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# Comparison of somatic mutation frequencies in the stamen hairs of one mutable and two stable clones of *Tradescantia* treated with small doses of gamma rays

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ABSTRACT

Induced somatic pink mutation frequencies in the stamen hairs of *Tradescantia* KU 20 clone, a blue/pink heterozygote highly mutable spontaneously at lower temperature, were studied after treating with relatively small doses of $^{60}$Co gamma rays (39 to 551 mGy or 3.9 to 55.1 rad), and were compared with those of two stable clones (non-mutable spontaneously), BNL 02 and KU 9, which are also blue/pink heterozygotes. It was found that the gamma-ray-induced mutation frequency in KU 20 clone was comparable (18.8 pink mutant events per $10^4$ hair-cell divisions per Gy) to those in BNL 02 (12.2 and 21.2) and KU 9 (17.4) clones, when the spontaneous mutation frequencies of KU 20 clone were relatively low (at most about 5.7 and 2.3 times of BNL 02 and KU 9 clones, respectively). However, when the spontaneous mutation frequencies of KU 20 clone were much higher (up to about 65 and 27 times of BNL 02 and KU 9 clones, respectively), induced mutation frequency was significantly higher in KU 20 clone (58.8 pink mutant events per $10^4$ hair-cell divisions per Gy) than in BNL 02 and KU 9 clones. The extent of increase in the gamma-ray-induced mutation frequency in the latter case was nevertheless very much less than the increase in the spontaneous mutation frequency, suggesting different mechanisms of initiation and repair of radiation-induced and spontaneous mutations.

## 1. INTRODUCTION

*Tradescantia* KU 20 clone heterozygous for flower color has been confirmed to be a temperature-sensitive mutable clone which is highly mutable spontaneously especially at lower temperature (Takahashi and Ichikawa, 1976; Ichikawa, 1984; Imai et al., 1991). However, the gamma-ray-induced somatic mutation frequency in the stamen hairs of this clone was reported to be comparable to those in a stable (non-mutable) clone, KU 9 (Ichikawa and Takahashi, 1977). These findings suggested that different mechanisms are involved in the initiation and repair of spontaneous and radiation-induced mutations (Takahashi and Ichikawa, 1976).

The present paper describes the results of a series of experiments which have been carried out to re-examine the gamma-ray-induced mutation frequency in the stamen hairs of KU 20 clone and to compare that with those in two stable clones, BNL 02 and KU 9, which are also heterozygous for flower color and have been

used in a variety of studies of somatic mutation induction by ionizing radiations and various chemical mutagens (see Ichikawa, 1991).

## 2. MATERIALS AND METHODS

*Materials*: KU 20 clone is a triploid clone ($3x=18$) heterogyzous for flower color (blue/pink; the blue color being dominant). The origin of this clone remains unknown, but this clone possesses part of the characteristics of *T. ohiensis* Raf., thus appears to be a hybrid between this and an unidentified species (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1991). KU 20 clone has been reported to be highly mutable spontaneously especially at lower temperature, showing about a 23-fold difference in mutation frequency in the temperature range of 17.9 to 28.3°C (Takahashi and Ichikawa, 1976) or about a 40-fold difference in 17.5 to 27.5°C range (Imai et al., 1991), and has been therefore regarded as a temperature-sensitive mutable clone (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1991; Imai et al., 1991).

Two stable clones, BNL 02 and KU 9, which are also blue/pink heterozygotes, were used for comparison. BNL 02 clone is a diploid clone ($2n=2x=12$) thought to be derived from a hybrid between *T. occidentalis* (Britt.) Smyth. and *T. ohiensis* (Mericle and Mericle, 1971, 1973), and has been often used in studies of somatic mutations (see Ichikawa, 1991) as one of the most stable clones in terms of spontaneous somatic pink mutation frequency in the stamen hairs (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1991). KU 9 clone is a triploid hybrid ($3x=18$) between a blue-flowered diploid clone of *T. paludosa* And. et Woods. and a pink-flowered tetraploid clone of *T. ohiensis* (Ichikawa, 1972a; Ichikawa and Takahashi, 1977), and shows a relatively stable spontaneous mutation frequency which is about two times higher than that in BNL 02 clone (Ichikawa, 1984, 1991).

It has been demonstrated that all these three clones produce the identical blue-color pigment normally, and also the identical pink-color pigment when the dominant gene for the blue color underwent mutation (Kamigawara and Ichikawa, 1988).

*Gamma-ray treatments*: Seven serial gamma-ray irradiation experiments were performed. KU 20, BNL 02 and KU 9 clones were irradiated in six (except for Exp. 3), three (Exps. 3, 5 and 6) and two (Exps. 2 and 7) out of the seven experiments, respectively. Potted plants bearing young inflorescences just before initiating blooming were selected for these experiments from those which had been grown in a growth chamber (the Sherer CEL 38–15; see below for the environmental conditions), excepting Exp. 1 in which those plants having been grown in the greenhouse were used.

Irradiation treatments with $^{60}$Co gamma rays were carried out either in the gamma field of the Institute of Radiation Breeding, National Institute of Agricultural Science, Ohmiya, Ibaraki, for 20 hr (Exps. 1 and 5 to 7) or 4 hr (Exp. 2),

or in the $^{60}$Co irradiation facility of the Faculty of Engineering, Saitama University, for 18 hr (Exps. 3 and 4). All the exposure data (in R) were obtained by simultaneous dosimetry with the National UD-170L thermo-luminescence dosimeter (TLD) elements attached to individual potted plants (in the gamma field) or to individual inflorescences (in the $^{60}$Co facility), and with the National UD-502B thermo-luminescence reader. The exposure data obtained were converted into absorbed doses in Gy with a converting factor of $9.57\times10^{-3}$ (i.e., 1R=9.57 mGy or 0.957 rad).

During the irradiation treatments, control plants were placed either in the control field of the gamma field or in the operation room of the $^{60}$Co facility, in order to expose them to environmental conditions similar to those for irradiated plants except for gamma-ray exposures. The temperature during irradiation treatments were recorded with self temperature recorders, and the temperature ranges during transportations of the plants to and from the gamma field were measured with a maximum/minimum thermometer.

The gamma-rayed and control plants (excepting those in Exp. 1) were grown in the above growth chamber in which the temperature was maintained at $25\pm1^{\circ}$C during the day and $20\pm1^{\circ}$C at night, the humidity at 60%, and the day length was kept to be 16 hr with the maximum light intensity of 23 klx for 12 hr. The irradiated and control plants in Exp. 1 were grown in the greenhouse in which a 16-hr day length was maintained and the temperature and the humidity were continuously recorded with a self temperature/humidity recorder.

*Scoring methods*: The methods used for scoring pink mutations in the stamen hairs in the present study were essentially identical to those described elsewhere recently (Ichikawa et al., 1990; Ichikawa and Ishii, 1991; Ichikawa, 1991). All the flowers that opened during the experimental periods (for 21 days after irradiation) were collected daily from each potted plant or inflorescence used. The numbers of stamen hairs and of pink mutant events were scored for each flower, and the number of hair cells was also counted on ten representative hairs each of two oppositely located stamens per flower as described earlier (Ichikawa and Takahashi, 1977, 1978) to estimate the average number of cells per hair for calculating mutation frequency per hair-cell division. The definition of the pink mutant events has been described elsewhere (Ichikawa, 1981a, 1981b, 1984). The mutation frequency was expressed as the number of pink mutant events per $10^3$ hairs as well as that per $10^4$ hair-cell divisions (Ichikawa, 1984, 1991; Ichikawa et al., 1990; Ichikawa and Ishii, 1991), and the data were pooled for the 7-day peak period for each treatment (Ichikawa et al., 1990). The induced mutation frequencies per $10^4$ hair-cell divisions (rather than those per $10^3$ hairs) after subtracting each control frequency were used for the analyses of dose-response curves, since the average number of cells per hair differs between clones and may differ after different gamma-ray treatments (Ichikawa and Takahashi, 1977).

Table 1. Gamma-ray-induced pink mutation frequencies in the stamen hairs of KU 20 clone

| Dose in mGy | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs (±SE) | Minus control (±SE) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±SE) | Minus control (±SE) |
|---|---|---|---|---|---|---|---|
| Experiment 1[a] | | | | | | | |
| 0 | 24,047 | 2,305 | 95.9 ±1.9 | — | 24.56 | 40.7 ±0.9 | — |
| 39 | 11,633 | 1,199 | 103 ±3 | 7.22±3.40 | 25.12 | 42.7 ±1.2 | 2.05±1.49 |
| 87 | 9,550 | 993 | 104 ±3 | 8.13±3.66 | 24.21 | 44.8 ±1.4 | 4.11±1.65 |
| 137 | 7,724 | 930 | 120 ±4 | 24.6 ±4.2 | 25.10 | 50.0 ±1.6 | 9.28±1.84 |
| 188 | 8,550 | 1,103 | 129 ±4 | 33.2 ±4.1 | 25.03 | 53.7 ±1.6 | 13.0 ±1.8 |
| Experiment 2[b] | | | | | | | |
| 0 | 10,428 | 173 | 16.6 ±1.3 | — | 25.44 | 6.79±0.52 | — |
| 178 | 6,104 | 220 | 36.0 ±2.4 | 19.5 ±2.7 | 24.75 | 15.3 ±1.0 | 8.50±1.15 |
| Experiment 4[c] | | | | | | | |
| 0 | 10,985 | 97 | 8.83±0.89 | — | 25.66 | 3.58±0.36 | — |
| 140 | 2,708 | 44 | 16.2 ±2.4 | 7.42±2.59 | 26.77 | 6.31±0.95 | 2.72±1.02 |
| 180 | 3,405 | 64 | 18.8 ±2.3 | 9.97±2.49 | 26.30 | 7.43±0.93 | 3.85±1.00 |
| 513 | 2,425 | 83 | 34.2 ±3.7 | 25.4 ±3.8 | 24.67 | 14.5 ±1.6 | 10.9 ±1.6 |
| 551 | 1,996 | 69 | 34.6 ±4.1 | 25.7 ±4.2 | 25.25 | 14.3 ±1.7 | 10.7 ±1.8 |
| Experiment 5[d] | | | | | | | |
| 0 | 9,866 | 56 | 5.86±0.76 | — | 24.97 | 2.37±0.32 | — |
| 64 | 2,891 | 25 | 8.65±1.72 | 2.97±1.88 | 25.19 | 3.57±0.71 | 1.21±0.78 |
| 144 | 2,723 | 28 | 10.3 ±1.9 | 4.61±2.08 | 24.93 | 4.30±0.81 | 1.93±0.87 |
| 316 | 1,576 | 33 | 20.9 ±3.6 | 15.3 ±3.7 | 25.51 | 8.54±1.49 | 6.18±1.52 |
| Experiment 6[e] | | | | | | | |
| 0 | 7,894 | 31 | 3.93±0.70 | — | 23.18 | 1.77±0.32 | — |
| 63 | 1,182 | 8 | 6.77±2.38 | 2.84±2.49 | 22.89 | 3.09±1.09 | 1.32±1.14 |
| 147 | 1,758 | 17 | 9.67±2.33 | 5.74±2.44 | 24.40 | 4.13±1.00 | 2.36±1.05 |
| 315 | 2,320 | 35 | 15.1 ±2.5 | 11.2 ±2.6 | 23.14 | 6.81±1.15 | 5.04±1.19 |
| Experiment 7[e] | | | | | | | |
| 0 | 9,207 | 39 | 4.24±0.68 | — | 25.43 | 1.73±0.28 | — |
| 219 | 7,235 | 75 | 10.4 ±1.2 | 6.13±1.37 | 25.85 | 4.17±0.48 | 2.44±0.56 |
| 472 | 2,856 | 81 | 28.4 ±3.1 | 24.1 ±3.2 | 25.21 | 11.7 ±1.3 | 9.98±1.33 |

[a] Irradiated in the gamma field and grown in GH; 14.0 to 20.5°C (av. 16.8°C) during irradiation, 21.5 to 25°C during transportation, and 18.4 to 31.4°C (av. 23.8°C) in GH.

[b] Irradiated in the gamma field and grown in CEL; 6.0 to 11.0°C (av. 8.2°C) during irradiation, and 17 to 22.5°C during transportation.

[c] Irradiated in the $^{60}$Co facility and grown in CEL; 18.1 to 22.0°C (av. 19.8°C) during irradiation.

[d] Irradiated in the gamma field and grown in CEL; 15.5 to 28.0°C (av. 20.2°C) during irradiation, and 22.5 to 25.5°C during transportation.

[e] Irradiated in the gamma field and grown in CEL; 13.5 to 23.5°C (av. 17.2°C) during irradiation, and 20 to 24°C during transportation.

## 3. RESULTS

*Induced mutation frequencies in KU 20 clone*: The gamma-ray-induced somatic pink mutation frequencies in the stamen hairs of KU 20 clone obtained in Exps. 1, 2 and 4 to 7 are listed in Table 1. The induced mutation frequencies observed in Exps. 1 and 2 are apparently higher than those in Exps. 4 to 7, taking the gamma-ray doses applied into consideration. When the induced mutation frequencies in the numbers of pink mutant events per $10^4$ hair-cell divisions are plotted against gamma-ray dose on a log-log graph, the difference between the results in the former two experiments and those in the latter four experiments becomes much clearer, as shown in Fig. 1. It should be noted that the spon-

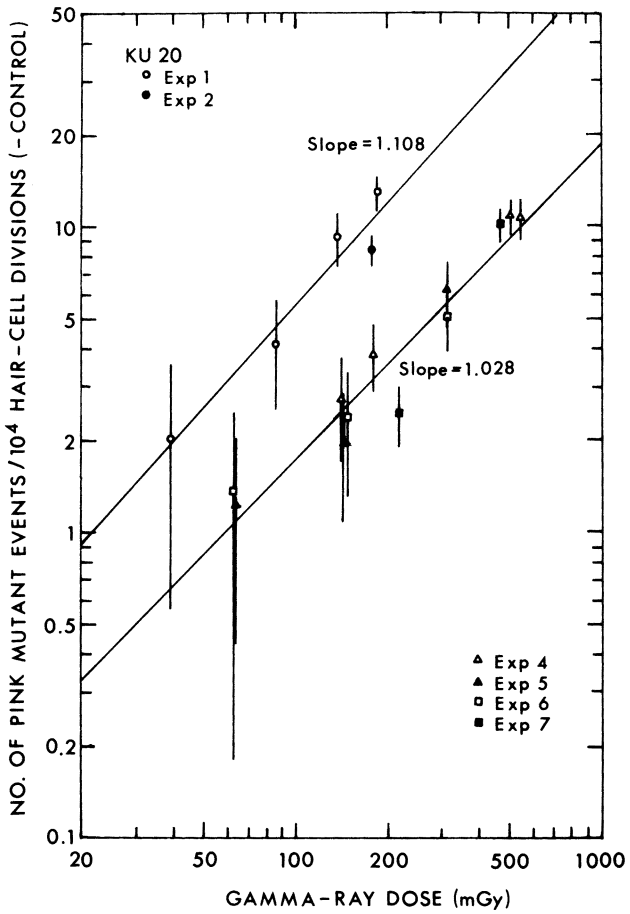


Fig. 1. Relationships between gamma-ray dose in mGy (log) and the number of pink mutant events per $10^4$ hair-cell divisions (log) in the stamen hairs of KU 20 clone. The best-fit regression lines were obtained by the least square method for Exps. 1 and 2 and Exps. 4 to 7. The longitudinal line attached to each point indicates standard error.

taneous mutation frequency in the control of Exp. 1 which was conducted in the greenhouse was very high (95.9±1.9 and 40.7±0.9 pink mutant events per $10^3$ hairs and per $10^4$ hair-cell divisions, respectively; see Table 1), and that in the control of Exp. 2 in which the plants used were exposed to much lower temperature than in other experiments (see the footnotes to Table 1) was also relatively high (16.1±1.3 and 6.79±0.52, respectively).

The regression lines drawn in Fig. 1, which were obtained by the least square method, have slopes of 1.108 (for Exps. 1 and 2) and 1.028 (for Exps. 4 to 7), both of which are not significantly different from 1. Therefore, it was considered not to be unreasonable to calculate the induced mutation frequency per Gy for each of the two groups, regarding the induced mutation frequency to be a linear function of dose. The resultant values for Exps. 1 and 2 and Exps. 4 to 7 are 58.8 and 18.8 pink mutant events per $10^4$ hair-cell divisions per Gy, respectively.

Table 2. Gamma-ray-induced pink mutation frequencies in the stamen hairs of BNL 02 clone

| Dose in mGy | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs (±SE) | Minus control (±SE) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±SE) | Minus control (±SE) |
|---|---|---|---|---|---|---|---|
| Experiment 3[a] | | | | | | | |
| 0 | 8,861 | 12 | 1.35±0.39 | — | 20.42 | 0.697±0.201 | — |
| 183 | 2,207 | 12 | 5.44±1.57 | 4.08±1.61 | 20.09 | 2.85 ±0.82 | 2.15±0.85 |
| 224 | 3,098 | 20 | 6.46±1.44 | 5.10±1.49 | 21.00 | 3.23 ±0.72 | 2.53±0.75 |
| 234 | 2,461 | 20 | 8.13±1.81 | 6.77±1.85 | 20.22 | 4.23 ±0.95 | 3.53±0.97 |
| 340 | 2,924 | 26 | 8.89±1.74 | 7.54±1.78 | 20.28 | 4.61 ±0.90 | 3.91±0.93 |
| 365 | 3,496 | 40 | 11.4 ±1.8 | 10.1 ±1.8 | 20.90 | 5.75 ±0.91 | 5.05±0.93 |
| 416 | 1,677 | 16 | 9.54±2.37 | 8.19±2.41 | 20.12 | 4.99 ±1.25 | 4.29±1.26 |
| 521 | 2,843 | 39 | 13.7 ±2.2 | 12.4 ±2.2 | 20.66 | 6.98 ±1.12 | 6.28±1.13 |
| Experiment 5[b] | | | | | | | |
| 0 | 13,560 | 14 | 1.03±0.28 | — | 20.47 | 0.530±0.142 | — |
| 64 | 5,690 | 23 | 4.04±0.84 | 3.01±0.89 | 20.11 | 2.12 ±0.44 | 1.58±0.46 |
| 144 | 5,634 | 43 | 7.63±1.16 | 6.60±1.19 | 20.58 | 3.90 ±0.59 | 3.37±0.61 |
| 316 | 5,158 | 67 | 13.0 ±1.6 | 12.0 ±1.6 | 19.27 | 7.11 ±0.87 | 6.58±0.88 |
| Experiment 6[c] | | | | | | | |
| 0 | 9,126 | 11 | 1.21±0.36 | — | 19.21 | 0.662±0.200 | — |
| 63 | 2,439 | 9 | 3.69±1.23 | 2.49±1.28 | 18.82 | 2.07 ±0.69 | 1.41±0.72 |
| 147 | 2,684 | 18 | 6.71±1.58 | 5.50±1.62 | 19.30 | 3.66 ±0.86 | 3.00±0.89 |
| 315 | 3,204 | 39 | 12.2 ±1.9 | 11.0 ±2.0 | 18.48 | 6.96 ±1.11 | 6.30±1.13 |

[a] Irradiated in the $^{60}$Co facility and grown in CEL; 17.8 to 21.6°C (av. 19.5°C) during irradiation.
[b] Irradiated in the gamma field and grown in CEL; 15.5 to 28.0°C (av. 20.2°C) during irradiation, and 22.5 to 25.5°C during transportation.
[c] Irradiated in the gamma field and grown in CEL; 13.5 to 23.5°C (av. 17.2°C) during irradiation, and 20 to 24°C during transportation.

*Induced mutation frequencies in BNL 02 and KU 9 clones*: The data of somatic pink mutation frequency in the stamen hairs of BNL 02 clone obtained in Exps. 3, 5 and 6 are presented in Table 2. The spontaneous mutation frequencies in the controls of the three experiments were all comparable to each other being stable and very low. However, the gamma-ray-induced mutation frequencies observed in Exp. 3 were apparently lower than those in Exps. 5 and 6. The difference is more clearly seen when the induced numbers of pink mutant events per $10^4$ hair-cell divisions are plotted against dose on a log-log graph, as shown in Fig. 2. The regression lines obtained for Exp. 3 and Exps. 5 and 6 have slopes of 0.935 and 0.912, respectively, both of which are not significantly different from 1. The induced mutation frequencies of 12.2 and 21.2 pink mutant events per $10^4$ hair-cell divisions per Gy can be calculated for Exp. 3 and Exps. 5 and 6, respectively.

The somatic pink mutation frequencies in the stamen hairs of KU 9 clone observed in Exps. 2 and 7 are presented in Table 3. The spontaneous mutation frequencies in the controls of the two experiments were about two to three times

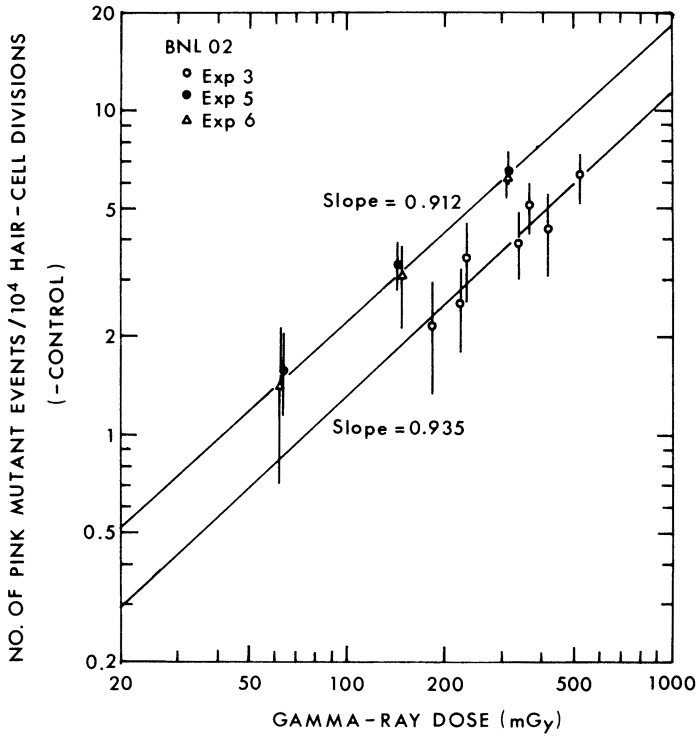


Fig. 2. Relationships between gamma-ray dose in mGy (log) and the number of pink mutant events per $10^4$ hair-cell divisions (log) in the stamen hairs of BNL 02 clone. The best-fit regression lines were obtained by the least square method for Exp. 3 and Exps. 5 and 6. The logitudinal line attached to each point indicates standard error.

Table 3. Gamma-ray-induced pink mutation frequencies in the stamen hairs of KU 9 clone

| Dose in mGy | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs (±SE) | Minus control (±SE) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±SE) | Minus control (±SE) |
|---|---|---|---|---|---|---|---|
| Experiment 2[a] | | | | | | | |
| 0 | 16,590 | 38 | 2.29±0.37 | — | 18.52 | 1.31±0.21 | — |
| 95 | 9,339 | 45 | 4.82±0.72 | 2.58±0.81 | 18.14 | 2.81±0.42 | 1.50±0.47 |
| 178 | 5,687 | 41 | 7.21±1.12 | 4.92±1.18 | 18.30 | 4.17±0.65 | 2.86±0.68 |
| 278 | 3,302 | 32 | 9.69±1.70 | 7.40±1.74 | 18.14 | 5.65±1.00 | 4.35±1.02 |
| 363 | 3,354 | 45 | 13.4 ±2.0 | 11.1 ±2.0 | 18.03 | 7.88±1.17 | 6.57±1.19 |
| Experiment 7[b] | | | | | | | |
| 0 | 31,702 | 101 | 3.19±0.32 | — | 19.15 | 1.76±0.17 | — |
| 219 | 3,626 | 33 | 9.10±1.58 | 5.92±1.61 | 18.85 | 5.10±0.89 | 3.34±0.90 |
| 472 | 2,921 | 58 | 19.9 ±2.6 | 16.7 ±2.6 | 18.98 | 11.0 ±1.4 | 9.26±1.46 |

[a] Irradiated in the gamma field and grown in CEL; 6.0 to 11.0°C (av. 8.2°C) during irradiation, and 17 to 22.5°C during transportation.

[b] Irradiated in the gamma field and grown in CEL; 13.5 to 23.5°C (av. 17.2°C) during irradiation, and 20 to 24°C during transportation.

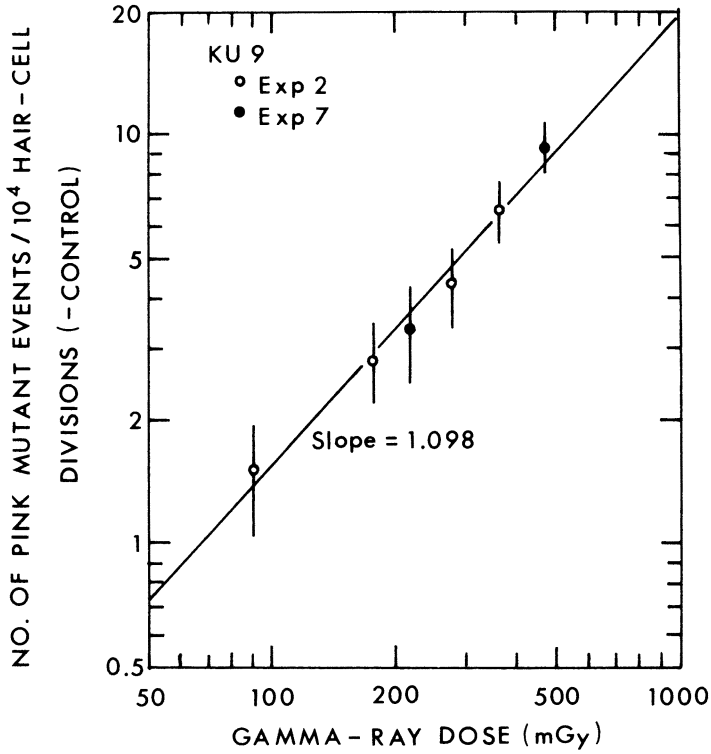


Fig. 3. Relationship between gamma-ray dose in mGy (log) and the number of pink mutant events per $10^4$ hair-cell divisions (log) in the stamen hairs of KU 9 clone. The best-fit regression line was obtained by the least square method. The longitudinal line attached to each point indicates standard error.

higher than those of BNL 02 clone (Table 2), and were about comparable to the spontaneous mutation frequencies of KU 20 clone at or near the lowest end (see Imai et al., 1991). When the gamma-ray-induced numbers of pink mutant events per $10^4$ hair-cell divisions in the two experiments are plotted against dose on a log-log graph, a regression line with a slope of 1.098 can be obtained as shown in Fig. 3, the value of the slope being also not different significantly from 1. The induced mutation frequency calculated from the linear dose-response relationship is 17.4 pink mutant events per $10^4$ hair-cell divisions per Gy.

## 4. DISCUSSION

The gamma-ray-induced pink mutation frequencies in the stamen hairs of KU 20 clone observed in Exps. 1 and 2 were apparently higher than those in Exps. 4 to 7 (Table 1 and Fig. 1). Grouping the data into Exps. 1 and 2 and Exps. 4 to 7, the dose-response relationships were judged to be linear based on the slopes of the best-fit regression lines on a log-log graph (Fig. 1), and the induced mutation frequencies of 58.8 and 18.8 pink mutant events per $10^4$ hair-cell divisions per Gy were calculated for Exps. 1 and 2 and Exps. 4 to 7, respectively. The two experiments in which the higher induced mutation frequency per Gy was obtained were those carried out either by growing irradiated and control plants in the greenhouse (Exp. 1) or by exposing plants to much lower temperature than in other experiments during the irradiation treatment (Exp. 2), and a very high or a relatively high spontaneous mutation frequencies were observed in these two experiments (Table 1), as expected from the findings by Imai et al. (1991).

The gamma-ray-induced mutation frequencies in BNL 02 clone (Table 2) were also grouped into Exp. 3 and Exps. 5 and 6, the frequencies in the latters being higher than in the former (Fig. 2). The induced mutation frequencies were judged to be linear functions of dose in the both groups, and the values of 12.2 and 21.2 pink mutant events per $10^4$ hair-cell divisions per Gy were obtained for Exp. 3 and Exps. 5 and 6, respectively.

The induced mutation frequencies in KU 9 clone (Table 3) were consistent in Exps. 2 and 7, although the plants used in Exp. 2 were exposed to much lower temperature during the irradiation treatments than in Exp. 7 (also no increase in the spontaneous mutation frequency in the control of Exp. 2). The induced frequencies were also judged to be a linear function of gamma-ray dose (Fig. 3), giving the induced mutation frequency of 17.4 pink mutant events per $10^4$ hair-cell divisions per Gy.

The linear relationships of pink mutation frequency with gamma-ray dose observed in the present study after semi-chronic irradiation treatments for 20, 18 or 4 hr agree well with earlier results obtained after similarly semi-chronic 16- or 20-hr treatments (Nayar and Sparrow, 1967; Ichikawa and Sparrow, 1968; Nauman et al., 1975; Ichikawa et al., 1978, 1981) or more chronic 8- to 30-day

treatments (Ichikawa, 1971, 1972b, 1973) of BNL 02, KU 9 or two other clones. The dose-response curve of the somatic pink mutations in *Tradescantia* stamen hairs has been proved to be linear at low dose (or exposure) rates (Nayar and Sparrow, 1967; Ichikawa and Sparrow, 1968; Ichikawa, 1971, 1972b, 1973; Nauman et al., 1975; Ichikawa et al., 1978, 1981), and also in the range of small doses even at higher dose rates (Sparrow et al., 1972; Nauman et al., 1975).

The dose-response regression line for KU 20 clone in Exps. 4 to 7 (the lower line in Fig. 1) was very close to that for KU 9 clone (Fig. 3), and was also located between the two regression lines for BNL 02 clone (Fig. 2). Comparing the dose-response relationships in another way, the lower induced mutation frequency of 18.8 pink mutant events per $10^4$ hair-cell divisions per Gy in KU 20 clone was close to the corresponding value of 17.4 in KU 9 clone, and was intermediate between the two corresponding values of 12.2 and 21.2 in BNL 02 clone. It seems therefore possible to consider that the induced mutation frequency of KU 20 clone obtained from Exps. 4 to 7 was comparable to those in BNL 02 and KU 9 clones. In these experiments, the spontaneous mutation frequencies of KU 20 clone were relatively low, being at most about 5.7 and 2.3 times of those of BNL 02 and KU 9 clones, respectively (Tables 1, 2 and 3).

On the other hand, the induced mutation frequency of KU 20 clone obtained from Exps. 1 and 2 (58.8 pink mutant events per $10^4$ hair-cell divisions per Gy) was about 3.1 times higher than that from Exps. 4 to 7 (18.8) (see also Fig. 1). In Exps. 1 and 2, the spontaneous mutation frequencies of KU 20 clone were very high and relatively high, respectiverly (Table 1).

Ichikawa and Takahashi (1977) reported that the induced numbers of pink mutant events per $10^3$ hairs per R in KU 20 and KU 9 clones were comparable (1.41 and 1.51, respectively) after acute (10-min) gamma-ray treatments with relatively small exposures comparable to the doses applied in the present study. They also reported that the induced number of pink mutant events per $10^4$ hair-cell divisions per R was smaller in KU 20 clone than in KU 9 clone (0.536 and 0.772, respectively; they can be converted into 56.0 and 80.7 per Gy, respectively). Although the above value of 58.8 pink mutant events per $10^4$ hair-cell divisions per Gy from Exps. 1 and 2 in the present study appears to agree with this value of 56.0 from the earlier study, the difference of dose rates between the earlier and the present studies must be considered, because clear effects of gamma-ray dose rate on mutation induction in the stamen hairs of *Tradescantia* have been reported (Nauman et al., 1975; Ichikawa and Takahashi, 1977; Ichikawa et al., 1978; Ichikawa, 1981b). Being different from the earlier 10-min acute gamma-ray treatments, semi-chronic 20-hr treatments with 39 to 188 mGy (Exp. 1) and a 4-hr irradiation with 178 mGy (Exp. 2) were conducted in the present study. According to Ichikawa and Takahashi (1977), such acute treatments resulted in an about three-fold higher induced mutation frequency per R as compared to such semi-chronic treatments. Therefore, the induced mutation

frequency per Gy obtained in Exps. 1 and 2 in the present study must be considered to be very high.

It seems possible to conclude that the induced mutation frequency in KU 20 clone becomes higher under the conditions under which the spontaneous mutation frequency of this mutable clone is much higher than those of stable clones. In fact, the spontaneous mutation frequencies of KU 20 clone in the controls of Exps. 1 and 2 ($40.7 \pm 0.9$ and $6.79 \pm 0.52$ pink mutant events per $10^4$ hair-cell divisions, respectively; Table 1) were about 65 and 11 times higher than that of BNL 02 clone, respectively (see Table 2), and about 27 and 4.4 times higher than that of KU 9 clone, respectively (see Table 3). However, the extent of increase in the gamma-ray-induced mutation frequency in KU 20 clone in Exp. 1 was very much less than the increase in spontaneous mutation frequency of this clone observed in the same experiment (Table 1).

Comparing the results from KU 20 clone in Exps. 1 and 2 with those in Exps. 4 to 7, and taking the above earlier results from this clone (Ichikawa and Takahashi, 1977) into consideration, it is very likely that different mechanisms are certainly involved in the initiation and repair of radiation-induced and spontaneous mutations in this clone, as suggested earlier (Takahashi and Ichikawa, 1976), although some part of the mechanisms are common in the both.

The majority of mutations induced by ionizing radiation are due to chromosomal breaks as reviewed earlier (Nauman et al., 1975; Ichikawa et al., 1978; Ichikawa, 1981b), while it is generally accepted that spontaneously occurring mutations are predominantly the results of errors during DNA replication thus being largely of base-change type. As for the induced somatic pink mutations in *Tradescantia* stamen hairs at lower dose rates and/or with small doses of radiation, the linear dose-response curves as discussed above and as obtained in the present study suggest that predominantly one-hit events occur at such lower dose rates and with small doses (Ichikawa et al., 1978; Ichikawa, 1981b), the one-hit events being those such as single chromosomal breaks leading to base-deletion type mutations (Ichikawa, 1981b). On the other hand, a significant part of the spontaneous somatic pink mutations in the stamen hairs of KU 20 clone must be due to somatic recombinations, as discussed by Imai et al. (1991). The importance of somatic recombination was demonstrated earlier in the stamen hairs of *T. hirsuticaulis* (BNL 2091 clone) by Christianson (1975) as the predominant mechanism of spontaneous mutations and a part of the mechanisms of gamma-ray-induced mutations. Therefore, the present results are considered to be reflecting such different (but probably common in part) mechanisms of producing radiation-induced and spontaneous mutations as well as the resultant difference in repair mechanism.

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