ムラサキツユクサ雄蕊毛における低線量率低線量域での体細胞突然変異率

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SOMATIC MUTATION RATE AT LOW LEVELS OF CHRONIC GAMMA-RAY EXPOSURES IN *TRADESCANTIA* STAMEN HAIRS[1)]

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INTRODUCTION

While utilization of atomic energy is growing rapidly, it is becoming increasingly important to obtain more basic data of genetic effects of radiations especially at low levels of exposures and/or exposure rates, in order to assist establishing how to protect ourselves from obviously increasing radiation hazard. The radiation hazard may be caused by accidental radiation leakage or contamination which results in relatively high acute exposures, or by non-accidental constant leakage or contamination at low levels. Both types of radiation hazard will increase more and more with the development of nuclear power plants, nuclear ships, and other facilities utilizing atomic energy. On the other hand, radiobiological researches have accumulated a considerable amount of data concerning the effects of various radiations in various organisms, and those data we now have can give us fairly detailed informations about biological responses to relatively high exposures at relatively high (or not very low) exposure rates. However, we do not have enough data of biological effects of low radiation exposures at very low exposure rates. Thus, it is absolutely necessary to add more data bearing this point in order to prevent the latter type of radiation hazard mentioned above. Furthermore, it is also important for us to establish a sensitive biometrical system by which we can check radiation leakage or contamination at low levels.

The present study was undertaken to obtain more informations on mutation rate at low levels of gamma-ray exposures and exposure rates, employing *Tradescantia* stamen hairs which have been demonstrated by us to be excellent experimental material for determining somatic mutation rate because of their essentially single-meristematic-cell nature (Nayar and Sparrow 1967; Ichikawa and Sparrow 1968, 1969; Ichikawa *et al.* 1969; Ichikawa 1970). The other purpose of this study was to test whether or not the *Tradescantia* stamen hair system can be used as a sensitive biometrical tool for detecting low levels of radiation exposures.

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MATERIAL AND METHODS

A clone (KU 7) of *Tradescantia ohiensis* Raf. (=*T. reflexa* Raf.) (4*x*, 2*n*=24) was used in the present study. This clone has flower petals and stamens (including stamen hairs) of a blue color but is heterozygous genetically for flower color (blue/pink, blue being dominant). Thus, if a mutation or a deletion of the dominant gene for the blue color was induced, the recessive character, a pink color, appears in daughter cells. The both colors are easily distinguishable from each other. Each inflorescence of this clone is composed of at least 50 buds, one or two (occasionally three) of which bloom daily. Each flower has six stamens and each stamen bears about 60 to 70 hairs. Each hair is a single chain of cells, and the average numbers of cells per hair at the basal, middle and distal thirds of the stamen filament are about 32, 28 and 23, respectively. Each hair is largely a product of a series of the lineally succeeding terminal cells, which are meristematic until full development of the hair. Most of the subterminal cells also divide but usually only once, and other interstitial cells divide rarely (see Ichikawa and Sparrow 1967b).

Irradiation treatments were performed in the gamma field of the National Institute of Radiation Breeding, M.A.F., Omiya, Ibaraki-ken, starting from July 25, 1970. Potted plants having several inflorescences of flowering size were placed at five different positions in the gamma field and were exposed to ^{60}Co gamma-rays chronically (Fig. 1). Exposure rates at these five positions were 18.5, 14.2, 9.5, 4.6 and 2.3 R/day (or 925, 710, 475, 230 and 115 mR/hr), respectively. Two potted plants were used for each exposure rate. Two other potted plants were placed on the protection earth bank of the gamma field (Fig. 1), where exposure rate of scattering radiation was calculated to be 280 mR/day (14 mR/hr) based on earlier measurement (see Kawara 1967). Two control plants were kept in a control field of the institution.

Observations of stamen hairs were carried out on the flowers which opened 11 and 12 days (Period 1) and 25 to 27 days (Period 2) after the start of irradiation. The stamen hairs of those flowers had been actively growing at the time of starting irradiation (for flowers observed at Period 1) or had started their development during the

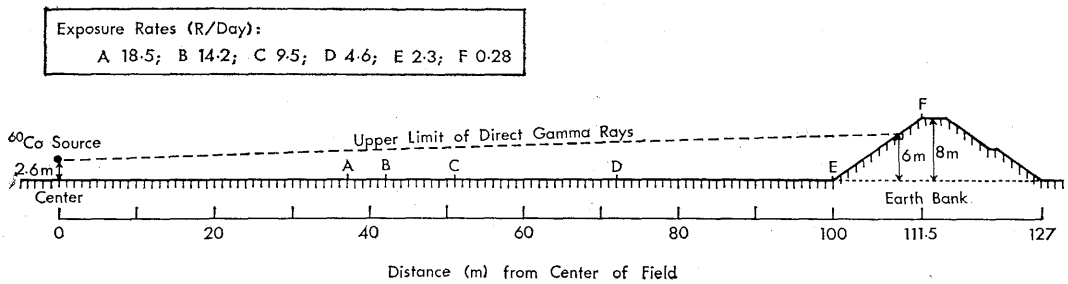

Fig. 1. Illustration of radiation treatments performed in the gamma field of the National Institute of Radiation Breeding. Potted plants of *Tradescantia ohiensis* were placed at positions A to E in the irradiation area in the gamma field or at position F on the protection earth bank, and were exposed chronically to ^{60}Co gamma-rays (A to E) or to scattering radiation (F).

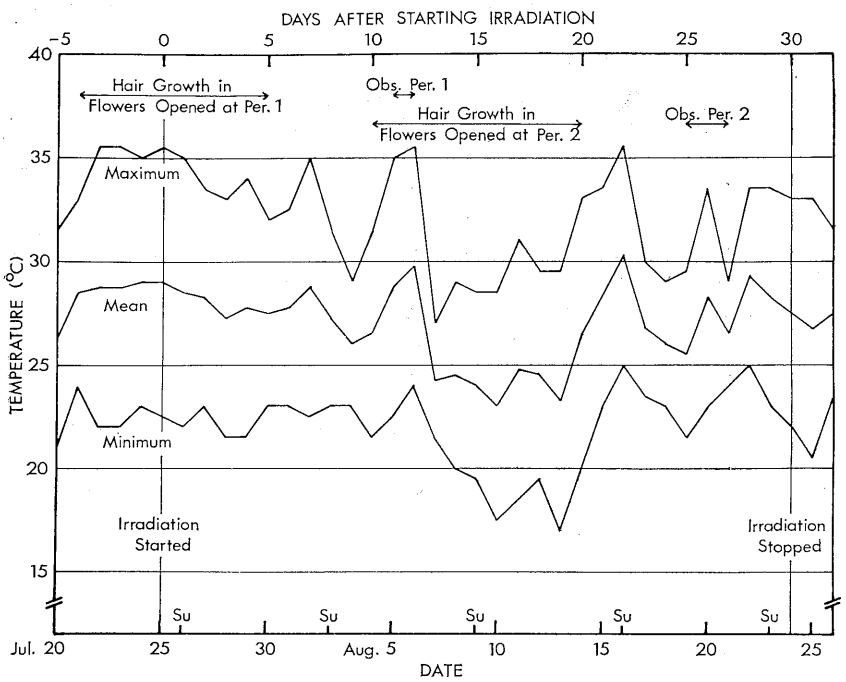

Fig. 2. The maximum, minimum and mean temperatures recorded in the gamma field during the experimental period (the data were supplied by the National Institute of Radiation Breeding). Irradiation period, two observation periods and developmental periods of the stamen hairs examined are indicated.

irradiation (for flowers observed at Period 2) (see Table 3 and Fig. 2). The number of flowers examined per day for each exposure and for the control was two to six. It means that about 800 to 2,400 stamen hairs or about 20,000 to 60,000 stamen hair cells were observed for each treatment per day. The number of stamen hairs was counted for each stamen and the positions of all somatic pink mutant cells which appeared in the terminal 30 cell positions of each hair were recorded. The cell numbers were also recorded for all of the hairs which contained mutant cells and were composed of less than 30 cells.

The temperature in the gamma field varied considerably during the experimental period as shown in Fig. 2.

RESULTS AND DISCUSSION

Evident genetic effects of the radiation treatments at low levels were clearly observed as seen in Tables 1 and 2. As will be described below in detail, it was possible to detect significant increases in somatic mutation rate in the stamen hairs even after gamma-ray treatment with about 8 R total exposure at 2.3 R/day exposure rate, or after treatment with scattering radiation at a level as low as only about 4.5 R total exposure at 280 mR/day (14 mR/hr) exposure rate.

Table 1. Average numbers of pink mutant events per hair determined at Periods 1 and 2

Exposure rate* (R/day)	Period 1			Period 2		
	No. hairs examined	No. pink mutant events	No. pink mutant events/hair ($\times 10^{-2}$) ±S.E.	No. hairs examined	No. pink mutant events	No. pink mutant events/hair ($\times 10^{-2}$) ±S.E.
18.5	2,922	78	2.67±0.09	3,410	278	8.15±0.11
14.2	2,521	54	2.14±0.10	2,422	137	5.66±0.15
9.5	3,188	49	1.54±0.10	2,932	152	5.18±0.15
4.6	2,920	35	1.20±0.08	2,589	57	2.20±0.09
2.3	3,064	22	0.72±0.08	2,884	36	1.25±0.09
0.28	1,607	5	0.31±0.03	2,409	30	1.25±0.08
0	2,207	11	0.50±0.07	2,446	12	0.49±0.06

* Total effective exposures at both periods are seen in Table 4.

Table 2. Frequencies of pink mutant cells in the hair cell population consisted of the terminal 30 cells of each hair determined at Periods 1 and 2

Exposure rate* (R/day)	Period 1			Period 2		
	No. hair cells examined	No. pink cells	% pink cells ±S.E.	No. hair cells examined	No. pink cells	% pink cells ±S.E.
18.5	64,284	374	0.58±0.06	80,818	2,390	2.96±0.08
14.2	57,978	295	0.51±0.06	59,128	1,143	1.97±0.09
9.5	79,062	269	0.34±0.05	73,300	1,265	1.73±0.10
4.6	72,517	283	0.39±0.08	63,432	366	0.58±0.07
2.3	77,520	156	0.20±0.03	72,965	380	0.52±0.07
0.28	43,549	68	0.16±0.02	65,285	318	0.49±0.06
0	58,488	121	0.21±0.05	64,820	116	0.18±0.05

* Total effective exposures at both periods are seen in Table 4.

The average numbers of somatic pink mutant events per hair at Periods 1 and 2 are presented in Table 1 and are plotted against daily exposure in Fig. 3. A *pink mutant event* is defined as two or more contiguous pink cells or a single pink cell, and is considered to have been derived from a mutation induced in a single meristematic hair cell (Ichikawa and Sparrow 1968; Ichikawa *et al.* 1969; Ichikawa 1970). The data collected on days 11 and 12 are pooled as those for Period 1 because there is no significant difference in the data between those days. The data on days 25 to 27 (Period 2) are also pooled from the same reason. It is clearly seen that the frequency of pink mutant events is much higher at Period 2 than at Period 1. It seems also evident from this figure that the number of pink mutant events per hair increases roughly linearly with increasing daily exposure at both the periods, excepting for the data from the lowest daily exposure, *i.e.* 280 mR of scattering radiation per day at the top of the earth bank (see Fig. 1).

Since no radiation effects are observed in the stamen hairs whose development had been completed by the time of irradiation (Ichikawa and Sparrow 1967a, 1967b, 1968; Ichikawa 1968, 1970; Ichikawa *et al.* 1969), gamma-ray exposures given after the com-

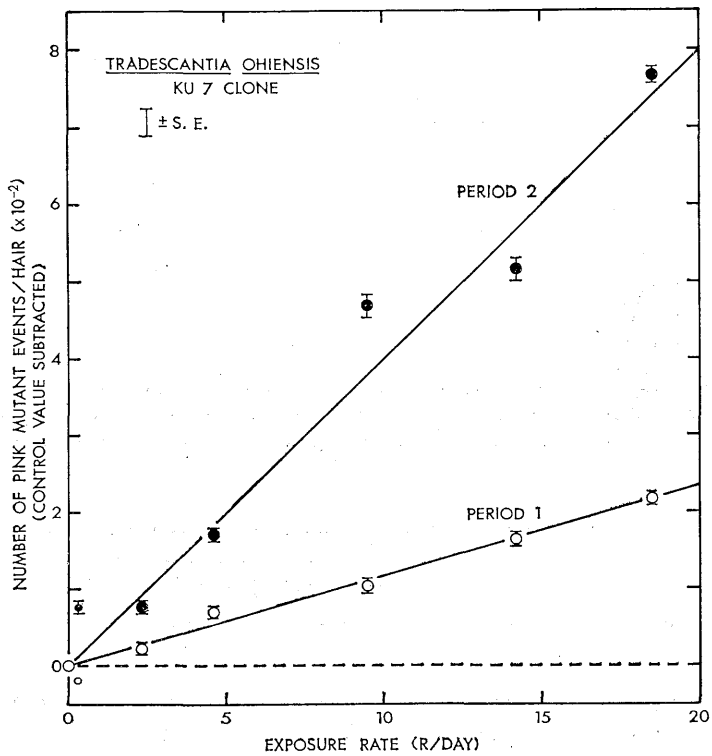

Fig. 3. Average numbers of pink mutant events per hair (minus control) determined at Periods 1 (open circles) and 2 (solid circles) for treatments with ^{60}Co gamma-rays at exposure rates of 2.3 to 18.5 R/day (larger circles) or with scattering radiation at exposure rate of 280 mR/day (smaller circles).

pletion of the hair development can be considered to be "ineffective exposures". In the clone used, the hair development is completed seven days before flowering. Also, no irradiation is performed on Sunday in the gamma field. Thus, in the present study, the number of days after the start of irradiation does not represent "effective exposure time". The effective exposure times are shown in Table 3. Based on the figures in this table and on the exposure rates, it is possible to calculate "total effective exposure" for each treatment for each day as shown in Table 4.

When the average numbers of pink mutant events per hair are plotted against total effective exposure in a log-log graph, it is clearly seen that the somatic mutation frequencies at Period 1 are higher than those at Period 2 (Fig. 4). Two best-fit lines with the slope of +1, one for Period 1 and the other for Period 2, are drawn in this figure, ignoring the data from the plants placed on the earth bank. From these lines in Fig. 4, mutation rates of 3.36×10^{-4} and 2.49×10^{-4} pink mutant events per hair per R effective exposure can be obtained for Periods 1 and 2, respectively. Comparing these two mutation rates, it is apparent that the gamma-ray exposure given before hair development (given only to flowers observed at Period 2, see Fig. 2) is less effective than that given during hair development in inducing somatic pink mutations. By a simple calcu-

Table 3. Total and effective exposure times in day

Period or day	Exposure time in day*				
	Before hair development**	During hair development**	After hair development**	Total	Effective exposure time***
Period 1					
Day 11		3	6	9	3
Day 12		4	6	10	4
Period 2					
Day 25	8	7	6	21	15
Day 26	9	7	6	22	16
Day 27	10	7	6	23	17

* 20-hr irradiation per day. No irradiation was performed on Sundays.
** Development of the stamen hairs of KU 7 clone starts and ends 15 and 7 days before flowering (=before observation), respectively, under natural condition in July and August.
*** See text.

Table 4. Total effective exposure in R for each treatment

Period or day	Exposure rate (R/day)					
	18.5	14.2	9.5	4.6	2.3	0.28
Period 1						
Day 11	55.5	42.6	28.5	13.8	6.9	0.84
Day 12	74.0	56.8	38.0	18.4	9.2	1.12
Average	*64.8*	*49.7*	*33.3*	*16.1*	*8.1*	*0.98*
Period 2						
Day 25	129.5(+148.0)*	99.4(+113.6)	66.5(+76.0)	32.2(+36.8)	16.1(+18.4)	1.96(+2.24)
Day 26	129.5(+166.5)	99.4(+127.8)	66.5(+85.5)	32.2(+41.4)	16.1(+20.7)	1.96(+2.52)
Day 27	129.5(+185.0)	99.4(+142.0)	66.5(+95.0)	32.2(+46.0)	16.1(+23.0)	1.96(+2.80)
Average	*129.5(+166.5)*	*99.4(+127.8)*	*66.5(+85.5)*	*32.2(+41.4)*	*16.1(+20.7)*	*1.96(+2.52)*

* Figures in parentheses show exposures before hair development.

lation based on the above mutation rates and on the exposure times before and during hair development (see Table 3), it is estimated that gamma-rays given before hair development produced somatic pink mutations in hairs at a rate of 1.81×10^{-4} pink mutant events per hair per R effective exposure. This mutation rate is about 0.54 times of the above rate of 3.36×10^{-4} pink mutant events per hair per R effective exposure obtained for Period 1, the mutation rate by gamma-ray exposure given during hair development only. It should be noted, however, that the effect of temperature can not be ignored, since the present experiment was carried out under an uncontrolled condition. The temperature data recorded in the gamma field during the experimental period are presented in Fig. 2. The data show that the stamen hairs observed at Period 1 accomplished their development at higher temperature than those observed at Period 2. Yamagata and Fujimoto (1970) found a higher somatic mutation frequency at 20°C than at 30°C in a strain of rice plants heterozygous for albinism after acute gamma-ray treatments of potted seedlings. It is unknown, however, whether or not

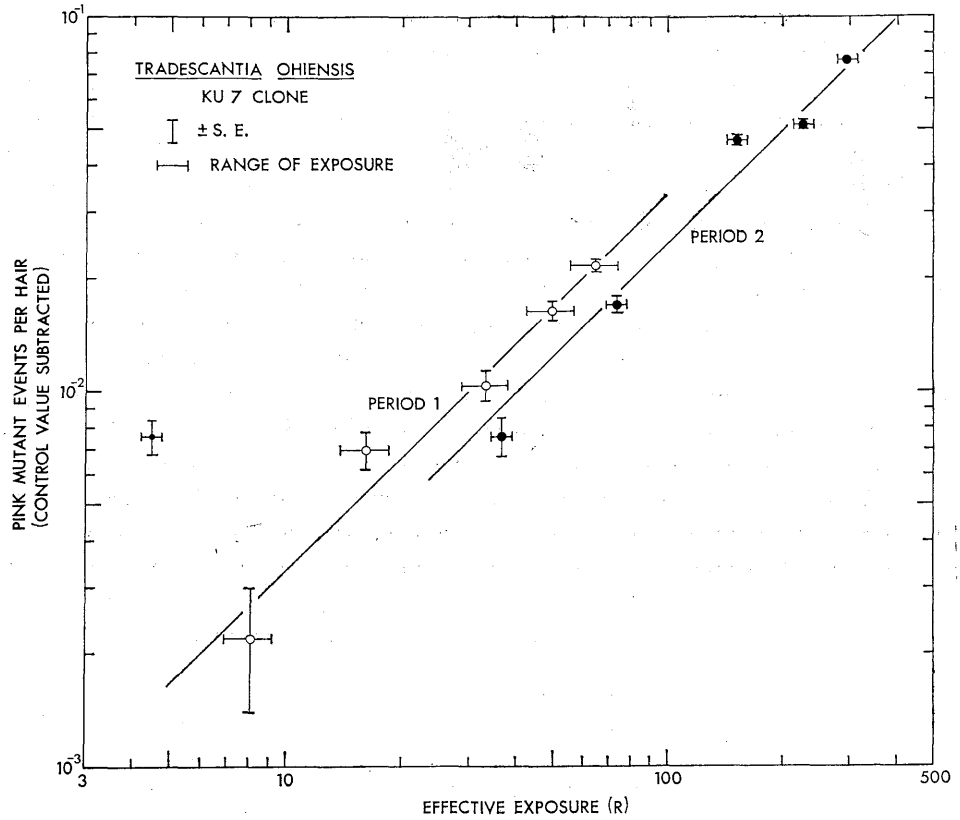

Fig. 4. Average nnmbers of pink mutant events per hair (minus control) at Periods 1 (open circles) and 2 (solid circles) plotted against total effective exposure. Two best-fit lines with the slope of +1 are drawn for both periods. The smaller solid circle at the leftmost is the result at Period 2 from the plants placed on the earth bank and is ignored for drawing the best-fit line (The result at Period 1 from the same plants can not be plotted).

the difference in temperature shown in Fig. 2 affects somatic mutation frequency in *Tradescantia* stamen hairs.

The above mutation rate of 3.36×10^{-4} pink mutant events per hair per R effective exposure obtained for Period 1 is much lower than the earlier value of 1.56×10^{-3} pink mutant events per hair per rad obtained from the same clone but after ^{137}Cs gamma-ray irradiation at very much higher dose rates of 18 to 45 rads/min (Ichikawa 1970). The present mutation rate is also significantly lower than the value of 9.03×10^{-4} pink mutant events per hair per R determined for a clone of *T. virginiana* (also tetraploid) irradiated with ^{137}Cs gamma rays at the exposure rates of 6.25 to 31.25 R/hr (Ichikawa and Sparrow 1968), about 30 to 60 times higher exposure rates than those in the present study. Therefore, the effect of dose rate seems to be evident in inducing somatic mutations as demonstrated by Ichikawa *et al.* (1969), although the effect of temperature can not be ignored as mentioned above.

The frequency of pink mutant cells in the hair cell population consisted of the

terminal 30 cells of each hair was also studied for Periods 1 and 2. The resultant values are shown in Table 2 and are plotted against daily exposure in Fig. 5. In this case, the data obtained for Period 1 are very much lower than those obtained for Period 2 in comparison with the case of the number of pink mutant events per hair shown in Fig. 3. Namely, the frequencies of pink mutant cells in the hair cell population per R effective exposure are calculated to be 5.71×10^{-5} and 8.78×10^{-5} for Periods 1 and 2, respectively (ignoring the data from the lowest daily exposure). The high value for Period 2 is considered to be due to the appearance of longer terminal pink mutant events at this period than at Period 1. A *terminal pink mutant event* is defined to be that derived from a somatic pink mutation induced in the terminal cell of an immature hair. Since such terminal pink mutant events are the products of (repeated) cell division of the terminal pink-mutated cells, the length (or the number of cells consisted) of the terminal pink mutant event depends on the number of times of cell division and thus on the length of period after the induction of mutation. Therefore, the higher frequency of pink mutant cells per R effective exposure for Period 2 than for Period 1 does not contradict with the above conclusion that the gamma-ray exposure given before hair development is less effective than that given during hair development.

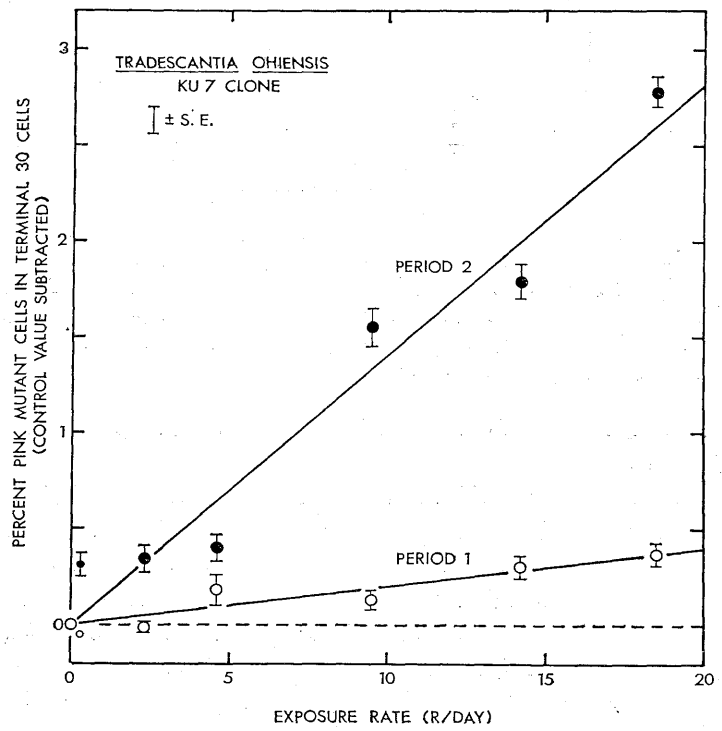

Fig. 5. Frequencies of pink mutant cells in the hair cell population consisted of the terminal 30 cells of each hair (minus control) determined at Periods 1 (open circles) and 2 (solid circles) for treatments with ^{60}Co gamma-rays at exposure rates of 2.3 to 18.5 R/day (larger circles) or with scattering radiation at exposure rate of 280 mR/day (smaller circles).

Somatic mutation rates observed in the plants placed on the earth bank of the gamma field and exposed to scattering radiation at the lowest exposure rate of 280 mR/day (14 mR/hr) do not fit to the mutation rate against daily exposure relationship obtained from the plants irradiated in the gamma field at 2.3 to 18.5 R/day exposure rates (Figs. 3 and 5). The data obtained for Period 2 are obviously much higher than the value expected from the results in the gamma field. The number of pink mutant events per hair per R effective exposure at this very low exposure rate at Period 2 is calculated to be 1.70×10^{-3}, and this value is about seven times as high as the value in the gamma field mentioned above (2.49×10^{-4}). Likewise, the frequency of pink mutant cells per R effective exposure at this exposure rate at this period, 6.92×10^{-4}, is almost eight times as high as the corresponding frequency of 8.78×10^{-5} in the gamma field. Although the data from the lowest exposure rate for Period 1 are somewhat lower than the control levels (Tables 1 and 2, see also Figs. 3 and 5), it should be remembered that the data are the results of the total exposure not exceeding 1 R (see Table 4). On the other hand, the data for Period 2 mentioned above are the results of about 4.5 R total exposure. Thus it seems reasonable to consider that the data for Period 2 are more reliable than those for Period 1. Anyhow, the results of the lowest daily exposure, 280 mR/day, at the top of the earth bank roughly correspond to the mutation frequencies at 2.3 R/day irradiation in the gamma field as seen in Figs. 3 and 5. It may be therefore suspected that the scattering radiation at the top of the earth bank is seven to eight times more efficient in inducing mutations than the direct gamma-rays from ^{60}Co.

The fact that the genetic effects of the scattering radiation at such a low level could be detected must be paid full attention. From the viewpoint of radiation control or health physics, we must not think little of this fact especially because this result was obtained at the top of the protection earth bank of the gamma field, which had been built to prevent radiation leakage.

The present study proved that the stamen hair system of *Tradescantia* heterozygous for flower color is a useful and sensitive biometrical system which shows a clear response to low levels of radiation exposures. Previously, Mericle and Mericle (1965) reported an increase in somatic mutation rate in the stamen hairs of a diploid *Tradescantia* clone by two-week exposure at a radiation level as low as 0.25 mR/hr. Therefore, it seems undoubted that we can detect radiation exposures at much lower levels than in the present study by employing the KU 7 clone of *Tradescantia*.

SUMMARY

Potted plants of *Tradescantia ohiensis* (=*T. reflexa*) were chronically exposed to ^{60}Co gamma-rays at exposure rates of 2.3 to 18.5 R/day (115 to 925 mR/hr) in the gamma field of the National Institute of Radiation Breeding. Other potted plants were placed on the protection earth bank surrounding the gamma field (Fig. 1) and were exposed to a low level (280 mR/day or 14 mR/hr) of scattering radiation. The genetic effects of these radiation treatments were studied in the stamen hairs (essentially single-meristematic-cell system) at two different periods.

Evident genetic responses to these radiation treatments were clearly observed.

Somatic mutation rates increased roughly linearly with increasing daily exposure of gamma rays. The gamma-ray exposure given during hair development induced somatic pink mutations at a rate of 3.36×10^{-4} pink mutant events per hair per R effective exposure. It was estimated that the gamma-ray exposure given during hair development was almost twice as efficient as those given before hair development. The frequency of pink mutant cells in the hair cell population also increased roughly linearly with increasing daily exposure. The frequency per cell per R effective exposure reached the value of 8.78×10^{-5}.

Genetic effects more than expected were observed in the stamen hairs irradiated with scattering radiation at a very low exposure rate of 280 mR/day at the top of the earth bank of the gamma field. Namely, the mutation rates by this treatment were almost as high as those by 2.3 R/day gamma-ray irradiation in the gamma field. It is suspected that the scattering radiation may have seven to eight times more efficiency than the direct ^{60}Co gamma rays.

The stamen hair system of *Tradescantia* heterozygous for flower color was proved to be a sensitive biometrical system for detecting low levels of radiation exposures (*e.g.*, *ca.* 2 R exposure during hair development plus *ca.* 2.5 R exposure before hair development at 280 mR/day exposure rate). The present results obtained by using *Tradescantia* stamen hairs seem to throw doubt on the safety standards for radiation facilities.

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