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Sectoring patterns of spontaneous and induced somatic pink mutations in the stamen hairs and petals of mutable and stable clones of *Tradescantia*

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ABSTRACT

The sectoring patterns of spontaneous and radiation- and EMS-induced somatic pink mutations were analyzed in the stamen hairs and petals of *Tradescantia* clones heterozygous for flower color (blue/pink). Spontaneous pink mutations were analyzed using clone KU 20 (a highly mutable clone especially at lower temperature) grown outdoors and clones KU 27 and BNL 02 (stable clones) grown under controlled environmental conditions, while induced pink mutations were analyzed using clones KU 27 and BNL 02 grown under the controlled environments. As for spontaneous mutations in the stamen hairs, the ratio of the number of single interstitial pink mutant events against that of single terminal pink mutant events was somewhat larger than 1 in all the three clones examined, indicating that somewhat more interstitial pink mutant events occur spontaneously than terminal pink mutant events. After treatments with X rays, gamma rays or EMS, however, the ratio increased to about 3 in the two clones examined, showing much more frequent inductions of interstitial pink mutant events than terminal pink mutant events by these mutagens. The daily changes of the sectoring patterns of radiation- and EMS-induced terminal pink mutant events in the stamen hairs showed a good accordance with the pattern of the stamen-hair development. Multiple pink mutant sectors in the same hairs were observed at much higher frequencies than expected from independent occurrences, especially in cases of spontaneous mutations in the mutable clone and of radiation-induced mutations in the two stable clones, suggesting the involvement of somatic recombinations. The sectoring patterns of radiation- and EMS-induced somatic pink mutations in the petals also showed daily changes which reflected the pattern of the flower-petal development.

1. INTRODUCTION

Recent analyses of the sectoring patterns of somatic pink mutations in the stamen hairs of *Tradescantia* clone KU 20, a temperature-sensitive mutable clone heterozygous for flower color (blue/pink) which is highly mutable spontaneously especially at lower temperature, suggested that somatic recombination is involved as one of the major causes of spontaneous mutations in this mutable clone

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(Ichikawa, 1994). Namely, the multiple pink mutant sectors in the same hairs occurred spontaneously more than four times frequently than expected from simultaneous and independent occurrences of two or more somatic mutations. It was revealed, however, that the sectoring patterns of gamma-ray-induced somatic pink mutations in the stamen hairs of this clone were clearly different from those of spontaneous mutations, especially as for the occurrences of multiple pink mutant sectors (Ichikawa, 1994). These results were considered to be related to the earlier findings that the mutational responses of clone KU 20 to gamma rays were rather similar to those of non-mutable or stable clones, KU 9 (Ichikawa and Takahashi, 1977; Ichikawa et al., 1991) and BNL 02 (Ichikawa et al., 1991).

In the present study, a series of experiments was conducted to investigate further the sectoring patterns of somatic pink mutations occurring spontaneously in the stamen hairs and petals of the mutable clone KU 20 and stable clones KU 27 and BNL 02, and of those induced by X rays, gamma rays or ethyl methanesulfonate (EMS) in the stamen hairs and petals of clones KU 27 and BNL 02.

2. MATERIALS AND METHODS

Clones used

Three *Tradescantia* clones, KU 20, KU 27 and BNL 02, were used in the present study. These clones are all blue/pink heterozygotes for flower color (the blue color being dominant), and have been continually propagated vegetatively (Ichikawa, 1992).

Clone KU 20 is a triploid clone ($3x=18$), but the origin of this clone is unknown. It possesses, however, part of the characteristics of *T. ohiensis* Raf., thus appears to be a hybrid involving this species (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992). This clone has been reported to be highly mutable spontaneously especially at lower temperature, showing as much as about 23- (Takahashi and Ichikawa, 1976) to 40-fold (Imai et al., 1991) differences in spontaneous mutation frequency in the stamen hairs in the temperature range of 17.5 to 28.3°C, and is therefore regarded as a temperature-sensitive mutable clone (Ichikawa and Takahashi, 1977; Ichikawa, 1984, 1992, 1994; Imai et al., 1991; Ichikawa et al., 1991).

Clones KU 27 and BNL 02 are diploid hybrids ($2n=12$) related to each other. Clone BNL 02 is thought to be derived from a hybrid between *T. occidentalis* (Britt.) Smyth. and *T. ohiensis* (Mericle and Mericle, 1971, 1973). This clone is one of those which have been most often used in studies of somatic mutations in the stamen hairs (Ichikawa, 1992), and is one of the most stable clones in terms of spontaneous somatic mutation frequency (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1992).

Clone KU 27, a segregant from clone BNL 02 (Sanda-Kamigawara et al., 1991), is as stable as the parental clone, but is about two to six times more sensitive to alkylating agents than the latter (Sanda-Kamigawara et al., 1991; Ichikawa, 1992;

Ichikawa et al., 1993). Also, the normal dominant blue color of this clone is somewhat darker than that of the parental clone, and the recessive mutant pink color is also generally deeper than that of the latter (Sanda-Kamigawara et al., 1991; Ichikawa et al., 1993).

The normal blue and mutant pink flower-color pigments of these three clones have been studied microspectrophotometrically, and these clones have been confirmed to produce the identical blue-color pigment normally, and also the identical pink-color pigment when the dominant gene for the blue color underwent mutation (Sanda-Kamigawara and Ichikawa, 1993).

Growing conditions

Potted plants of clone KU 20 were grown outdoors in order to obtain a higher spontaneous mutation frequency (Takahashi and Ichikawa, 1976; Ichikawa, 1984; Imai et al., 1991). The temperature data during the 16-week experimental period (plus preceding two weeks) were taken from the records by the Urawa Branch of the Kumagaya Weather Bureau, and the maximum and the minimum temperatures were 34.4 and 10.5°C, respectively, averaging 21.4°C.

Potted plants of clones KU 27 and BNL 02 were grown in either a growth chamber (Sherer CEL 38–15) or a growth room. The environmental conditions in the growth chamber were 25.0 ± 0.5°C during the day and 20.0 ± 0.5°C at night, 60% humidity, and a 16-hr day length with the maximum light intensity of 23 klx from Sylvania VHO cool white fluorescent tubes (22 klx) and incandescent bulbs (1 klx). The environmental conditions in the growth room were 23.0 ± 0.5°C (constant), 50% humidity, and a 16-hr day length with the light intensity of 6 klx from Toshiba DR400/T(L) metal-halide sunlamps plus white fluorescent tubes.

Radiation and EMS treatments

X-ray treatments were carried out using a Hitachi MBR-1505R X-ray generator. Potted plants of clones KU 27 and BNL 02 were exposed to X rays for 1 min at 150 kVp and 4 mA with a 0.5 mm Al+0.1 mm Cu filter at 23.0 ± 0.5°C. The exposure data were obtained simultaneously with thermoluminescence dosimeter (TLD) elements (National UD-170L) attached to individual inflorescences, and with a thermoluminescence reader (National UD-502B). The exposure data obtained in R were converted into absorbed doses in Gy with a converting factor of 9.57×10^{-3} (i.e., 1 R=9.57 mGy). The X-ray doses applied were 289 to 1,110 mGy for clone KU 27 and 247 to 870 mGy for clone BNL 02.

Gamma-ray treatments were carried out using either a 6.66 GBq ^{60}Co source at the Faculty of Engineering, Saitama University, or a 4.81×10^{3} GBq ^{137}Cs source at the gamma greenhouse of the Institute of Radiation Breeding, National Institute of Agricultural Science, Ohmiya, Ibaraki. Potted plants of clone KU 27 were exposed to ^{60}Co gamma rays for 18 hr at room temperature (recorded to have varied between 19 and 23°C) or to ^{137}Cs gamma rays for 20 hr at 20 ± 2°C.

The ^{60}Co and ^{137}Cs gamma-ray doses measured simultaneously with the TLD elements attached were 153 to 472 mGy and 139 to 475 mGy, respectively.

Young inflorescences of clones KU 27 and BNL 02 were treated with 0.1 to 0.4% (by volume) aqueous solutions of EMS for 12 or 16 hr. The methods used for the EMS treatments have been described elsewhere (Ichikawa and Takahashi, 1978; Ichikawa et al., 1990, 1993; Sanda-Kamigawara et al., 1991; Ichikawa, 1992; Shima and Ichikawa, 1994). The EMS doses applied were 1.2 to 6.4%×hr.

Scoring of mutations

All the flowers that opened during the scoring periods were collected daily from each inflorescence. The scoring period for induced mutations was three weeks after treatments, but those for spontaneous mutations were 16 weeks for clone KU 20 grown outdoors and up to 12 weeks for clones KU 27 and BNL 02 grown under controlled environmental conditions.

For scoring pink mutations in the stamen hairs, the numbers of stamen hairs and of pink mutant events were scored on each of six stamens, recording the sectoring pattern of every pink mutant event detected. A pink mutant event has been defined to represent the result of a single mutation (Ichikawa, 1981a, 1992, 1994).

For scoring pink mutations in the petals, on the other hand, the number of pink mutant spots detected on the upper surface was recorded (Sanda-Kamigawara et al., 1991). The sectoring patterns of pink mutant spots were analyzed by estimating the number of epidermal cells composing each pink mutant spot. The estimation of cell number was made based on the fact that their cell walls wave longitudinally about five times on the average (see the last section of Results and Discussion).

3. RESULTS AND DISCUSSION

Spontaneous and induced mutation frequencies

Most of the data of spontaneous pink mutation frequencies in the stamen hairs of clone KU 20 obtained have been analyzed earlier (Imai et al., 1991), and most of the spontaneous and induced pink mutation frequencies determined in the stamen hairs and petals of clones KU 27 and BNL 02 have been reported earlier (Sanda-Kamigawara et al., 1991).

The spontaneous pink mutation frequency in the stamen hairs of clone KU 20 grown outdoors was considerably high (42.6 ± 1.0 pink mutant events per 10^3 hairs), when the data were pooled for 16 weeks. However, the fluctuation of the mutation frequency was quite large when the data (but not all) were pooled for every two weeks (4.03 ± 1.21 to 99.9 ± 4.7: Imai et al., 1991).

The spontaneous pink mutation frequencies in the stamen hairs of clones KU 27

and BNL 02 grown in either the growth chamber or the growth room were 1.25 ± 0.10 to 1.66 ± 0.25 pink mutant events per 10^3 hairs (Sanda-Kamigawara et al., 1991). These spontaneous mutation frequencies in the two stable clones were much lower than that in clone KU 20 grown in the growth chamber (7.53 ± 1.14: Imai et al., 1991).

The spontaneous pink mutation frequency in the petals of clone KU 20 grown outdoors was 1.72 ± 0.10 pink mutant spots per petal, being also higher than those in clones KU 27 and BNL 02 grown in the growth room (0.553 ± 0.021 and 0.515 ± 0.042, respectively: Sanda-Kamigawara et al., 1991). However, the differences between the mutable and stable clones were much smaller than the case of the spontaneous mutation frequency in the stamen hairs. Such a clear difference found between petals and stamen hairs may represent the difference between the multi-cellular petal tissue and the stamen hair, an essentially single-meristematic-cell system (Ichikawa and Sparrow, 1967, 1968; Ichikawa et al., 1969), and pink mutant cells might be eliminated at a much higher frequency in the multi-cellular petal tissues than in the stamen hairs.

X-ray-, gamma-ray- and EMS-induced somatic pink mutation frequencies in the stamen hairs and petals of clones KU 27 and BNL 02 increased with increasing doses of these mutagens (Sanda-Kamigawara et al., 1991).

Sectoring patterns of spontaneous pink mutations in stamen hairs

As many as 1,601 stamen hairs of clone KU 20 grown outdoors were found to have pink mutant events occurred spontaneously, whereas 115 and 82 hairs of clones KU 27 and BNL 02, respectively, grown under controlled environmental conditions had spontaneous pink mutant events.

These hairs with spontaneous pink mutations were classified into four categories; namely, those with single terminal, single interstitial, whole-hair and multiple pink mutant events or sectors (Ichikawa, 1994), as shown in Table 1. A single terminal pink mutant event is defined as a single pink terminal cell or a row of pink cells including the terminal cell of a hair, without any other pink cells in the hair. A single interstitial pink mutant event means a single pink cell or two or more contiguous pink cells between blue cells in a hair, without any other pink cells in the hair. A whole-hair pink mutant event means an entirely pink hair without any blue cell. Multiple pink mutant sectors are defined as two or more pink mutant sectors occurred in the same hair, and they can be further classified into double, triple and quadruple pink mutant sectors (Ichikawa, 1994).

The frequencies of single terminal pink mutant events occurred spontaneously were somewhat lower than those of single interstitial pink mutant events, as seen in Table 1. The ratio of the number of single interstitial pink mutant events against that of single terminal pink mutant events was 1.14 in clone KU 20, and those in clones KU 27 and BNL 02 were 1.56 and 1.13, respectively. The present value for clone KU 20 is smaller than 1.35 reported earlier (Ichikawa, 1994), but

Table 1. Sectoring patterns of spontaneous somatic pink mutations in the stamen hairs of clone KU 20 grown outdoors, and of spontaneous and induced somatic pink mutations in the stamen hairs of clones KU 27 and BNL 02 grown under controlled environmental conditions

Clone	Grown (in)[1]	Source	Period[2]	Single terminal	Single interstitial	I/T[3]	Whole-hair	Multiple	Total
KU 20	OD	spont.	——	663	758	1.14	34	146	1,601
KU 27	GR	spont.	——	36	56	1.56	16	7	115
	GC	X rays	during	203	644	3.17	3	106	956
			after	94	125	1.33	115	58	392
	GR	gamma	during	36	96	2.67	2	15	149
			after	22	16	0.73	2	8	48
	GR	EMS	during	51	142	2.78	7	8	208
			after	6	9	1.50	6	4	25
BNL 02	GR	spont.	——	23	26	1.13	3	8	82
	GC	X rays	during	49	155	3.16	4	32	240
			after	23	45	1.96	26	20	114
	GR	EMS	during	19	57	3.00	2	5	83
			after	15	25	1.67	25	9	74

[1]OD: grown outdoors; GR: grown in growth room; GC: grown in growth chamber.
[2]During or after the 7-day peak period of mutation frequency.
[3]The ratio of the number of single interstitial pink mutant events against that of single terminal pink mutant events.

all these values larger than 1 must be reflecting the nature of cell increase in *Tradescantia* stamen hairs (Ichikawa and Sparrow, 1967; Ichikawa et al., 1969; Ichikawa, 1981b, 1992). That is, the hair cells are largely the products of repeated divisions of the lineally succeeding terminal cells which are meristematic until the hair is fully developed, while many of the subterminal cells also divide but usually only once (Ichikawa and Sparrow, 1967). Other interstitial cells divide only occasionally (Ichikawa and Sparrow, 1967), except for early stage of hair growth (Mericle and Hazard, 1980). Owing to such nature, single interstitial pink mutant events result from both chromosome- and chromatid-type mutations occurred in the subterminal (and occasionally interstitial) cells and also from 50% of chromatid-type mutations in the terminal cells, while single terminal pink mutant events are derived solely from mutations (all of chromosome-type and 50% of chromatid-type) in the terminal cells (Ichikawa, 1994).

The distribution patterns of the number of pink cells per single terminal pink mutant event and of that per single interstitial pink mutant event in clone KU 20 are drawn in Fig. 1. The distribution pattern for single terminal pink mutant events showed a wide variation in the number of pink cells composing the mutant events, while that for single interstitial pink mutant events showed a very much smaller variation, more than 80% of the mutant events being composed of one or

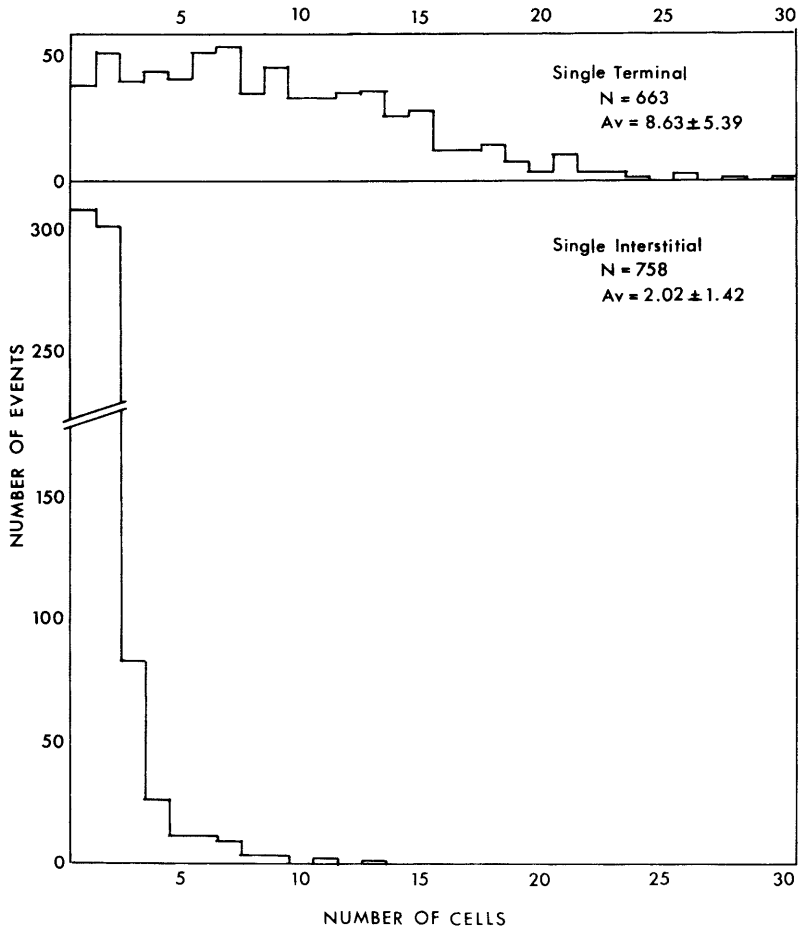

Fig. 1. Distribution patterns of the number of pink cells per single terminal or single interstitial pink mutant event occurred spontaneously in the stamen hairs of clone KU 20 grown outdoors.

two pink cells. These results agree well with those reported earlier (Ichikawa, 1994).

Multiple pink mutant sectors in the same hairs were observed much more frequently than expected from simultaneous occurrences of two or more independent mutations. For the mutable clone KU 20, a mutation frequency of 35.3 single pink mutant events per 10^3 hairs can be calculated, based on the total number of single terminal and single interstitial pink mutant events (663 and 758, respectively, totaling 1,421: see Table 1) and the number of stamen hairs observed (40,285). The simultaneous occurrences of two, three and four independent somatic mutations in a hair are therefore expected to be realized at frequencies of 1.25, 0.0440 and 0.00155 per 10^3 hairs, respectively, totaling 1.30 per 10^3 hairs. The observed frequency of 3.62 per 10^3 hairs (146 out of 40,285 hairs observed had

multiple pink mutant sectors) is about 2.8-fold higher than the expectation. Although the present value is smaller than about 4 folds reported earlier (Ichikawa, 1994), the occurrences of such multiple pink mutant sectors at a much higher frequency than expected suggest that somatic recombinations, which produce pairs of homozygous blue and homozygous pink daughter cells from blue/pink heterozygous cells, are involved in producing a significant part of spontaneous mutations as well as of such multiple pink mutant sectors in this mutable clone, as discussed earlier (Ichikawa, 1994) and as will be discussed below.

The observed frequencies of multiple pink mutant sectors in the stamen hairs of clones KU 27 and BNL 02 were also much higher than the expectations, although the sample sizes were limited.

Sectoring patterns of induced pink mutations in stamen hairs

X-ray, gamma-ray and EMS treatments of clone KU 27 resulted in producing 1,348, 197 and 233 hairs with pink mutant events, respectively, and X-ray and EMS treatments of clone BNL 02 produced 354 and 157 hairs with pink mutant events, respectively (see Table 1). These hairs with pink mutant events are those observed on and after post-treatment day 6, since the mutation frequency started to increase on post-treatment day 6 (pink mutant events observed before post-treatment day 6 were regarded as those occurred spontaneously).

These hairs with induced pink mutant events were also classified into the above four categories, and are presented in Table 1. It is conspicuous that single interstitial pink mutant events were much more frequently induced than single terminal pink mutant events. Namely, in clone KU 27 exposed to X rays, the ratio of the number of single interstitial pink mutant events against that of single terminal pink mutant events was 3.17, being quite different from the value of 1.56 for spontaneous mutations. The value of the ratio for clone BNL 02 exposed to X rays was 3.16, being almost identical to that in clone KU 27 and also being much larger than 1.13 for spontaneous mutations in this clone. The values for clone KU 27 treated with gamma rays and EMS were 2.67 and 2.78, respectively, and that for clone BNL 02 treated with EMS was 3.00. These results of much more frequent inductions of single interstitial pink mutant events than single terminal pink mutant events agree well with earlier result obtained in clone KU 20 exposed to gamma rays (Ichikawa, 1994).

The great differences between these radiation- or EMS-induced mutations and spontaneous mutations seem to indicate that much more mutations are induced in the subterminal cells than in the terminal cells of young stamen hairs, and/or that much more chromatid-type mutations than chromosome-type ones are induced, as discussed earlier (Ichikawa, 1994). One of the important keys to verify these possibilities is the proportion of single-cell interstitial pink mutant events among single interstitial pink mutant events during the 7-day peak period of mutation frequency. The distribution patterns (during the 7-day peak period) of the

number of pink cells per single terminal pink mutant event and of that per single interstitial pink mutant event in clone KU 27 treated with X rays (which produced the largest sample size: see Table 1) are shown in Fig. 2, and it is clear in this figure that the proportion of single-cell interstitial pink mutant events was very high, being 72.8%. The values in clone KU 27 treated with gamma rays and EMS were 65.6 and 78.2%, respectively, and those in clone BNL 02 treated with X rays and EMS were 71.0 and 63.2%, respectively. Taking these results and also similar results obtained earlier (Ichikawa, 1994) into consideration, it is most likely that more chromatid-type mutations are induced in the subterminal cells by these mutagens, since such single-cell interstitial pink mutant events are the results of chromatid-type mutations occurred in the subterminal cells (see Fig. 2 in Ichikawa, 1994). The loss of reproductive integrity (Ichikawa and Sparrow, 1967, 1968; Ichikawa et al., 1969, 1978; Ichikawa, 1972) caused in the subterminal cells by these mutagens might also be involved (Ichikawa, 1994).

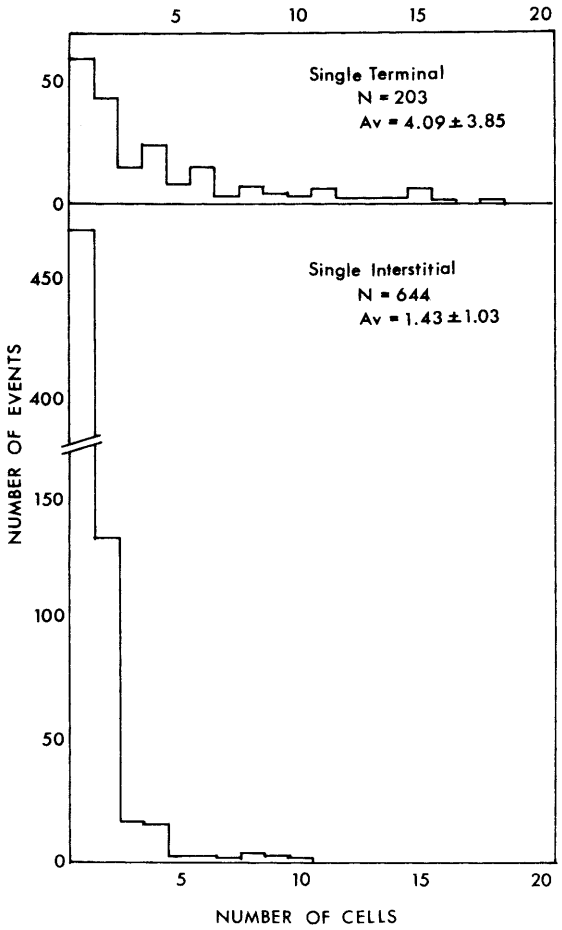

Fig. 2. Distribution patterns of the number of pink cells per single terminal or single interstitial pink mutant event induced by X rays in the stamen hairs of clone KU 27 grown in the growth chamber (the data collected during the 7-day peak period of the mutation frequency).

The average number of pink cells per single terminal pink mutant event increased gradually as the number of post-treatment days proceeded, as demonstrated earlier (Ichikawa and Sparrow, 1968; Ichikawa et al., 1969; Ichikawa, 1994), then was stabilized at the level of spontaneous mutations at the end of the scoring period of three weeks, agreeing well with earlier results (Ichikawa, 1994). The average number of pink cells per single interstitial pink mutant event, on the other hand, also increased as the number of post-treatment days proceeded, but the increases were very much smaller as compared with the case of single terminal pink mutant events, reflecting the pattern of cell increase in *Tradescantia* stamen hairs (Ichikawa, 1981b, 1992, 1994).

Whole-hair pink mutant events induced were mostly observed after the peak period of mutation frequency (Table 1), as reported earlier (Ichikawa, 1994). Such entirely pink hairs result from somatic mutations induced in the epidermal cells of stamen filaments before hair initiation, and this result thus reflects the hair initiation pattern reported earlier (Mericle and Hazard, 1980).

Multiple pink mutant sectors induced in the same hairs were also analyzed to know whether or not they were induced more frequently than expected. Because of applying many different doses, especially with X rays and gamma rays, it was difficult to analyze the data directly in the same way as for spontaneous mutations, or as in earlier study (Ichikawa, 1994). Therefore, the ratio of the number of hairs with multiple mutant sectors against that with single (terminal and interstitial) mutant events was compared between spontaneous mutations in clone KU 20 (in which multiple mutant sectors occurred spontaneously 2.8 times more frequently than expected; see above) and induced mutations by each mutagen in other clones, as an attempt to clarify whether or not the induction of multiple pink mutant sectors was more than expected. The ratio for spontaneous mutations in clone KU 20 was 0.103 (146/1,421: see Table 1), while that for X-ray-induced mutations in clone KU 27 during the peak period of mutation frequency was 0.125 (106/847: see Table 1), the latter being larger than the former. The ratios for X-ray-induced mutations in clone BNL 02 and for gamma-ray-induced mutations in clone KU 27 were 0.157 and 0.114, respectively, being also larger than 0.103, while those for EMS-induced mutations in clones KU 27 and BNL 02 were much smaller, being 0.041 and 0.066, respectively. Therefore, it is certain that multiple pink mutant sectors were much more frequently induced than expected at least by ionizing radiations in these stable clones, differing from the case of gamma-ray-induced mutations in the mutable clone KU 20 reported earlier (Ichikawa, 1994).

These results, together with the results for spontaneous mutations in the mutable clone KU 20 obtained in the present study (see above) and reported earlier (Ichikawa, 1994), suggest that somatic recombinations are involved, not only in producing significant parts of spontaneous pink mutations and of multiple pink mutant sectors occurring spontaneously in clone KU 20 (Ichikawa, 1994), but

also in producing multiple pink mutant sectors in stable clones KU 27 and BNL 02 after treatments especially with ionizing radiations.

The possibility of involvement of somatic recombinations in producing multiple pink mutant sectors in the stamen hairs of clone BNL 02 was suggested earlier (Mericle and Mericle, 1967, 1973), and the occurrence of somatic recombinations was confirmed in the stamen hairs of a purple-flowered clone of *T. hirsuticaulis* Small (BNL 2091, also stable) that produces blue/pink twin spots when a somatic recombination occurs (Christianson, 1975). The latter study, analyzing both spontaneous and gamma-ray-induced mutations, concluded that the predominant cause of spontaneous twin spots and an important cause of induced twin spots were somatic recombinations. Similar conclusion was also reported earlier in *Glycine max* (Vig, 1973, 1975). Therefore, it is conclusive that somatic recombination plays an important role in producing multiple pink mutant sectors in *Tradescantia* stamen hairs.

Considering that the frequency of gamma-ray-induced multiple pink mutant sectors in the stamen hairs of the mutable clone KU 20 was not higher than the expectation (Ichikawa, 1994), and also that gamma-ray-induced somatic pink mutation frequency in this mutable clone was rather similar to those in stable clones BNL 02 (Ichikawa et al., 1991) and KU 9 (Ichikawa and Takahashi, 1977; Ichikawa et al., 1991), absence and presence of somatic recombinations in radiation-induced pink mutations in clone KU 20 and in other stable clones, respectively, seem to be related to the rather similar induced mutation frequencies reported earlier.

Sectoring patterns of spontaneous and induced pink mutations in petals

The epidermal cells of flower petals of *Tradescantia* have a specific morphology to form waved cell walls between adjacent cells as shown in Fig. 3. Also, the petal tissue is composed of many layers of cells. Under stereoscope, one epidermal cell thus appears as if it is composed of several cells because of its specific morphology. Accordingly, it was often difficult to count the exact number of epidermal cells in a pink spot as a measure of the size of the pink spot. In the present study, therefore, the numbers of epidermal cells composing individual pink spots were mostly estimated based on the fact that the cell walls of epidermal cells wave longitudinally about five times on the average (Fig. 3).

Average numbers of epidermal cells composing spontaneously occurred pink mutant spots in the petals of clone KU 20 grown outdoors and clones KU 27 and BNL 02 grown under controlled environmental conditions are presented in Table 2. The figures in this table showed a variation from 5.44 in clone KU 27 grown in the growth chamber to 8.65 in clone KU 20 grown outdoors. However, when especially larger pink mutant sectors composed of more than 40 cells (estimated) were excluded, the figures decreased to 4.92 to 6.07 and showed a much smaller variation (Table 2).

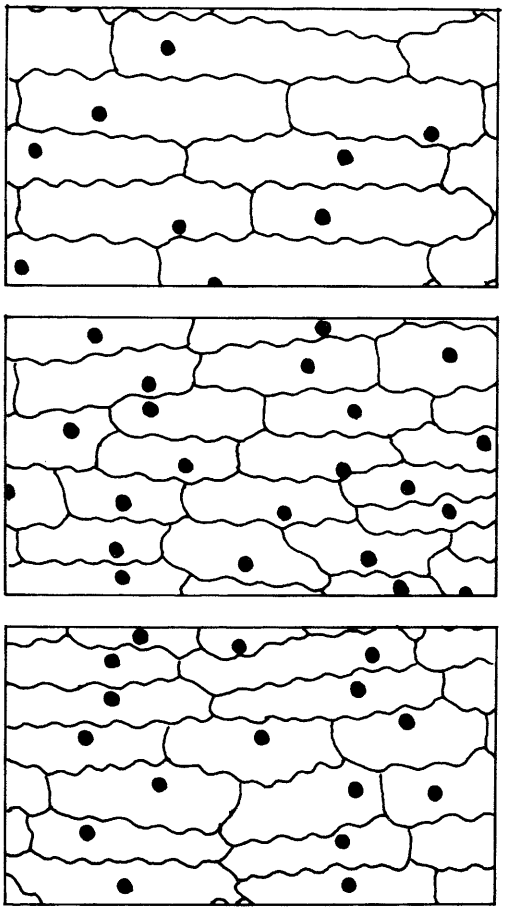

Fig. 3. Sketches of epidermal cells on the upper surface of a flower petal of clone KU 27 observed under a microscope (×120). Since the petal tissue is composed of many layers of cells, one epidermal cell appears as if it is composed of several cells because of its specific morphology when observed under stereoscope. The cell size differs depending on positions in the petal, but the cell walls wave longitudinally about five times on the average.

Average numbers of epidermal cells composing pink mutant spots induced by gamma rays or EMS in the petals of clones KU 27 and BNL 02 are also presented in Table 2. It is evident that the average cell number decreased during the peak period of mutation frequency (10-day peak period for petals differing from 7-day peak period for stamen hairs; see Sanda-Kamigawara et al., 1991), namely, to 3.13 in clone KU 27 treated with EMS, 4.05 in the same clone exposed to gamma rays, and 4.50 (4.31 excluding one especially large sector) in clone BNL 02 treated with EMS. The number then increased greatly after the peak period as seen in Table 2. These results show a good accordance with the earlier analyses of mutant spots in the petals of *Antirrhinum majus* (Sparrow et al., 1961), and also reflect well the pattern of the development of *Tradescantia* flower petals reported earlier (Mericle and Hazard, 1980).

Table 2. Average estimated numbers of epidermal cells per spontaneous or induced pink mutant spot in the petals of clones KU 20, KU 27 and BNL 02

Clone	Grown (in)[1]	Source	Period[2]	No. of pink spots observed	Total estimated no. of cells	Average no. of cells /pink spot	Av. no. of cells /pink spot except large sectors[3]
KU 20	OD	spont.	——	391	3,384	8.65	6.07
KU 27	GR	spont.	——	725	4,006	5.53	4.92
	GC	spont.	——	397	2,160	5.44	5.35
	GR	gamma	during	424	1,717	4.05	4.05
			after	134	990	7.39	6.03
	GR	EMS	during	212	663	3.13	3.13
			after	85	691	8.13	7.13
BNL 02	GR	spont.	——	150	931	6.21	5.93
	GR	EMS	during	197	886	4.50	4.31
			after	81	1,086	13.4	6.11

[1]See the footnote to Table 1.

[2]During or after the 10-day peak period of mutation frequency.

[3]Large pink mutant sectors composed of more than 40 epidermal cells were excluded, since the rare occurrences of such large sectors affect greatly the average number of cells per pink mutant spot.

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