Genes Genet. Syst. (1996) **71**, p. 159–165

Variation of spontaneous somatic mutation frequency in the stamen hairs of *Tradescantia* clone BNL 02

Sadao Ichikawa*, Naoko Shima, Chizu Ishii, Hiromi Kanai, Marie Sanda-Kamigawara and Chie Matsuura-Endo†

Laboratory of Genetics, Department of Regulation Biology, Faculty of Science, Saitama University, Urawa 338, Japan

(Received 5 April 1996)

Variation of spontaneous somatic mutation frequency was studied in the stamen hairs of *Tradescantia* clone BNL 02 heterozygous for flower color (blue/pink; the blue color being dominant), one of the most stable clones in terms of spontaneous mutation frequency. Young inflorescence-bearing potted plants of this clone were grown under several different controlled environmental conditions, and the spontaneous pink mutation frequency in the stamen hairs was scored daily for three (partly two) weeks. The average number of hairs per stamen decreased as inflorescences became older, especially during the first one week or so after the inflorescences initiated flowering. The average number of cells per hair also decreased with the age of inflorescences, but the decreases were much smaller than those in the number of hairs. On the other hand, the spontaneous mutation frequency expressed as the number of pink mutant events per 10^4 hair-cell divisions did not show any significant changes with the age of inflorescences. The spontaneous mutation frequency varied, however, depending on the controlled environmental conditions and showing nearly significant negative and positive correlations with average temperature and diurnal temperature difference, respectively, although the variation observed was very much smaller than those reported earlier in a temperature-sensitive mutable clone KU 20 and also smaller than those in other clones.

INTRODUCTION

The stamen-hair system of *Tradescantia* heterozygous for flower color has proven to be one of the most suitable materials to study the frequency of mutations induced by low doses of various ionizing radiations and chemical mutagens as reviewed earlier (Underbrink et al., 1973; Schairer and Sautkulis, 1982; Schairer et al., 1983; Ichikawa, 1992). The system has also been used successfully for detecting mutagenic synergisms among chemical mutagens and X rays (Cebulska-Wasilewska et al., 1981; Ichikawa, 1992; Ichikawa et al., 1993; Shima and Ichikawa, 1994, 1995; Xiao and Ichikawa, 1995), as well as for studying the variations of spontaneous mutation frequency (Sparrow and Sparrow, 1976; Takahashi and Ichikawa, 1976; Mericle et al., 1976; Nauman et al., 1978; Ichikawa et al., 1981, 1995, 1996; Ichikawa, 1984; Imai et al., 1991).

Ichikawa (1984) studied the frequencies of spontaneously occurring pink mutations in the stamen hairs of 14 different blue/pink heterozygous *Tradescantia* clones, and found that the spontaneous mutabilities could be classified into six different classes, and that clone BNL 02 was the most stable clone belonging to the class with the lowest mutation frequency. It has been demonstrated later that clone KU 27, a segregant from clone BNL 02, also shows a low spontaneous mutation frequency very comparable to that in the parental clone (Sanda-Kamigawara et al., 1991).

This paper describes the variation of spontaneous mutation frequency observed in the stamen hairs of clone BNL 02, one of such most stable clones, by analyzing the data accumulated during earlier studies (Ichikawa, 1984, 1992; Ichikawa et al., 1990; Ichikawa and Ishii, 1991; Sanda-Kamigawara et al., 1991) and those not published yet, to determine whether or not and how much, if any, the spontaneous mutation frequency is affected by the environmental conditions and the age of inflorescences.

* Corresponding author.
† Present address: Crop Quality Research Team, Upland Agriculture Research Center, Hokkaido National Agricultural Experiment Station, Memuro, Kasai 082, Japan.

MATERIALS AND METHODS

Materials used. The potted plants of clone BNL 02 bearing young inflorescences before initiating flowering were selected as materials. This clone is a diploid hybrid ($2n$=12) thought to be derived from a hybrid between *T. occidentalis* (Britt.) Smyth. and *T. ohiensis* Raf. (Mericle and Mericle, 1971, 1973), and has often been used in studies of somatic mutations (Underbrink et al., 1973; Ichikawa, 1992) as one of the most stable clones in terms of spontaneous mutation frequency (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1992).

Growing conditions. The potted plants were grown in either a sun-beamed growth chamber (Koitotron 3S-135), one of three indoor-type growth chambers (Sherer CEL 38-15, Conviron E8, or Conviron EF7), or the growth room of our laboratory.

The environmental conditions in the Koitotron were 25±2°C during the day and 20±1°C at night (shifting quickly), and 16-h day length being supplemented with white fluorescent tubes before sunrise and after sunset.

In the Sherer growth chamber, the environmental conditions were more strictly controlled in the following three ways: (a) 25.0±0.5°C during the day and 20.0±0.5°C at night (shifting quickly), 60% humidity, and 16-h day length with the maximum light intensity of 23 klx from Sylvania VHO cool white fluorescent tubes and incandescent bulbs for 13 h; (b) 23.0±0.5/20.0±0.5°C day/night shift and 17-h day length with maximum 17 klx for 14 h; and (c) 21.0±0.5/19.0±0.5°C day/night shift and 16-h day length with maximum 23 klx for 12 h. The humidity and the light sources in (b) and (c) were identical to those in (a).

In the two Convirons, the temperature was controlled to change gradually between 21.0±0.5°C (at 2 pm) and 19.0±0.5°C (at 2 am) with a sine curve, 50% humidity, and the day length was 17 h with the maximum light intensity of 15 (E8) or 13 klx (EF7) for 13 h and 40 min, with gradual changes also in the light intensity from the light sources similar to those in the Sherer growth chamber.

The environmental conditions in the growth room were 23.0±0.5°C (constant), 50% humidity, and a 16-h day length with a light intensity of 6 klx from Toshiba DR400/T(L) metal-halide sunlamps plus white fluorescent tubes.

The plants were acclimated to each of these environmental conditions for at least 10 days prior to initiating flowering (starting scoring of mutations), excepting one case (Exp. 11; see Results). They were watered every morning, and a 1/1,000 Hyponex solution was applied as fertilizer once a week.

Scoring methods. All the flowers that opened during the scoring periods of three (partly two) weeks were collected daily. The methods used for scoring pink mutations in stamen hairs in the present study were identical to those described earlier (Ichikawa et al., 1990, 1991, 1993; Ichikawa and Ishii, 1991; Sanda-Kamigawara et al., 1991; Ichikawa, 1992). Briefly, the numbers of stamen hairs and of pink mutant events (PMEs) were scored on each of six stamens of every flower; and the number of hair cells was also counted on 10 representative hairs each of two oppositely located stamens per flower (Ichikawa and Ishii, 1991), to estimate the average number of cells per hair for calculating mutation frequency per hair-cell division (Ichikawa and Takahashi, 1977, 1978; Ichikawa, 1984, 1992; Ichikawa and Ishii, 1991). A PME has been defined to represent the result of a single mutation (Ichikawa 1981, 1992, 1994). The mutation frequency was expressed as the number of PMEs per 10^4 hair-cell divisions, as the most accurate mutation frequency in the stamen hairs (Ichikawa, 1992). In the present study, the data were pooled for every week.

Differences of mutation frequencies between different weeks or experiments were examined by chi-square tests. Correlations of mutation frequencies with environmental factors were also examined by the absolute values of correlation coefficients and the degree of freedom.

RESULTS

Experiments in Koitotron. Three experiments (Exps. 1 to 3) were conducted in the sun-beamed Koitotron. During the scoring periods, about 8 to 15% decreases in the average number of hairs per stamen were observed in the second and third weeks as shown in Table 1. Decreases in the average number of cells per hair were also observed in the second and third weeks, but the decreases were much smaller than those in the hair number. The spontaneous mutation frequencies scored showed some fluctuations between weeks in each experiment and also between experiments, but the values of 0.744±0.171 to 1.01±0.13 PMEs per 10^4 hair-cell divisions did not show any significant differences among them or any clear changes with the age of inflorescences (Table 1).

Experiments in Sherer growth chamber. Six experiments (Exps. 4 to 9) were performed in the Sherer growth chamber under three different environmental conditions. Under the condition of 25/20°C day/night shift, 23 klx and 16-h day length (Exps. 4 and 5), about 7 to 11% decreases in the average number of hairs per stamen were observed in the second and/or third weeks of the scoring periods as shown in Table 2. Some decreases in the average number of cells per hair with the age of inflorescences were also observed. Although the spontaneous mutation frequencies fluctuated (0.567±0.284 to 1.03±0.39 PMEs per 10^4 hair-cell divisions), neither significant differences among them nor obvious age-dependent changes were observed (Table 2).

Under the condition of 23/20°C day/night shift, 17 klx

Table 1. Spontaneous pink mutation frequencies in the stamen hairs of clone BNL 02 determined in the Koitotron

Scoring week	No. of flowers observed	No. of hairs scored	Av. no. of hairs /stamen	No. of PMEs[a] scored	Av. no. of cells /hair	No. of PMEs /10^4 cell divisions (±SE)
Exp. 1						
1	50	14,562	48.54(1.00)[b]	28	20.75(1.00)[b]	0.974±0.184
2	51	13,488	44.08(0.91)	23	20.02(0.96)	0.897±0.187
Total	101	28,050	46.29	51	20.40	0.937±0.131
Exp. 2						
1	150	36,937	41.04(1.00)	68	19.73(1.00)	0.983±0.119
2	150	31,565	35.07(0.85)	58	19.22(0.97)	1.01 ±0.13
3	150	33,889	37.65(0.92)	59	19.40(0.98)	0.946±0.123
Total	450	102,391	37.92	185	19.45	0.979±0.072
Exp. 3						
1	50	14,455	48.18(1.00)	23	21.22(1.00)	0.787±0.164
2	50	13,166	43.89(0.91)	19	20.39(0.96)	0.744±0.171
3	50	12,746	42.49(0.88)	22	20.46(0.96)	0.887±0.189
Total	150	40,367	44.85	64	20.71	0.804±0.101

[a] Pink mutant events.
[b] Ratio against the value in the first week.

Table 2. Spontaneous pink mutation frequencies in the stamen hairs of clone BNL 02 determined in the Sherer growth chamber

Scoring week	No. of flowers observed	No. of hairs scored	Av. no. of hairs /stamen	No. of PMEs[a] scored	Av. no. of cells /hair	No. of PMEs /10^4 cell divisions (±SE)
Exp. 4 (25/20°C)						
1	18	5,164	47.81(1.00)[b]	7	20.94(1.00)[b]	0.680±0.257
2	17	4,552	44.63(0.93)	6	20.59(0.98)	0.673±0.275
3	15	3,817	42.41(0.89)	4	19.47(0.93)	0.567±0.284
Total	50	13,533	45.11	17	20.38	0.648±0.157
Exp. 5 (25/20°C)						
1	34	8,138	39.89(1.00)	12	18.97(1.00)	0.821±0.237
2	20	4,605	38.38(0.96)	5	18.47(0.97)	0.622±0.278
3	18	3,987	36.92(0.93)	7	18.10(0.95)	1.03 ±0.39
Total	72	16,730	38.73	24	18.61	0.815±0.166
Exp. 6 (23/20°C)						
1	16	4,222	43.98(1.00)	7	20.32(1.00)	0.858±0.324
2	19	4,874	42.75(0.97)	9	20.25(1.00)	0.959±0.320
3	16	3,526	36.73(0.84)	5	19.55(0.96)	0.764±0.342
Total	51	12,622	41.25	21	20.05	0.873±0.191
Exp. 7 (23/20°C)						
1	12	2,821	39.18(1.00)	4	20.53(1.00)	0.726±0.363
2	20	4,591	38.26(0.98)	8	20.72(1.01)	0.884±0.312
3	9	2,018	37.37(0.95)	6	20.05(0.98)	1.56 ±0.64
Total	41	9,430	38.33	18	20.52	0.978±0.230
Exp. 8 (23/20°C)						
1	22	5,459	41.36(1.00)	9	20.09(1.00)	0.864±0.288
2	18	4,278	39.61(0.96)	10	19.44(0.97)	1.27 ±0.40
3	16	3,698	38.52(0.93)	8	19.93(0.99)	1.14 ±0.40
Total	56	13,435	39.99	27	19.84	1.07 ±0.21
Exp. 9 (21/19°C)						
1	24	7,898	54.85(1.00)	14	21.83(1.00)	0.851±0.227
2	21	6,639	52.69(0.96)	12	20.86(0.96)	0.910±0.263
3	21	5,933	47.09(0.86)	9	20.57(0.94)	0.775±0.258
Total	66	20,470	51.69	35	21.12	0.850±0.144

[a] Pink mutant events.
[b] Ratio against the value in the first week.

and 17-h day length (Exps. 6 to 8), about 5 to 16% decreases in the average hair number per stamen were observed in the third week, while the average cell number per hair showed only slight decreases (Table 2). The spontaneous mutation frequencies obtained in these experiments also showed a considerable fluctuation (0.726±0.363 to 1.56±0.64 PMEs per 10^4 hair-cell divisions) and were generally higher than those obtained in Exps. 4 and 5, but neither significant differences among them nor obvious age-dependent changes were observed also in these experiments (Table 2).

In Exp. 9 performed under the conditions of 21/19°C day/night shift, 23 klx and 16-h day length, about 14 and 6% decreases in the average hair number per stamen and the average cell number per hair, respectively, were observed in the third week (Table 2). The frequencies of 0.775±0.258 to 0.910±0.263 PMEs per 10^4 hair-cell divisions obtained (Table 2) were apparently higher than those in Exp. 4 (but not significantly different), and were roughly comparable to those in Exps. 5 and 6.

Other experiments. In Exp. 10 conducted in the Conviron EF7 growth chamber, an about 8% decrease in the average hair number per stamen was observed in the third week, but the average cell number per hair showed only slight reductions (Table 3). Relatively high mutation frequencies (0.977±0.230 to 1.43±0.41 PMEs per 10^4 hair-cell divisions) were obtained in this experiment (Table 3), as compared with the above experiments.

In Exp. 11, the plants which had been grown in the Conviron E8 growth chamber were transferred to the Sherer growth chamber (23/20°C day/night shift) when their inflorescences were just initiating flowering, and the spontaneous mutation frequency was studied starting scoring on the next day of transferring. An about 8% decrease in the average hair number per stamen occurred in the second week, but only a slight reduction in the average cell number per hair was observed (Table 3). The mutation frequencies of 0.792±0.251 to 1.07±0.48 PMEs per 10^4 hair-cell divisions obtained (Table 3) were somewhat lower than those in Exp. 10, and were very close to those in Exps. 6 and 9 in the Sherer growth chamber (see Table 2).

Exp. 12 was performed in the growth room kept at a constant temperature of 23°C. About 10 to 12% decreases in the average hair number per stamen were observed, while only slight reductions in the average cell number per hair occurred (Table 3). Relatively low mutation frequencies of 0.533±0.202 to 0.736±0.233 PMEs per 10^4 hair-cell divisions were observed in this experiment (Table 3).

DISCUSSION

Stamen-hair growth and age of inflorescence. In all the experiments conducted, at least some and often considerable (up to 16%) decreases in the average number of hairs per stamen were observed as scoring period proceeded. The differences were especially conspicuous between the first and second weeks in most cases, and there were rather small differences between the second and third weeks in general (Tables 1 to 3). These results show that the stamen-hair development is related to the age of inflorescences. That is, the differentiation of hairs from the epidermal cells of stamen filament, as demonstrated by Mericle and Hazard (1980) with an optical microscope and a scanning electron microscope, was most active in several to at most 10 older flower buds of an inflorescence. These flower buds started their development when the inflorescence was younger, and opened

Table 3. Spontaneous pink mutation frequencies in the stamen hairs of clone BNL 02 determined in the Conviron EF7 and the growth room, or in the Sherer growth chamber transferring the plants from the Conviron E8

Scoring week	No. of flowers observed	No. of hairs scored	Av. no. of hairs /stamen	No. of PMEs[a] scored	Av. no. of cells /hair	No. of PMEs /10^4 cell divisions (±SE)
Exp. 10 (Conviron)						
1	38	9,677	42.44(1.00)[b]	18	20.04(1.00)[b]	0.977±0.230
2	23	5,524	40.03(0.94)	11	19.55(0.98)	1.07 ±0.46
3	19	4,431	38.87(0.92)	12	19.91(0.99)	1.43 ±0.41
Total	80	19,632	40.90	41	19.87	1.11 ±0.17
Exp. 11 (Sherer; plants from Conviron)						
1	22	6,597	49.98(1.00)	10	20.13(1.00)	0.792±0.251
2	9	2,496	46.22(0.92)	5	19.72(0.98)	1.07 ±0.48
Total	31	9,093	48.89	15	20.01	0.868±0.224
Exp. 12 (Growth room)						
1	26	7,501	48.08(1.00)	10	20.81(1.00)	0.673±0.213
2	27	7,030	43.40(0.90)	10	20.34(0.98)	0.736±0.233
3	27	6,858	42.33(0.88)	7	20.14(0.97)	0.533±0.202
Total	80	21,389	44.56	27	20.44	0.649±0.125

[a] Pink mutant events.
[b] Ratio against the value i n the first week.

during the early period of one week or so after the initiation of flowering of the inflorescence. The results obtained agree well with our recent findings in the cuttings of BNL 02 and other clones (Ichikawa et al., 1995).

Some decreases in the average number of cells per hair with the age of inflorescences were also observed in all the experiments conducted. However, the decreases were mostly much smaller than those in the average number of hairs per stamen (Tables 1 to 3). The smaller decreases in the cell number per hair than in the hair number per stamen also agree well with our findings in the cuttings of BNL 02 and other clones (Ichikawa et al., 1995).

These results show that the reductions in stamen-hair development or growth with the age of inflorescences are mainly caused by the decreases in the hair number per stamen, rather than those in the cell number per hair.

Spontaneous mutation frequency and age of inflorescence. In each of the 12 experiments conducted, some to considerable fluctuations in the spontaneous pink mutation frequency in the stamen hairs were observed between scoring weeks (Tables 1 to 3). However, the fluctuated frequencies had no consistent relationship with the age of inflorescences, and no significant difference was found among the fluctuated mutation frequencies in each experiment, indicating that there was no clear relationship between the spontaneous mutation frequency and the age of inflorescences. Some differences in spontaneous mutation frequency between young and old inflorescences have been reported earlier in clones BNL 02 (Mericle and Mericle, 1969) and KU 20 (Takahashi and Ichikawa, 1976), but negative results have also been reported in clones BNL 4430 (Sparrow and Sparrow, 1976) and KU 9 (Takahashi and Ichikawa, 1976). Clones BNL 4430 and KU 9 are fairly stable clones (Ichikawa, 1984, 1992), while clone KU 20 is a temperature-sensitive mutable clone (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992, 1994; Imai et al., 1991; Ichikawa et al., 1991; Sanda-Kamigawara et al., 1995). The present study shows that no effect of the age of inflorescences on spontaneous mutation frequency appears in clone BNL 02 at least within the first three weeks.

Some to considerable fluctuations in the mutation frequency were also found among or between the experiments under the same environmental conditions, i.e., among Exps. 1 to 3 in the Koitotron (Table 1), between Exps. 4 and 5 (25/20°C day/night) and among Exps. 6 to 8 (23/20°C day/night) in the Sherer growth chamber (Table 2). No significant difference was found, however, among or between the fluctuated mutation frequencies under the same conditions (see below), proving the stable nature of clone BNL 02 in terms of spontaneous mutation frequency (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1992).

Spontaneous mutation frequencies under different conditions. Out of the 12 spontaneous mutation frequencies based on the pooled data for the whole scoring periods of three (partly two) weeks (totals in Tables 1 to 3), three obtained in Exps. 1 to 3 in the Koitotron (Table 1) have been reported by Ichikawa (1984), Ichikawa and Ishii (1991) and Ichikawa et al. (1990), respectively; two in Exps. 4 and 9 in the Sherer growth chamber (Table 2) by Sanda-Kamigawara et al. (1991) and Ichikawa (1992), respectively; and the frequency in Exp. 12 in the growth room (Table 3) by Sanda-Kamigawara et al. (1991). The remaining six pooled mutation frequencies obtained in Exps. 5 to 8, 10 and 11 are reported here for the first time.

These 12 spontaneous mutation frequencies are graphed in Fig. 1. As seen in this figure, they showed considerable fluctuations, being relatively low in Exps. 4 and 12 and high in Exps. 10 and 8. When these differences were examined by the chi-square tests, statistically significant differences at 5% level were detected between the mutation frequencies in Exps. 10 and 12 and between those in Exps. 2 and 12, and none of other differences were found to be significant.

Since Exp. 12 was conducted in the growth room at the fixed temperature of 23.0±0.5°C, whereas Exp. 10 was carried out in the Conviron at 21.0±0.5 to 19.0±0.5°C changed

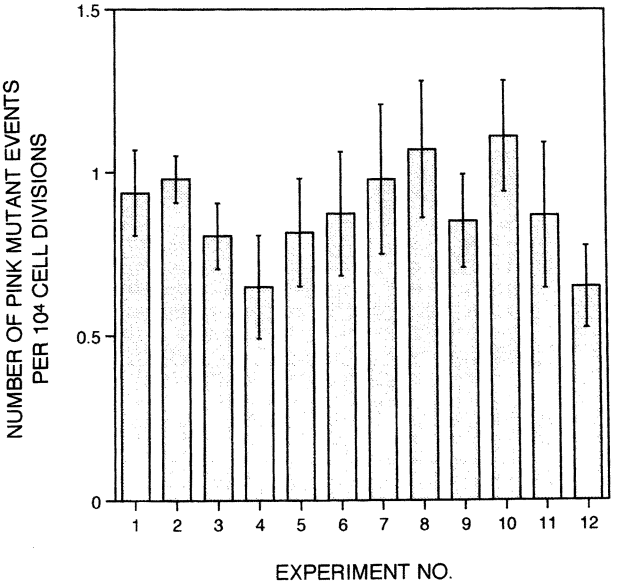

Fig. 1. Spontaneous pink mutation frequencies in the stamen hairs of clone BNL 02, obtained by pooling the data of whole scoring period in each of 12 experiments. Exps. 1 to 3 at 25±2/20±1°C day/night shift in the Koitotron; Exps. 4 and 5 at 25±0.5/20±0.5°C, Exps. 6 to 8 at 23±0.5/20±0.5°C and Exp. 9 at 21±0.5/19±0.5°C day/night shift in the Sherer; Exp. 10 at 21±0.5 to 19±0.5°C gradual change in the Conviron EF7; Exp. 11 at 23±0.5/20±0.5°C day/night shift in the Sherer transferring the plants from the Conviron E8; and Exp. 12 at 23±0.5°C constant in the growth room. The vertical line at the top of each column shows standard error.

gradually and Exp. 2 in the Koitotron at 25±2/20±1°C day/night temperatures shifted quickly with the largest diurnal temperature difference, the relationships of the spontaneous mutation frequency with the average temperature or with the diurnal temperature difference were analyzed.

As shown in Fig. 2 (the frequency from Exp. 11 is excluded because the plants were transferred from the Conviron to the Sherer), there was a tendency to result in higher spontaneous mutation frequencies at lower average temperatures. The correlation coefficient between average temperature and mutation frequency was calculated to be –0.5592, showing that there is a negative correlation between them. However, the absolute value of this correlation coefficient is smaller than (92.9% of) the value with which we can conclude that the negative correlation is statistically significant at 5% level (the degree of freedom is 9).

Fig. 2 also suggests that the spontaneous mutation frequency might be higher with larger diurnal temperature difference even at the same average temperature (compare three mutation frequencies obtained in the Koitotron with two those in the Sherer at 25/20°C). The correlation coefficient between diurnal temperature difference and mutation frequency calculated for all the points plotted in this figure is very small (0.0558), but the correlation coefficient for the 5 points at the average temperature of 22.5°C is calculated to be 0.7740, showing that there is a positive correlation between diurnal temperature difference and spontaneous mutation frequency. However, this figure is also smaller than (88.1% of) the value with which we can conclude that the correlation is significant at 5% level (the degree of freedom is 3).

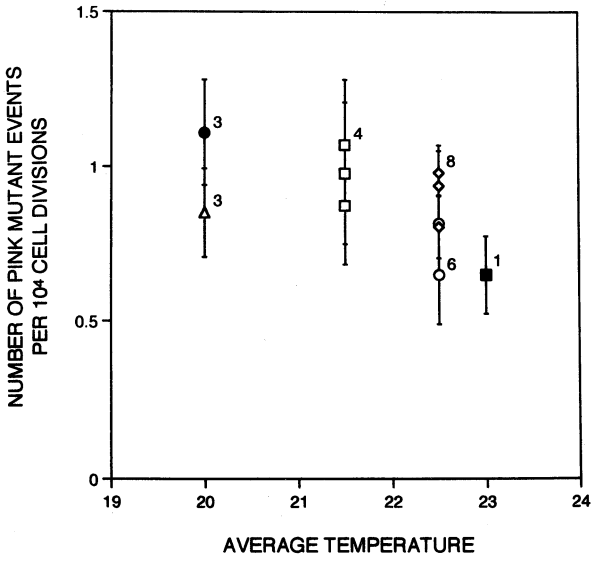

Fig. 2. Spontaneous pink mutation frequencies plotted against average temperature. ◇ Exps. 1 to 3 in the Koitotron (25±2/20±1°C day/night shift); ○ Exps. 4 and 5 in the Sherer (25±0.5/20±0.5°C day/night shift); □ Exps. 6 to 8 in the Sherer (23±0.5/20±0.5°C day/night shift); △ Exp. 9 in the Sherer (21±0.5/19±0.5°C day/night shift); ● Exp. 10 in the Conviron EF7 (21±0.5 to 19±0.5 gradual change); and ■ Exp. 12 in the growth room (23±0.5°C constant). The frequency from Exp. 11 is not included in this figure. The figure at the shoulder of each type of mark indicates the maximum diurnal temperature difference under each controlled condition. The standard errors for points plotted are overlapping to each other.

These nearly significant correlations with temperature factors, together with the detection of some significant variations in the spontaneous mutation frequency in this stable clone BNL 02 as described above (see Fig. 1), seem to agree with an earlier report (Mericle et al., 1976) but contradict another report (Sparrow and Sparrow, 1976) on the same clone. Namely, the tendency of higher spontaneous mutation frequencies with larger diurnal temperature difference found in the present study agrees with the earlier finding in the same clone (Mericle et al., 1976), but the negative correlation between spontaneous mutation frequency and average temperature observed in the present study contradicts the earlier report of higher spontaneous mutation frequencies at higher temperature in the same clone (Sparrow and Sparrow, 1976). However, the tendencies of higher mutation frequencies at lower temperature, similar to the present results, have been observed earlier in two stable clones KU 7 (Takahashi and Ichikawa, 1976; Ichikawa et al., 1981; Ichikawa, 1984) and KU 9 (Takahashi and Ichikawa, 1976; Ichikawa et al., 1981, 1996; Ichikawa, 1984) and three mutable clones KU 20 (Takahashi and Ichikawa, 1976; Ichikawa, 1984; Imai et al., 1991), KU 13 and KU 24 (Ichikawa, 1984). The variations of spontaneous mutation frequency found in clone BNL 02 in the present study were, however, smaller and much smaller than those in these stable and mutable clones, respectively. The rather small variations, which were nearly significantly correlated negatively and positively with average temperature and diurnal temperature difference, respectively, might have also been affected by other environmental factors.

The present finding of the variations of spontaneous mutation frequency (in the range of only 3°C difference in average temperature with 1 to 8°C diurnal temperature differences) in clone BNL 02 has an importance, since this clone had been most often used previously (Underbrink et al., 1973; Ichikawa, 1992) as one of the most stable clones in terms of spontaneous mutation frequency (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1992).

The present study was supported in part by Grants-in-Aid for Scientific Research (Nos. 358017 and 05454607) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Cebulska-Wasilewska, A., Leenhouts, H. P. and Chadwick, K. H. (1981) Synergism between EMS and X-rays for the induction of somatic mutations in *Tradescantia*. Int. J. Radiat. Biol.

40, 163–173.

Ichikawa, S. (1981) *In situ* monitoring with *Tradescantia* around nuclear power plants. Environ. Health Perspect. **37**, 145–164.

Ichikawa, S. (1984) Spontaneous somatic mutation frequencies in the stamen hairs of 14 different *Tradescantia* clones heterozygous for flower color. Environ. Exp. Bot. **24**, 259–266.

Ichikawa, S. (1992) *Tradescantia* stamen-hair system as an excellent botanical tester of mutagenicity: Its responses to ionizing radiations and chemical mutagens, and some synergistic effects found. Mutat. Res. **270**, 3–22.

Ichikawa, S. (1994) Sectoring patterns of spontaneous and radiation-induced somatic pink mutations in the stamen hairs of a temperature-sensitive mutable clone of *Tradescantia*. Jpn. J. Genet. **69**, 577–591.

Ichikawa, S. and Ishii, C. (1991) Validity of simplified scoring methods of somatic mutations in *Tradescantia* stamen hairs. Environ. Exp. Bot. **31**, 247–252.

Ichikawa, S. and Takahashi, C. S. (1977) Somatic mutation frequencies in the stamen hairs of stable and mutable clones of *Tradescantia* after acute gamma-ray treatments with small doses. Mutat. Res. **45**, 195–204.

Ichikawa, S. and Takahashi, C. S. (1978) Somatic mutations in *Tradescantia* stamen hairs exposed to ethyl methanesulfonate. Environ. Exp. Bot. **18**, 19–25.

Ichikawa, S., Imai, T. and Nakano, A. (1991) 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. Jpn. J. Genet. **66**, 513–525.

Ichikawa, S., Kanai, H. and Harada, H. (1990) Somatic mutation frequencies in *Tradescantia* stamen hairs treated with aqueous solutions of ethyl methanesulfonate and methyl methanesulfonate. Jpn. J. Genet. **65**, 309–321.

Ichikawa, S., Takahashi, C. S. and Nagashima-Ishii, C. (1981) Somatic mutation frequency in the stamen hairs of *Tradescantia* KU 7 and KU 9 clones exposed to low-level gamma rays. Jpn. J. Genet. **56**, 409–423.

Ichikawa, S., Yamaguchi, A. and Okumura, M. (1993) Synergistic effects of methyl methanesulfonate and X rays in inducing somatic mutations in the stamen hairs of *Tradescantia* clones, KU 27 and BNL 4430. Jpn. J. Genet. **68**, 277–292.

Ichikawa, S., Shima, N., Xiao, L. Z., Matsuura-Endo, C., Harada, H., Yogo, A. and Okumura, M. (1995) Flower production, stamen-hair growth, and spontaneous and induced somatic mutation frequencies in *Tradescantia* cuttings and shoots with roots cultivated with nutrient solutions. Jpn. J. Genet. **70**, 585–600.

Ichikawa, S., Nakano, A., Kenmochi, M., Yamamoto, I., Murai, M., Takahashi, E., Yamaguchi, A., Watanabe, K., Tomiyama, M., Sugiyama, K., Yogo, A., Yazaki, T., Okumura, M., Shima, N., Satoh, M., Yoshimoto, M. and Xiao, L. Z. (1996) Yearly variation of spontaneous somatic mutation frequency in the stamen hairs of *Tradescantia* clone KU 9 grown outdoors, which showed a significant increase after the Chernobyl accident. Mutat. Res. **349**, 249–259.

Imai, T., Ichikawa, S. and Sanda-Kamigawara, M. (1991) Variation of spontaneous somatic mutation frequency in the stamen hairs of a mutable clone of *Tradescantia*, KU 20. Jpn. J. Genet. **66**, 501–511.

Mericle, L. W. and Hazard, R. M. (1980) Stamen hair initiation and development in *Tradescantia*, clone 02. Environ. Exp. Bot. **20**, 233–241.

Mericle, L. W. and Mericle, R. P. (1969) Induced somatic mutations for interpreting floral development and inflorescence aging. In: Induced Mutations in Plants, editor pp. 591–600, IAEA, Vienna.

Mericle, L. W. and Mericle, R. P. (1971) Somatic mutations in clone 02 *Tradescantia*: A search for genetic identity. J. Hered. **62**, 323–328.

Mericle, L. W. and Mericle, R. P. (1973) Resolving the enigma of multiple mutant sectors in stamen hairs of *Tradescantia*. Genetics **73**, 575–582.

Mericle, R. P., Mericle, L. W. and Nunez, B. (1976) Environmental modulation of somatic mutations. In: Biological and Environmental Effects of Low-Level Radiation, vol. 1, pp. 31–38, IAEA, Vienna.

Nauman, C. H., Schairer, L. A. and Sparrow, A. H. (1978) Influence of temperature on spontaneous and radiation-induced somatic mutations in *Tradescantia* stamen hairs. Mutat. Res. **50**, 207–218.

Sanda-Kamigawara, M., Ichikawa, S. and Watanabe, K. (1991) Spontaneous, radiation- and EMS-induced somatic pink mutation frequencies in the stamen hairs and petals of a diploid clone of *Tradescantia*, KU 27. Environ. Exp. Bot. **31**, 413–421.

Sanda-Kamigawara, M., Tomiyama, M. and Ichikawa, S. (1995) Sectoring patterns of spontaneous and induced somatic pink mutations in the stamen hairs and petals of mutable and stable clones of *Tradescantia*. Jpn. J. Genet. **70**, 339–353.

Schairer, L. A. and Sautkulis, R. C. (1982) Detection of ambient levels of mutagenic atmospheric pollutants with higher plant *Tradescantia*. In: Environmental Mutagenesis, Carcinogenesis, and Plant Biology, vol. 2 (ed.: E. J. Klelowski Jr.), pp. 155–194, Praeger, New York.

Schairer, L. A., Sautkulis, R. C. and Tempel, N. R. (1983) A search for the identity of gaseous agents in the ambient air using the *Tradescantia* bioassay. In: Short-Term Bioassay in the Analysis of Complex Environmental Mixtures, vol. 3 (eds.: M. D. Waters, S. S. Sandhu, J. Lewtas, L. D. Claxton and S. Nesnow), pp. 211–228, Plenum, New York.

Shima, N. and Ichikawa, S. (1994) Synergisms detected among methyl methanesulfonate, ethyl methanesulfonate and X-rays in inducing somatic mutations in the stamen hairs of *Tradescantia* clone BNL 4430. Environ. Exp. Bot. **34**, 393–408.

Shima, N. and Ichikawa, S. (1995) Mutagenic synergism detected between dimethyl sulfate and X-rays but not found between *N*-methyl-*N*-nitrosourea and X-rays in the stamen hairs of *Tradescantia* clone BNL 4430. Mutat. Res. **331**, 79–87.

Sparrow, A. H. and Sparrow, R. C. (1976) Spontaneous somatic mutation frequencies for flower color in several *Tradescantia* species and hybrids. Environ. Exp. Bot. **16**, 23–43.

Takahashi, C. S. and Ichikawa, S. (1976) Variation of spontaneous mutation frequency in *Tradescantia* stamen hairs under natural and controlled environmental conditions. Environ. Exp. Bot. **16**, 287–293.

Underbrink, A. G., Schairer, L. A. and Sparrow, A. H. (1973) *Tradescantia* stamen hairs: A radiobiological test system applicable to chemical mutagenesis. In: Chemical Mutagens: Principles and Methods for their Detection, vol. 3 (ed.: A. Hollaender), pp. 171–207, Plenum, New York.

Xiao, L. Z. and Ichikawa, S. (1995) Mutagenic interactions between maleic hydrazide and X rays in the stamen hairs of *Tradescantia* clone BNL 4430. Jpn. J. Genet. **70**, 473–485.