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Variation of spontaneous somatic mutation frequency in the stamen hairs of a mutable clone of *Tradescantia*, KU 20

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ABSTRACT

Variation of spontaneous somatic pink mutation frequency was studied in the stamen hairs of *Tradescantia* KU 20 clone, a highly mutable blue/pink heterozygote. The spontaneous mutation frequency varied greatly from 4.03 ± 1.21 to 120 ± 7 and from 18.8 ± 3.1 to 110 ± 5 pink mutant events per 10^3 hairs when the plants were grown outdoors and in the greenhouse, respectively, being generally higher at lower temperature and also in the greenhouse than in outdoors. The spontaneous mutation frequency under controlled environmental conditions also varied from 3.06 ± 0.37 to 40.8 ± 3.1 pink mutant events per 10^3 hairs, showing a clearer negative correlation with temperature. It was found that the spontaneous mutation frequency under controlled environmental conditions increased when day/night temperature shifts were applied, especially with a 5°C shift than with 3°C shifts. The difference between the highest and the lowest mutation frequencies reached almost 40 times, and this clone was confirmed to be a temperature-sensitive mutable clone. A repair mechanism of DNA damages occurring spontaneously, which is more effective at higher temperature, thus presumably an enzymatic one, is very likely involved in the mutable nature of this clone.

1. INTRODUCTION

KU 20 clone of *Tradescantia* was originally found by the second author among several clones which had been grown in the campus of Kyoto University of Education, Kyoto (see Ichikawa, 1984a), as one of those proven to be heterozygous for flower color, and was soon confirmed to be highly mutable spontaneously especially at lower temperature (Takahashi and Ichikawa, 1976; Ichikawa, 1984a).

Diversely different spontaneous mutabilities have been reported among many different *Tradescantia* clones heterozygous for flower color (Sparrow and Sparrow, 1976; Takahashi and Ichikawa, 1976; Ichikawa, 1984a), and, besides KU 20 clone, at least five other highly mutable clones, BNL 0106 (Sparrow and Sparrow, 1976; Nauman et al., 1978), KU 13, KU 14, KU 17 and KU 24 (Ichikawa, 1984a), have been described. Among these highly mutable clones, KU 20 clone is the most mutable (Ichikawa, 1984a), being followed by BNL 0106 and KU 17 clones.

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The present paper describes the results of a series of experiments in which KU 20 plants were cultivated under a number of different environmental conditions in the field, greenhouse and controlled environment facilities in order to study further the variation of the spontaneous mutation frequency in the stamen hairs, and discusses the mutable nature of this clone based on the experimental results.

2. MATERIALS AND METHODS

Materials: KU 20 clone is a triploid clone ($3x=18$) heterozygous for flower color (blue/pink; the blue color being dominant), but the origin of this clone remains unknown. It possesses, however, 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, 1984a, 1991). This 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), and has been therefore regarded as a temperature-sensitive mutable clone (Ichikawa and Takahashi, 1977; Ichikawa, 1984a, 1991). It has been further confirmed that KU 20 clone is the most mutable spontaneously among 14 different *Tradescantia* clones heterozygous for flower color examined (Ichikawa, 1984a).

The blue-color pigment produced in the stamen-hair cells of KU 20 clone normally and the pink-color pigment produced when the dominant gene for the blue color underwent mutation have been both confirmed microspectrophotometrically to be identical to those produced by BNL 02, KU 27 and KU 9 clones (Kamigawara and Ichikawa, 1988) which have been used in studies of somatic mutations as stable (non-mutable) clones (see Ichikawa, 1991).

In the present study, potted plants of KU 20 clone bearing young inflorescences were used as materials in most cases. The only exception was using young inflorescences of KU 20 plants grown in the field for studying the spontaneous mutation frequencies in outdoors.

Scoring methods: For scoring spontaneously occurring pink mutations in the stamen hairs, one or two flowers were collected daily at random from the inflorescences used as materials, and the numbers of stamen hairs and of pink mutant events were scored for each flower. The definition of the pink mutant event has been described elsewhere (Ichikawa, 1981a, 1981b, 1984a). The spontaneous mutation frequency was expressed as the number of pink mutant events per 10^3 hairs, pooling the data for each of the scoring periods not exceeding two weeks in cases of studies under uncontrolled environmental conditions, or pooling the data for whole scoring periods in cases of studies under controlled environmental conditions, excepting two experiments in whcich the mutation frequency was determined for each day to know the daily change in the frequency.

Studies of spontaneous mutation frequency under uncontrolled environmental

conditions: Cultivating several KU 20 plants outdoors (in the field; OD hereafter) and several potted plants of KU 20 clone in the greenhouse (GH hereafter; steam-heated during late October through early April and the day length kept to be 16 hr), the spontaneous mutation frequencies were investigated for a few months. The temperature in GH was continuously recorded with a self temperature recorder, whereas the temperature data in OD were taken from the records by the Urawa Branch of the Kumagaya Weather Bureau.

Studies of changes in spontaneous mutation frequency after moving plants from GH to gorwth chambers: One potted plant of KU 20 clone bearing young inflorescences, which had been grown in GH, was moved in a growth chamber (the Sherer CEL 38–15; CEL hereafter) in which the temperature was maintained at 24 ± 1°C during the day and 21 ± 1°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 spontaneous mutation frequency in this plant was scored daily for 21 days.

Similar experiment was repeated by moving one potted plant of KU 20 clone from GH to another growth chamber (LPH-200-RD of the Nippon Medical and Chemical Instruments Co., Ltd.; LPH hereafter) in which the temperature was changed between 25 ± 0.5 and 20 ± 0.5°C (at 2 p.m. and 2 a.m., respectively) with a sine curve, the humidity was kept at 55%, and the day length was 16 hr with a light intensity of 6 klx. Scoring of the spontaneous mutation frequency was made daily for 14 days.

Studies of spontaneous mutation frequency under controlled environmental conditions: Growing potted plants of KU 20 clone in either of the above two growth chambers (CEL or LPH) or in the plant growth room (GR hereafter), the spontaneous mutation frequencies under controlled environmental conditions were examined for 14 or 21 days. The temperature was either kept constant (at 17.5 ± 0.5, 20 ± 0.5 or 22.5 ± 0.5°C in LPH, or at 23 ± 1°C in GR), changed with a sine curve (22.5 to 17.5 ± 0.5 or 25 to 20 ± 0.5°C in LPH), or shifted in a 12-hr day/12-hr night cycle ($25/20\pm1$, $24/21\pm1$, $23/20\pm1$ or $22/19\pm1$°C day/night cycles in CEL). The humidity was kept at 55, 50 or 60% in LPH, GR and CEL, respectively. The day length was 16 hr in all of the three facilities, and the light intensity was 6, 5 or 23 klx in LPH, GR and CEL, respectively. Scoring of mutation frequency was started after acclimating the plants for ten days in each facility.

3. RESULTS

The spontaneous somatic pink mutation frequencies obtained in the stamen hairs of KU 20 clone grown OD and in GH are presented in Table 1, together with temperature data. The temperature data listed in this table are those of the periods starting and ending nine days earlier than corresponding scoring periods, considering the fact that the stamen hairs are most affected by environmental

Table 1. Variation in spontaneous pink mutation frequency in the stamen hairs of KU 20 clone grown outdors or in the greenhouse

Grown in[a]	Scoring period	Temperature (°C)[b]			No. of hairs observed	No. of pink mutant events	No. of pink mutant events /10^3 hairs (±SE)
		Max.	Min.	Av. (±SD)			
OD	82/5/17–18	27.4	11.6	19.2±5.1	1,989	238	120 ±7
	6/23–7/2	27.7	14.6	20.7±3.9	3,852	105	27.3 ±2.6
	7/10–23	32.5	14.9	22.2±4.2	10,296	324	31.5 ±1.7
	7/24–30	28.1	18.7	22.1±2.7	3,069	55	17.9 ±2.4
	83/5/9–20	27.3	11.2	17.5±4.8	4,074	407	99.9 ±4.7
	5/24–6/6	28.7	10.5	18.5±5.1	4,647	245	52.7 ±3.3
	6/7–17	31.4	11.5	19.6±5.0	3,174	151	47.6 ±3.8
	6/23–7/1	29.7	15.1	20.6±3.9	2,211	35	15.8 ±2.7
	7/7–19	28.9	14.9	20.1±3.5	1,896	117	61.7 ±5.5
	7/27–8/9	34.4	19.3	25.9±4.0	2,355	36	15.3 ±2.5
	8/18–27	34.3	22.8	27.5±3.4	2,727	11	4.03±1.21
GH	81/5/23–30	32.0	11.9	20.0±5.0	3,254	359	110 ±5
	6/1–8	37.5	14.0	22.9±5.6	8,103	687	84.8 ±3.1
	82/4/2–14	32.7	15.4	21.3±5.3	1,545	71	46.0 ±5.3
	5/6–10	33.3	14.7	21.6±5.8	2,715	123	45.3 ±4.0
	5/13–18	42.0	16.3	26.4±7.1	1,920	36	18.0 ±3.1

[a] OD: outdors; GH: greenhouse.

[b] Those of the periods nine days earlier than scoring periods (see text).

factors about ten days before flowering, and because the nine-day earlier temperature data showed the highest absolute correlation coefficients with the mutation frequencies (−0.875 in OD and −0.805 in GH). The variation of the mutation frequency observed was quite large, ranging from 4.03±1.21 to 120±7 and from 18.8±3.1 to 110±5 pink mutant events per 10^3 hairs in OD and GH, respectively. The average temperature also varied from 17.5 to 27.5°C in OD and from 20.0 to 26.4°C in GH. The mutation frequency was generally higher at lower temperature, and also tended to be higher in GH than in OD. When the logarithms of the spontaneous mutation frequencies are plotted against average temperature as done by Takahashi and Ichikawa (1976), such relationships can be realized, although there are considerably large fluctuations (Fig. 1).

In the two experiments to study how the spontaneous mutation frequency changes after moving the potted plants of KU 20 clone from GH to CEL or LPH, it was found that the mutation frequency (which was very high being around 100 or 40 pink mutant events per 10^3 hairs at the beginning) decreased sharply or gradually and reached a considerably stable low level (around 5) ten days after moving, as shown in Fig. 2. The results showed that it is necessary to acclimate plants for ten days, prior to starting scoring of spontaneous mutation frequency

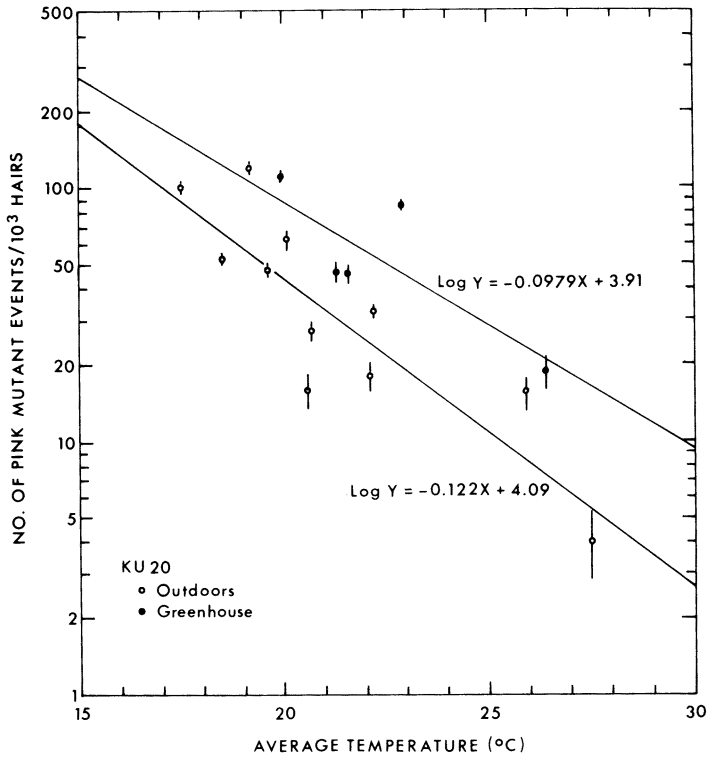

Fig. 1. Relationships between average temperature (of the period nine days earlier than each scoring period of mutations) and spontaneous pink mutation frequency (log) in the stamen hairs of KU 20 clone grown outdoors or in the greenhouse. The regression lines were obtained by the least square method for the data from outdoors and the greenhouse. The longitudinal line attached to each point indicates standard error.

under a certain controlled environment, in the growth chamber in which the experiment is going to be carried out.

The spontaneous mutation frequencies of KU 20 clone under ten different controlled environmental conditions in LPH, CEL and GR are presented in Table 2. The mutation frequencies observed ranged from 3.06 ± 0.37 to 40.8 ± 3.1 pink mutant events per 10^3 hairs, and a negative correlation between the mutation frequency and temperature is also seen in this table. It is clear, however, that the spontaneous mutation frequencies obtained under the conditions shifting temperature in the day/night cycle, especially with a 5°C shift than with 3°C shifts, were obviously higher than those obtained under constant temperatures or sine-curved gradual temperature changes. These relationships are more clearly seen in Fig. 3 in which the logarithms of the mutation frequencies are plotted against average temperature.

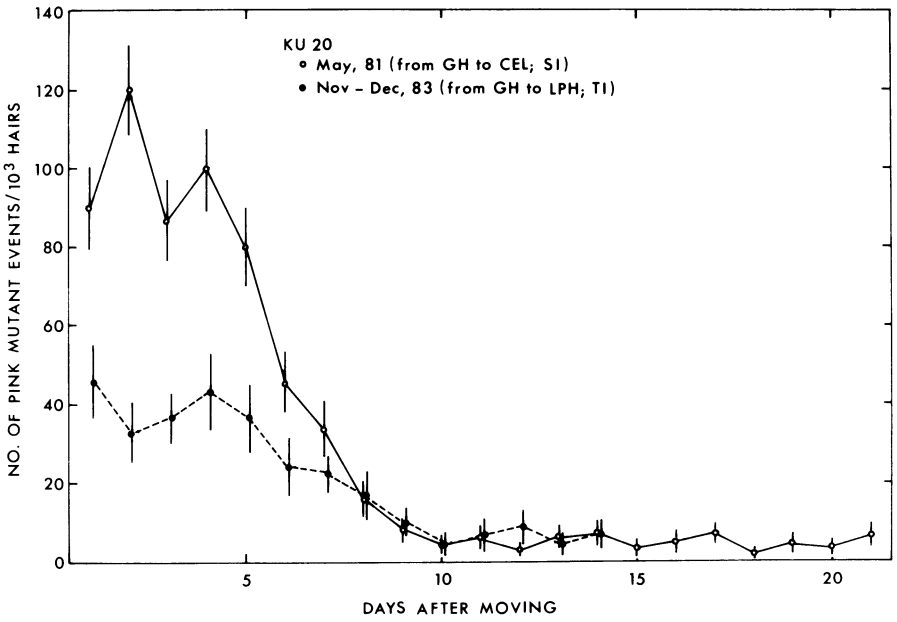

Fig. 2. Daily changes in spontaneous pink mutation frequency after moving KU 20 plants from the greenhouse (GH) to growth chambers (CEL and LPH; see text). The longitudinal line attached to each point indicates standard error.

Table 2. Spontaneous pink mutation frequencies in the stamen hairs of KU 20 clone under controlled environmental conditions

Facility[a]	Temperature (°C)	No. of hairs observed	No. of pink mutant events	No. of pink mutant events /10^3 hairs (±SE)
Constant temperature				
LPH	17.5±0.5	4,165	170	40.8 ±3.1
	20.0±0.5	12,925	87	6.73±0.72
	22.5±0.5	21,893	67	3.06±0.37
GR	23 ±1	15,304	55	3.59±0.48
Sine-curved gradual change				
LPH	17.5–22.5±0.5	5,298	31	5.85±1.05
	20.0–25.0±0.5	13.301	44	3.31±0.50
Day/night shift				
CEL	25(D)/20(N)±1	10,875	67	6.16±0.75
		5,712	43	7.53±1.14
	24(D)/21(N)±1	8,092	38	4.70±0.76
	23(D)/20(N)±1	17,881	136	7.61±0.65
	22(D)/19(N)±1	18,206	207	11.4 ±0.8

[a] LPH: LPH-200-RD growth chamber; GR: plant growth room; GEL: Sherer CEL 38–15 growth chamber.

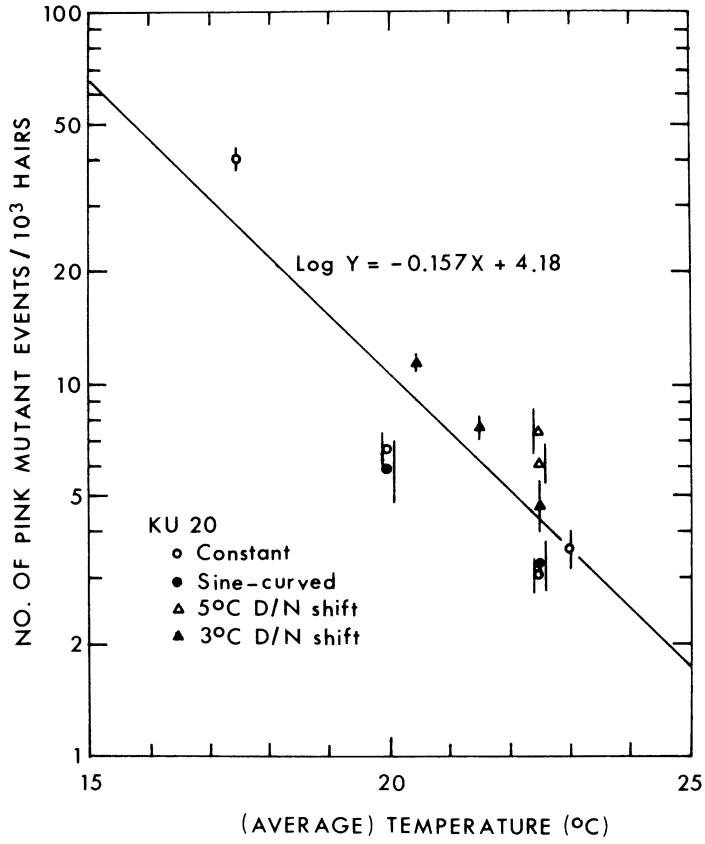

Fig. 3. Relationship between temperature or average temperature and spontaneous pink mutation frequency (log) in the stamen hairs of KU 20 clone grown under controlled environmental conditions. The regression line was obtained by the least square method. The longitudinal line attached to each point indicates standard error.

4. DISCUSSION

The frequency of spontaneously occurring pink mutations in the stamen hairs of KU 20 clone varied greatly from 4.03 ± 1.21 to 120 ± 7, from 18.8 ± 3.1 to 110 ± 5, and from 3.06 ± 0.37 to 40.8 ± 3.1 pink mutant events per 10^3 hairs in OD, GH (Table 1), and in CEL, LPH or GR (Table 2), respectively. The difference between the highest (in OD) and the lowest (in LPH) spontaneous mutation frequencies observed in the present study exceeded 39 times, being much larger than the 23-fold difference reported earlier in this clone (Takahashi and Ichikawa, 1976). The spontaneous mutation frequencies collected from the control plants in six serial gamma-ray irradiation experiments with KU 20 clone, which were performed in parallel with the present study, also ranged from 3.93 ± 0.70 to 16.6 ± 1.3 pink mutant events per 10^3 hairs in CEL, with a much higher value of

95.9±1.9 in GH (Ichikawa et al., 1991). Including the highest value ever reported (133±2; Takahashi and Ichikawa, 1976), KU 20 clone has exhibited a greater than 43-fold variation of spontaneous pink mutation frequency in its stamen hairs.

The spontaneous mutation frequency was negatively correlated to temperature, being higher at lower temperature in general, although considerably large fluctuations were observed (Figs. 1 and 3). The relationship with temperature in OD (Fig. 1) was similar to that in OD and the air-conditioned GH reported by Takahashi and Ichikawa (1976). However, all of the five points from GH in Fig. 1 were plotted above the regression line for the data from OD in the same figure, and were also located above the regression line reported earlier for the data from OD and the air-conditioned GH putting together (Takahashi and Ichikawa, 1976), indicating that the spontaneous mutation frequency becomes higher in GH than in OD or in the air-conditioned GH.

Since the fluctuation of temperature in the period nine days earlier than each scoring period tended to be larger in GH (17.3 to 25.7°C differences between the maximum and the minimum, averaging 21.0°C) than in OD (9.4 to 19.9°C, averaging 15.0°C) (see Table 1), the larger fluctuations of temperature in GH might be related to the higher spontaneous mutation frequencies. In fact, the spontaneous mutation frequencies of this clone under controlled environmental conditions (0 to 5°C diurnal temperature differences) were significantly lower (Table 2 and Fig. 3) than in OD (Table 1 and Fig. 1), and also the frequency decreased sharply and stabilized at a low level after moving this clone from GH to CEL or LPH (3 and 5°C diurnal differences, respectively) (Fig. 2). It has been demonstrated that larger diurnal temperature differences resulted in higher spontaneous mutation frequencies in BNL 02 clone (Mericle et al., 1976), and also in KU 20 clone and at least two other clones, KU 13 and KU 24 (Ichikawa, 1984a).

In the present study, however, 5°C diurnal temperature differences by changing the temperature gradually with a sine curve in LPH were found not to affect the spontaneous mutation frequency of KU 20 clone, the frequencies being kept at almost identical levels with those under no diurnal temperature differences (constant temperatures) in LPH (Table 2 and Fig. 3). On the other hand, the diurnal temperature differences of 3 and 5°C (especially the latter) by shifting the temperature suddenly in a 12-hr day/12-hr night cycle in CEL were found to be effective in increasing the spontaneous mutation frequency of this clone (Table 2 and Fig. 3). It seems therefore likely that such sudden or rapid changes of temperature are related to increasing spontaneous mutations, as the true cause of the effect of diurnal temperature differences.

It was reconfirmed that KU 20 clone is a temperature-sensitive mutable clone which possesses a characteristic of being highly mutable spontaneously at lower temperature. It is considered that a repair mechanism of DNA damages occurring spontaneously, which is more effective at higher temperature, thus presum-

ably an enzymatic one, is very likely involved in the mutable nature of this clone, as suggested earlier (Ichikawa, 1981b). A similar relationship reported earlier for gamma-ray-induced mutations in *Tradescantia* BNL 02 clone (Sparrow et al., 1971) and in rice plants (Yamagata and Fujimoto, 1970), that is, higher induced mutation frequency at lower temperature, may also be related to such repair mechanism.

While the repair mechanisms in higher plants are much less known at molecular level as compared to those in microorganisms, some evidences have been accumulated from not a few representatives of higher plants (Veleminsky and Gichner, 1978; Caldwell, 1981; Ichikawa, 1981b). The examples of enzymatic repair mechanisms demonstrated to be operative in higher plants are: the photoreactivation of UV-induced DNA damage found in maize (see Ikenaga et al., 1974), the excision repair of UV damages in wild carrot protoplasts (Howland, 1975) and grass pea, *Lathyrus sativa* (Soyfer and Cieminis, 1977), the rejoining repair of gamma-ray-induced DNA single-stranded breaks in wild carrot protoplasts (Howland et al., 1975) and barley embryos (Tano and Yamaguchi, 1977), and the repair of DNA single-stranded breaks induced by alkylating agents in barley (see Veleminsky and Gichner, 1978).

However, it is not clear to what extent each of these repair mechanisms is involved in repairing spontaneously occurring DNA damages. The nature of spontaneously occurring mutations has not yet been understood in detail because of their scarce occurrence in most of experimental organisms, although it is generally accepted that the proportion of spontaneous mutations caused by naturally existing UV, ionizing radiations or mutagenic chemicals among all spontaneous mutations must be rather small.

The present material, KU 20 clone of *Tradescantia*, gives a rare opportunity to conduct studies on spontaneous mutations, and our recent study on the sectoring patterns of spontaneous pink mutations in the stamen hairs, as compared to those of gamma-ray-induced pink mutations, revealed that somatic recombination must be the mechanism of a significant part of the spontaneously occurring mutational events in this clone (Ichikawa, 1984b, unpublished data). The importance of somatic recombination has been also demonstrated in the stamen hairs of *T. hirsuticaulis* (BNL 2091 clone) by Christianson (1975) as the predominant mechanism of spontaneous mutations. If such somatic recombination is playing an important role in producing spontaneous mutations, it seems likely that a recombinational or postreplication repair, which has not yet been proved to be operative in higher plants, is involved in the mutable nature of KU 20 clone.

It should be mentioned that one of other mutable clones of *Tradescantia*, BNL 0106, a segregant from BNL 02 clone, has been described to show a variation in spontaneous mutation frequency ranging from 13.6 ± 1.4 to 118 ± 4 pink mutant events per 10^3 hairs (Sparrow and Sparrow, 1976; Nauman et al., 1978). However, the mutation frequency of this clone was higher at a higher temperature

(26.5°C) than in a lower temperature range (18 to 20°C) (Nauman et al., 1978), in contrast to KU 20 clone.

On the other hand, KU 13 and KU 24 clones (both also being mutable) showed responses to temperature similar to that of KU 20 clone, their spontaneous mutation frequencies being higher at lower temperature (Ichikawa, 1984a). Similar tendencies but at less extents have been also observed in three non-mutable clones, KU 7 (Takahashi and Ichikawa, 1976; Ichikawa et al., 1981; Ichikawa, 1984a), KU 9 (Takahashi and Ichikawa, 1976; Ichikawa, 1984a) and KU 16 (Ichikawa, 1984a). There are therefore two opposite responses to temperature and two different types of temperature-sensitive mutable clones in *Tradescantia*.

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