeding, and discusses about informations accumulated through a series of such experiments.

### 2. MATERIALS AND METHODS

The materials used were two triploid clones (2n=18) of *Tradescantia*, KU 7 and KU 9, both heterozygous for flower and stamen color (blue/pink; the blue
color being dominant). The KU 7 clone of *T. ohiensis* Raf. (= *T. reflexa* Raf.) has been described to be a tetraploid (Ichikawa 1970, 1971, 1972b, 1974; Takahashi and Ichikawa 1976) but is noted here as a triploid, since our recent chromosome counts on the stock plants as well as on the materials used in the present and other studies have proved that all of those examined cytologically have 18 chromosomes (Ichikawa 1981a). The morphological characteristics of this clone are, however, essentially unchanged from those described earlier (Ichikawa 1970, 1974). The KU 9 is a hybrid clone between *T. ohiensis* and *T. paludosa* And. et Woods. as reported earlier (Ichikawa 1972a). The both clones are vigorous in growth and are sterile because of their triploid nature, being suitable materials for outdoor experiments. All the plants used were those propagated vegetatively from the stock clones, and were grown in 24 cm clay pots.

Irradiation treatments were performed in the gamma field of the Institute of Radiation Breeding. In the first experiment, potted plants of KU 7 clone having young inflorescences of flowering size were placed on May 28, 1974, at five different points in the gamma field, i.e., 100, 70, 50 and 40 m apart from the $^{60}$Co source and 40 m apart but behind an earth bank built in the gamma field to shield from direct gamma rays. The plants were removed from the gamma field on the next day, except a part of the plants placed behind the earth bank, which were removed one more day later. These plants were thus exposed to 2.9, 6.5, 13.7 and 22.5 R of gamma rays (plus scattering radiation) and to 1.0 and 2.0 R of scattering radiation only. All these exposure data and also those described below are based on the dosimetry made by the personnel of the institute with thermo-luminescence dosimeters. The control plants were placed in a control field (ca. 640 m apart from the center of the gamma field; ca. 0.05 mR/hr radiation level) of the institute. The irradiated and control plants were carried back to Kyoto University, and mutation data were collected 10 to 16 days after irradiation.

The second irradiation experiment was carried out with KU 9 clone on August 5, 1974, following the same procedures as described above, and the potted plants were exposed to 2.9, 6.4, 13.5 and 22.2 R of gamma rays (plus scattering radiation) and to 1.0 and 2.0 R of scattering radiation only. In this experiment, the irradiated and control plants were not carried back to Kyoto, but were left in the control field after treatments. Thus the mutation scoring was made at Ohmiya 10 to 14 days after irradiation.

In the third irradiation experiment which was carried out on September 17, 1976, both KU 7 and KU 9 clones were employed. Potted plants were placed at five different points in the gamma field, i.e., 100, 70, 50, 35 and 25 m apart from the $^{60}$Co source, and were removed on the next day. With such overnight (20-hr) treatments, the plants of both clones were exposed to 2.1, 4.8, 10.1, 21.6 and 42.3 R of gamma rays (plus scattering radiation). In this experiment, the irradiated and control plants were not carried back to Kyoto, but were left in the control field after treatments. Thus the mutation scoring was made at Ohmiya 10 to 14 days after irradiation.
For collecting mutation data, occurrence of somatic mutations from blue to pink in stamen hairs was recorded regarding a single pink cell or two or more contiguous pink cells as one pink mutant event (see Ichikawa and Sparrow 1968; Ichikawa et al. 1969). The number of stamen hairs was counted on each stamen (each flower has three antipetalous and three antisepalous stamens) to determine mutation frequency per hair, and the number of hair cells was also counted on ten representative hairs (distal three, middle four and basal three hairs) each of one antipetalous and one antisepalous stamens per flower (see Ichikawa and Takahashi 1978) in order to obtain mutation frequency per cell division.

Besides the above irradiation experiments, spontaneous somatic pink mutation frequency in stamen hairs was examined in late July and late October of 1975 for the KU 7 plants which had been kept in the control field of the Institute of Radiation Breeding since May, 1970, in order to know the variation of spontaneous mutation frequency level.

3. RESULTS

The data obtained from the first experiment with KU 7 clone are presented in Table 1. The mutation frequency in the control plants, 9.11±1.31 pink mutant events per 10^3 hairs, was much higher than those determined as control levels in earlier experiments in the gamma field (4.91±1.41 to 6.87±1.21 pink mutant events per 10^3 hairs; see Table 5), but it was apparently observed that the mutation frequency per 10^3 hairs tended to increase more with higher exposures. Similar increases are also seen for the mutation frequency expressed as the number of pink mutant events per 10^4 hair-cell divisions.

The mutation data collected in the second experiment in which KU 9 clone was employed are presented in Table 2. The mutation frequency in the stamen

<table>
<thead>
<tr>
<th>Exposure (R)</th>
<th>No. of hairs observed</th>
<th>No. of pink mutant events</th>
<th>No. of pink mutant events /10^3 hairs (±SE)</th>
<th>Average no. of cells/hair</th>
<th>No. of pink mutant events /10^4 cell divisions (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5,286</td>
<td>48</td>
<td>9.11±1.31</td>
<td>27.3</td>
<td>3.46±0.50</td>
</tr>
<tr>
<td>1.0*</td>
<td>5,783</td>
<td>54</td>
<td>9.34±1.26</td>
<td>27.8</td>
<td>3.45±0.47</td>
</tr>
<tr>
<td>2.0*</td>
<td>6,981</td>
<td>80</td>
<td>11.46±1.27</td>
<td>29.4</td>
<td>4.04±0.45</td>
</tr>
<tr>
<td>2.9</td>
<td>5,561</td>
<td>69</td>
<td>12.41±1.48</td>
<td>29.7</td>
<td>4.92±0.52</td>
</tr>
<tr>
<td>6.5</td>
<td>4,524</td>
<td>53</td>
<td>11.72±1.60</td>
<td>28.2</td>
<td>4.31±0.59</td>
</tr>
<tr>
<td>18.7</td>
<td>4,677</td>
<td>67</td>
<td>14.33±1.74</td>
<td>30.1</td>
<td>4.92±0.60</td>
</tr>
<tr>
<td>22.5</td>
<td>5,592</td>
<td>108</td>
<td>19.31±1.84</td>
<td>28.8</td>
<td>6.95±0.67</td>
</tr>
</tbody>
</table>

* Exposure to scattering radiation. Other exposures are those to gamma rays.
Mutation frequency at low radiation level

Table 2. Somatic pink mutation frequencies in the stamen hairs of KU 9 clone determined 10 to 16 days after exposures to gamma rays or scattering radiation

<table>
<thead>
<tr>
<th>Exposure (R)</th>
<th>No. of hairs observed</th>
<th>No. of pink mutant events</th>
<th>No. of pink mutant events /10^6 hairs (±SE)</th>
<th>Average no. of cells/hair</th>
<th>No. of pink mutant events /10^4 cell divisions (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4,660</td>
<td>14</td>
<td>3.00±0.80</td>
<td>18.7</td>
<td>1.70±0.45</td>
</tr>
<tr>
<td>1.0*</td>
<td>6,915</td>
<td>30</td>
<td>4.34±0.79</td>
<td>19.9</td>
<td>2.30±0.42</td>
</tr>
<tr>
<td>2.0*</td>
<td>5,027</td>
<td>24</td>
<td>4.77±0.97</td>
<td>20.2</td>
<td>2.49±0.51</td>
</tr>
<tr>
<td>2.9</td>
<td>411**</td>
<td>1</td>
<td>2.43±2.43</td>
<td>19.2</td>
<td>1.34±1.34</td>
</tr>
<tr>
<td>6.4</td>
<td>1,084**</td>
<td>6</td>
<td>5.54±2.25</td>
<td>19.5</td>
<td>2.99±1.22</td>
</tr>
<tr>
<td>18.5</td>
<td>5,988</td>
<td>42</td>
<td>7.02±1.06</td>
<td>20.3</td>
<td>3.64±0.56</td>
</tr>
<tr>
<td>22.2</td>
<td>18,794</td>
<td>181</td>
<td>13.12±0.97</td>
<td>19.2</td>
<td>7.21±0.54</td>
</tr>
</tbody>
</table>

* Exposures to scattering radiation. Other exposures are those to gamma rays.
** Plant were damaged on the way to take them back to Kyoto, thus sufficient data could not be collected.

Table 3. Somatic pink mutation frequencies in the stamen hairs of KU 7 clone determined 10 to 14 days after exposures to gamma rays

<table>
<thead>
<tr>
<th>Exposure (R)</th>
<th>No. of hairs observed</th>
<th>No. of pink mutant events</th>
<th>No. of pink mutant events /10^6 hairs (±SE)</th>
<th>Average no. of cells/hair</th>
<th>No. of pink mutant events /10^4 cell divisions (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32,149</td>
<td>242</td>
<td>7.53±0.48</td>
<td>28.3</td>
<td>2.76±0.18</td>
</tr>
<tr>
<td>2.1</td>
<td>10,778</td>
<td>73</td>
<td>6.77±0.79</td>
<td>27.3</td>
<td>2.58±0.80</td>
</tr>
<tr>
<td>4.8</td>
<td>5,891</td>
<td>59</td>
<td>10.02±1.30</td>
<td>25.4</td>
<td>3.66±0.48</td>
</tr>
<tr>
<td>10.1</td>
<td>2,445</td>
<td>28</td>
<td>11.45±2.15</td>
<td>27.9</td>
<td>4.26±0.80</td>
</tr>
<tr>
<td>21.6</td>
<td>2,094</td>
<td>34</td>
<td>16.72±2.84</td>
<td>28.0</td>
<td>6.19±1.06</td>
</tr>
<tr>
<td>42.3</td>
<td>2,390</td>
<td>71</td>
<td>29.71±3.47</td>
<td>28.2</td>
<td>10.92±1.30</td>
</tr>
</tbody>
</table>

hairs of control plants was reasonably low being 3.00±0.80 pink mutant events per 10^6 hairs or 1.70±0.45 pink mutant events per 10^4 cell divisions (KU 9 shows a lower spontaneous mutation frequency than KU 7; see Takahashi and Ichikawa 1976). Insufficient data could be collected from plants irradiated with 2.9 and 6.4 R gamma rays, because those plants were damaged on the way to take them back to Kyoto. Excepting the two exposures, evidently higher mutation frequencies were observed with higher exposures.

The mutation frequencies determined for KU 7 and KU 9 clones in the third irradiation experiment are presented in Tables 3 and 4, respectively. In this experiment, greater numbers of stamen hairs were observed for control plants and for plants treated with smaller exposures. The control level of mutation frequency of KU 7 in this experiment (7.53±0.48 pink mutant events per 10^6 hairs) was lower than in the first experiment (see Table 1) but was still higher
than those reported earlier (see Table 5). The mutation frequency determined for 2.1 R gamma-ray exposure was not higher than the control level, but obviously higher frequencies were observed for higher exposures (Table 3). On the other hand, almost linearly increased mutation frequencies with increasing exposure were observed for KU 9, the control plants of which showed a rela-

Table 4. Somatic pink mutation frequencies in the stamen hairs of KU 9 clone determined 10 to 14 days after exposures to gamma rays

<table>
<thead>
<tr>
<th>Exposure (R)</th>
<th>No. of hairs observed</th>
<th>No. of pink mutant events</th>
<th>No. of pink mutant events $/10^6$ hairs ($\pm$SE)</th>
<th>Average no. of cells/hair</th>
<th>No. of pink mutant events $/10^4$ cell divisions ($\pm$SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11,419</td>
<td>38</td>
<td>3.33$\pm$0.54</td>
<td>19.9</td>
<td>1.76$\pm$0.29</td>
</tr>
<tr>
<td>2.1</td>
<td>11,010</td>
<td>58</td>
<td>5.27$\pm$0.69</td>
<td>19.8</td>
<td>2.80$\pm$0.37</td>
</tr>
<tr>
<td>4.8</td>
<td>5,467</td>
<td>34</td>
<td>6.22$\pm$1.06</td>
<td>20.1</td>
<td>3.26$\pm$0.56</td>
</tr>
<tr>
<td>10.1</td>
<td>3,077</td>
<td>27</td>
<td>8.77$\pm$1.68</td>
<td>19.7</td>
<td>4.69$\pm$0.90</td>
</tr>
<tr>
<td>21.6</td>
<td>2,402</td>
<td>33</td>
<td>13.74$\pm$2.38</td>
<td>19.2</td>
<td>7.55$\pm$1.81</td>
</tr>
<tr>
<td>42.3</td>
<td>2,481</td>
<td>59</td>
<td>28.78$\pm$3.06</td>
<td>19.5</td>
<td>12.85$\pm$1.67</td>
</tr>
</tbody>
</table>

Fig. 1. The numbers of pink mutant events per $10^6$ stamen hairs of KU 7 clone (minus each control) plotted against exposure. Vertical lines attached to the points plotted indicate standard errors. The best-fit line drawn was obtained by calculating weighted average slope but ignoring the points for scattering radiation exposures.
Mutation frequency at low radiation level

A relatively low mutation frequency of $3.33 \pm 0.54$ pink mutant events per $10^3$ hairs (Table 4).

To see the exposure-response relationships of the mutation data, the numbers of pink mutant events per $10^3$ hairs (minus each control) observed in these experiments are plotted together against exposure in Figs. 1 and 2 for KU 7 and KU 9 clones, respectively. It is apparent that the mutation frequency increases with increasing radiation exposure in the both clones. Lines drawn in these figures are those obtained by calculating weighted average slopes ignoring the points for scattering radiation. The slopes of the lines indicate that 0.437 and 0.468 pink mutant events were induced per $10^3$ hairs per R of gamma rays in KU 7 and KU 9, respectively, and it seems possible to regard that the both clones exhibit similar genetic responses to relatively small exposures of gamma rays when mutation frequency is expressed as the number of pink mutant events per $10^3$ hairs. However, when the mutation frequency is converted into the number of pink mutant events per $10^4$ cell divisions, mutational events are calculated to occur more frequently in KU 9 stamen hairs than in KU 7 per unit exposure, since the average cell number per hair
is smaller in KU 9 than in KU 7 (see Tables 1 to 4; the average number of cell divisions per hair corresponds to one less than the average number of cells per hair).

For analyzing further the exposure-response relationships, the mutation data are plotted against exposure on log-log graphs as shown in Figs. 3 and 4. The best-fit lines obtained by the least square method (but based on the points for 4.8 R and higher exposures since the negative data for 2.1 R exposed KU 7 and 2.9 R exposed KU 9 can not be plotted on the log-log graphs) are drawn in these figures, and their slopes (1.039 for KU 7 and 0.961 for KU 9), which are not significantly different from the +1 slope, indicate that the mutation frequency increases linearly with gamma-ray exposure.

The spontaneous mutation frequencies determined for KU 7 in late July and late October of 1975 were 4.95 ± 0.66 and 8.71 ± 0.92 pink mutant events per 10^9 hairs, respectively (see Table 5), showing a considerable difference between

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**Fig. 3.** The numbers of pink mutant events per 10^9 stamen hairs of KU 7 clone (minus each control) plotted against exposure on a log-log graph. Vertical lines attached to the points plotted indicate standard errors. The regression line drawn was obtained by the least square method but based on the points for 4.8 R and higher exposures (see text).
4. DISCUSSION

The present results of obtaining the mutation frequency of 0.437 pink mutant events per $10^6$ hairs per R of gamma rays for KU 7 clone shows a fairly good accordance with earlier results of 0.336 (Ichikawa 1971), 0.488, 0.388 (Ichikawa 1972b) and 0.396 (Ichikawa 1974) pink mutant events per $10^6$ hairs per R, all of which were obtained from the experiments in the same gamma field. The corresponding value determined for KU 9 clone in this study (0.468) also roughly fits to these values for KU 7. Therefore, it seems pertinent to plot all the mutation data on the same graph for obtaining information from larger amount of data.

All the corresponding data reported earlier, namely, the data for Period I reported by Ichikawa (1971, 1972b) and all the data reported by Ichikawa (1973),
are plotted on a log-log graph together with the data being reported in this paper as shown in Fig. 5. The data reported in two earlier papers (Ichikawa 1971, 1972b) were subdivided into those for each day of collecting data, because

![Graph showing relationships with gamma-ray and scattering radiation exposures on a log-log graph of the numbers of pink mutant events per 10^3 stamen hairs of KU 7 and KU 9 clones (minus each control) determined in the present study and earlier experiments in the gamma field (Ichikawa 1971, 1972b, 1973). The regression lines drawn were obtained by the least square method.]

**Fig. 5.** The relationships with gamma-ray and scattering radiation exposures on a log-log graph of the numbers of pink mutant events per 10^3 stamen hairs of KU 7 and KU 9 clones (minus each control) determined in the present study and earlier experiments in the gamma field (Ichikawa 1971, 1972b, 1973). The regression lines drawn were obtained by the least square method.
the data reported in those papers were pooled ones from the stamen hairs exposed to radiations for different number of days (thus exposed to different doses). The data plotted in Fig. 5 are thus those obtained from exposures ranging from 2.1 to 201.6 R of gamma rays and from 0.72 to 57.6 R of scattering radiation. A considerable fluctuation of mutation frequency is seen in the figure especially in small-exposure range, reflecting an inevitable consequence of outdoor experiments and also too small sample sizes for some points, but a clear correlation between exposure and mutation frequency is evidently seen. The regression lines for gamma rays and scattering radiation calculated by the least square method are drawn in the figure, and their slopes, 1.010 and 0.938 respectively, do not differ significantly from the +1 slope. It is therefore conclusive that somatic pink mutation frequency increases linearly with increasing exposure of gamma rays and scattering radiation.

The regression lines in Fig. 5 show that 0.40 pink mutant events per $10^6$ hairs are induced per R of gamma rays, and about 0.9 is the corresponding value for scattering radiation. It means that scattering radiation has a genetic efficiency more than two times higher than that of gamma rays. The higher efficiency of scattering radiation as compared to gamma rays seems to be due to the lower energy (thus higher linear energy transfer or LET) of the former.

Variation of spontaneous mutation frequency in the stamen hairs of KU7 clone was conspicuous in the present study and also in comparison with earlier studies. The number of pink mutant events per $10^6$ hairs in the control (or non-irradiated) plants placed in the control field of the Institute of Radiation Breeding varied from 4.95±0.66 to 9.11±1.31 in the present study and from 4.91±1.41 to 6.87±1.21 in earlier studies (Ichikawa 1971, 1972b, 1973) as listed.

Table 5. Variation of spontaneous somatic pink mutation frequency in the stamen hairs of KU7 clone placed in a control field of the Institute of Radiation Breeding

<table>
<thead>
<tr>
<th>Scoring period</th>
<th>No. of hairs observed</th>
<th>No. of pink mutant events</th>
<th>No. of pink mutant events /10^6 hairs (±SE)</th>
<th>Average temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/5-6, 70</td>
<td>2,207</td>
<td>11</td>
<td>4.38±1.50</td>
<td>29.0</td>
<td>Ichikawa (1971)</td>
</tr>
<tr>
<td>8/19-21, 70</td>
<td>2,446</td>
<td>12</td>
<td>4.91±1.41</td>
<td>24.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>8/11-13, 71</td>
<td>2,246</td>
<td>12</td>
<td>5.34±1.54</td>
<td>28.5</td>
<td>Ichikawa (1972b)</td>
</tr>
<tr>
<td>8/19-27, 71</td>
<td>2,242</td>
<td>14</td>
<td>6.24±1.66</td>
<td>27.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>9/20-10/6, 71</td>
<td>4,659</td>
<td>32</td>
<td>6.87±1.21</td>
<td>21.1</td>
<td>Ichikawa (1973)</td>
</tr>
<tr>
<td>6/7-13, 74</td>
<td>5,268</td>
<td>43</td>
<td>9.11±1.31</td>
<td>18.6</td>
<td>This paper</td>
</tr>
<tr>
<td>7/20-31, 75</td>
<td>11,310</td>
<td>56</td>
<td>4.95±0.66</td>
<td>25.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>10/21-23, 75</td>
<td>10,219</td>
<td>89</td>
<td>8.71±0.92</td>
<td>17.0</td>
<td>&quot;</td>
</tr>
<tr>
<td>9/27-10/1, 76</td>
<td>32,149</td>
<td>242</td>
<td>7.58±0.68</td>
<td>19.4</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* Data of 12 days before scoring periods (see text).
in Table 5. Since the main cause of the variation of spontaneous mutation frequency in *Tradescantia* stamen hairs has been proved to be attributable to temperature variation (Takahashi and Ichikawa 1976; Yamashita 1976), i.e., the mutation frequency being inversely correlated to temperature, it is necessary to examine if the variation of spontaneous mutation frequency observed in the series of experiments at Ohmiya can be also attributable to different temperatures.

Temperature data are also listed in Table 5 together with the data of spontaneous mutation frequency in the present and earlier studies. The average temperatures listed are those of 12 days before the scoring periods, since the KU 7 stamen hairs show their maximum development thus are most affected by environmental factors about 12 days before flowering (Ichikawa 1970; Takahashi and Ichikawa 1976). It is obvious in this table that higher spontaneous mutation frequencies were obtained when temperature was lower. In order to see the relationship more clearly, the data are plotted on a semi-log graph as shown in Fig. 6. The reason of plotting data on the semi-log graph is based on earlier finding of a higher correlation with temperature of the logarithm of mutation frequency than of mutation frequency in arithmetic scale (Takahashi and Ichikawa 1976). The broken line drawn in the figure is the best-fit regression line for the data listed in Table 5.

![Fig. 6. The relationship of the logarithm of spontaneous pink mutation frequency in the stamen hairs of KU 7 clone with average temperature 12 days before flowering. The data from Kyoto are those reported earlier (Takahashi and Ichikawa 1976). Vertical lines attached to the points plotted indicate standard errors. The regression lines drawn were obtained by the least square method (solid line for all the points plotted; broken line only for the points from Ohmiya).](image-url)
When the data of spontaneous mutation frequency in KU 7 stamen hairs which were collected in Kyoto and reported earlier (Takahashi and Ichikawa 1976) are also plotted on the same graph, they show a fairly good accordance with the data taken at Ohmiya as seen in Fig. 6. The regression line (solid line in the figure) calculated for all the data is very close to that only for Ohmiya data. Therefore, the variation of spontaneous mutation frequency observed at Ohmiya is considered to be primarily reflecting the variation in temperature. The higher spontaneous mutation frequency at lower temperature may be dependent upon a repair mechanism which is less effective at lower temperature thus presumably an enzymatic one (Takahashi and Ichikawa 1976; Ichikawa 1981b).

Mericle et al. (1976) demonstrated that a large diurnal temperature difference (≥22.2°C) was very effective in increasing spontaneous somatic pink mutations in Tradescantia clone 02. However, the diurnal temperature difference under natural condition at Ohmiya was almost similar being about 10°C in the three experiments in this study and also in earlier experiments (Ichikawa 1971, 1972b, 1973). Therefore, the variation of spontaneous mutation frequency observed in the present study can not be attributed to the diurnal temperature difference.

The frequency of pink mutant events per hair can be converted into that per cell division in hairs by dividing the former by one less than the average number of cells per hair (Sparrow and Sparrow 1976; Ichikawa and Takahashi 1977, 1978), and the data converted are presented in Tables 1 to 4. The number of pink mutant events per hair-cell division was first determined for spontaneous pink mutations in order to compare the mutation frequency in Tradescantia stamen-hair system with those in other organisms (Sparrow and Sparrow 1976). The validity of this way of expressing mutation frequency depends on the assumption that spontaneous mutations occur randomly throughout the period of hair growth. Therefore, this method is applicable, besides to spontaneous mutations, to cases of chronic irradiations in which exposure times are long enough to cover the whole period of hair growth. In cases of shorter exposure times, as in the present case (one- or two-day exposures), on the other hand, the number of pink mutant events per cell division merely expresses a relative mutation frequency, although it represents a more precise relative value than the frequency per hair since the cell number per hair often differs between different clones (e.g., ca. 27 to 30 in KU 7 and ca. 19 to 20 in KU 9 on the average; see Tables 1 to 4) or after different treatments. In fact, KU 7 and KU 9 clones show very similar mutation frequencies per hair (compare Figs. 1 and 2 or 3 and 4), but more pink mutations occur per cell division in KU 9 than in KU 7. In this sense, KU 9 is judged to be more radiosensitive than KU 7 in terms of pink mutation induction.
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