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# Somatic mutation frequencies in *Tradescantia* stamen hairs treated with aqueous solutions of ethyl methanesulfonate and methyl methanesulfonate

Sodao ICHIKAWA, Hiromi KANAI and Hiroshi HARADA

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

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ABSTRACT

Young inflorescences of *Tradescantia* BNL 02 clone heterozygous for flower color (blue/pink; the blue color being deminant) were treated with 0.1 to 0.5% aqueous solutions of ethyl methanesulfonate (EMS) for 2 to 16 hr, or with 0.005 and 0.01% aqueous solutions of methyl methanesulfonate (MMS) for 16 hr. Somatic pink mutations induced in the stamen hairs were scored, and the mutation frequencies expressed as the number of pink mutant events per $10^3$ hairs and per $10^4$ hair-cell divisions were calculated. The mutation frequencies increased with increasing EMS or MMS dose in %·hr, and the slopes of the dose-response curves on log-log graphs were about 1.2 when the mutation frequencies (minus controls) pooled for 7-day peak periods (post-treatment days 8–14 to 10–16) were plotted agaist EMS dose. It was also found that MMS was about ten times more effective than EMS in inducing pink mutations. The mutation frequencies observed were roughly comparable to those induced by 4 to 300 mGy (0.4 to 30 rad) of acute X rays.

## 1. INTRODUCTION

Since Ichikawa (1971, 1972) and Sparrow et al. (1972) demonstrated that the somatic pink mutation frequency in the stamen-hairs of *Tradescantia* heterozygous for flower color was proportional to the radiation dose even in a small-dose range below 10 mGy (1 rad) or 1R, the stamen-hair system has proven to be an excellent botanical test system for detecting the genetic effects of ionizing radiations and various chemicals at such low levels as human beings may actually be exposed (Underbrink et al., 1973; Schairer et al., 1978, 1983; Ichikawa, 1981a, 1981b, 1990; Schairer and Sautkulis, 1982).

For testing various chemicals, four different methods of treating *Tradescantia* inflorescences with chemicals have been used so far. The first method is treating *Tradescantia* cuttings bearing young inflorescences with gaseous or vaporized chemicals in a special equipment in which the pressure is greatly decreased (Sparrow and Schairer, 1974; Sparrow et al., 1974; Nauman et al., 1976, 1979). The second method is treating inflorescences with aqueous solutions of chemicals, by covering each inflorescence with a small piece of mutagen-soaked absorbent

cotton and sealing it in a small plastic bag (Ichikawa and Takahashi, 1978). The third one is preparing cuttings bearing young inflorescences, and immersing the inflorescences in mutagen solutions (Gichner et al., 1980, 1982). The fourth is a rather indirect one to immerse the stems of cuttings bearing inflorescences in mutagen solutions (Gichner et al., 1980, 1982; Cebulska-Wasilewska et al., 1981).

The chemicals tested so far with *Tradescentia* stamen hairs include alkylating agents such as ethyl methanesulfonate (EMS) (Sparrow and Schairer, 1974; Sparrow et al., 1974; Nauman et al., 1976; Ichikawa and Takahashi, 1978; Cebulska-Wasilewska et al., 1981; Ichikawa et al., 1981a; Sanda and Ichikawa, 1984) and 1,2-dibromoethane (DBE) (Sparrow and Schairer, 1974; Sparrow et al., 1974; Nauman et al., 1976, 1979), some specific air pollutants (Sparrow and Schairer, 1974), gaseous agents in ambient air (Schairer et al., 1978, 1983; Schairer and Sautkulis, 1982), some pesticides (Sparrow and Schairer, 1974), herbicides (Gichner et al., 1982), a promutagen (Gichner et al., 1980), and others (see Schairer and Sautkulis, 1982).

In the present study, young inflorescences of *Tradescantia* were treated with aqueous solutions of EMS and of methyl methanesulfonate (MMS) using the second treating method mentioned above, and the mutagenic effects of EMS and MMS were compared. Testing the mutagenic effect of MMS with *Tradescantia* stamen hairs was tried for the first time. The results obtained in the present study have been reported but only briefly (Ichikawa et al., 1981a).

## 2. MATERIALS AND METHODS

The material used in the present study was *Tradescantia* BNL 02 clone, a diploid hybrid ($2n=12$) heterozygous for flower color (blue/pink; the blue color being dominant). This clone has been frequently used in studies of induced somatic mutations (see Ichikawa, 1990), and its genetic nature has been investigated (Mericle and Mericle, 1967, 1971; Mericle et al., 1974; Kamigawara and Ichikawa, 1988).

In all of the following four experiments, young inflorescences just before initiating blooming were selected from potted plants and were treated with EMS or MMS, or used as controls. They were allowed to grow on the potted plants throughout the experimental periods, except for Experiment 4.

For EMS or MMS treatments, bracts of each inflorescence were cut off, and older flower buds (one or two days before flowering) were removed. Small pieces of absorbent cotton were dipped into EMS or MMS solutions which were freshly prepared, the pH value being adjusted to 7.0. Each inflorescence was carefully covered with the mutagen-soaked cotton, covered by a small polyethylene bag ($27\times40$ mm), and sealed tightly with a scotch tape. After the treatments, inflorescences were washed with tap water for 3 min.

*Experiment 1*: Fifty inflorescences were treated with 0.1 or 0.5% (by volume)

aqueous solutions of EMS (Nakarai Chemicals, Ltd., Kyoto) for 2, 4, 8 or 16 hr, and eight others were used as the controls. The number of inflorescences used for each of the eight different EMS treatments varied from four to nine, using more for lower EMS doses in %·hr. The control inflorescences were not treated in any particular way. The potted plants bearing these inflorescences were grown in a sun-beamed growth chamber (Koitotron 3S-135) throughout the experimental period including 2 weeks prior to the treatments. The temperature in the growth chamber was maintained at 25±2°C during the day and 20±1°C at night, and the day length was kept to be 16 hr.

*Experiment 2*: Twelve inflorescences were treated with 0.2 or 0.4% aqueous solutions of EMS for 8 or 16 hr (four of them with 0.2% for 8 hr, three each with 0.2% for 16 hr and with 0.4% for 8 hr, and two with 0.4% for 16 hr). Thirteen other inflorescences were used as the controls, treating seven and six of them with water (instead of EMS solutions) for 8 and 16 hr, respectively, with the same method as used for the EMS treatments. The potted plants bearing these inflorescences were grown in a growth chamber (the Sherer CEL 38–15) throughout the experimental period including 2 weeks prior to the treatments. The conditions in the growth chamber were maintained as 25±1°C during the day and 20±1°C at night, 60% humidity, and 16-hr day length with the maximum intensity of 23 klx for 12 hr.

*Experiment 3*: Three inflorescences each were treated with 0.005 and 0.01% aqueous solutions of MMS (Nakarai Chemicals) for 16 hr, and five others were treated with water for 16 hr as the controls. The reason for applying MMS at much lower concentrations than EMS was that MMS had been reported to be highly mutagenic in soybean at such low concentrations (Vig et al., 1976). The potted plants bearing these inflorescences were grown in the Sherer CEL 38–15 growth chamber under the same conditions and for the same period as in Experiment 2.

*Experiment 4*: Two inflorescences of axillary shoots (of the potted plants that had been grown in the Sherer growth chamber) were treated with 0.5% aqueous solution of EMS for 16 hr, and two others with water for 16 hr as the controls. Immediately after the treatments, the axillary shooots were cut off at their basal (branching) nodes, making them as cuttings. The basal nodes of the cuttings were immediately immersed in a 1% solution of a commercial plant hormone activator containing $Fe^{2+}$ ("Menedael", Menedael Chemical Laboratory, Osaka) for 2 hr to promote rooting. The cuttings were cultivated with the Knop solution in a growth chamber (LPH-200-RD of the Nippon Medical and Chemical Instruments Co., Ltd.) in which the temperature was changed between 25°C (at 2 p.m.) and 20°C (at 2 a.m.) with a sine curve, the humidity was kept at 55%, and the day length was 16 hr with a light intensity of 6 klx.

For scoring pink mutations in the stamen hairs in these experiments, all the flowers that opened during the scoring periods (post-treatment days 3–21) were

collected daily from each inflorescence used. The number of stamen hairs was counted on each stamen, and the number of hair cells was also counted on ten representative hairs (distal three, middle four and basal three hairs) of one antipetalous and one antisepalous stamens per flower as described earlier (Ichikawa and Takahashi, 1977, 1978). A single pink cell, or two or more contiguous pink cells in a hair was regarded as one pink mutant event, following the earlier definition (Ichikawa et al., 1969). The number of pink mutant events per $10^3$ hairs was calculated for each treatment for each day, and the number of pink mutant events per $10^4$ hair-cell divisions was also obtained by dividing the mutation frequency per hair by one less than the average number of cells per hair (see Sparrow and Sparrow, 1976; Ichikawa and Takahashi, 1977, 1978).

For each treatment, the mutation frequencies expressed in the above two ways were pooled for 7 continuous days (7-day peak period), when the highest 7-day mutation frequencies were observed, and the pooled frequencies were used for the analyses of dose-response relationships.

Table 1. EMS-induced somatic mutation frequencies in the stamen hairs pooled for

| EMS concentration (%) | Duration treated (hr) | Peak period (days after treatment) | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs (±S.E.) |
|---|---|---|---|---|---|
| 0 | — | — | 40,367 | 64 | 1.59±0.20 |
| 0.1 | 2 | 8–14 | 15,434 | 28 | 1.81±0.34 |
| 0.1 | 4 | 8–14 | 14,386 | 27 | 1.88±0.36 |
| 0.1 | 8 | 9–15 | 12,357 | 33 | 2.67±0.46 |
| 0.1 | 16 | 9–15 | 10,208 | 39 | 3.82±0.61 |
| 0.5 | 2 | 9–15 | 12,176 | 29 | 2.38±0.44 |
| 0.5 | 4 | 9–15 | 9,328 | 36 | 3.86±0.64 |
| 0.5 | 8 | 9–15 | 7,597 | 63 | 8.29±1.04 |
| 0.5 | 16 | 10–16 | 7,180 | 123 | 17.13±1.53 |

Table 2. EMS-induced somatic mutation frequencies in the stamen hairs pooled for

| EMS concentration (%) | Duration treated (hr) | Peak period (days after treatment) | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs (±S.E.) |
|---|---|---|---|---|---|
| 0 | 8 | (9–15) | 11,853 | 9 | 0.759±0.253 |
| 0.2 | 8 | 9–15 | 7,101 | 27 | 3.80 ±0.73 |
| 0.4 | 8 | 9–15 | 6,119 | 76 | 12.42 ±1.42 |
| 0 | 16 | (8–14) | 10,342 | 16 | 1.55 ±0.39 |
| | | (10–16) | 10,069 | 14 | 1.39 ±0.37 |
| 0.2 | 16 | 8–14 | 5,190 | 62 | 11.95 ±1.51 |
| 0.4 | 16 | 10–16 | 3,552 | 62 | 17.45 ±2.20 |

## 3. RESULTS

In Experiments 1–3, the frequencies of pink mutant events in stamen hairs started to increase 6 or 7 days after EMS or MMS treatments, reached the highest values on the post-treatment days 10 to 13, and decreased gradually thereafter. The appearance of the peaks of the mutation frequencies tended to be delayed with higher mutagen doses in %·hr. Thus, the 7-day peak period for which data were pooled differed (post-treatment days 8–14 to 10–16) depending on the treatment.

On the other hand, the appearance of pink mutations in stamen hairs was much more delayed in Experiment 4, in which the inflorescences treated with the highest dose of EMS were grown as cuttings, resulting in the 7-day peak period of post-treatment days 13–19.

The results obtained in Experiment 1 are summarized in Table 1. The control inflorescences in this experiment were not treated in any particular way, thus the

7-day peak periods in Experiment 1

| Minus control (±S.E.) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±S.E.) | Minus control (±S.E.) |
|---|---|---|---|
| — | 20.71 | 0.804±0.101 | — |
| 0.229±0.396 | 20.68 | 0.922±0.174 | 0.118±0.201 |
| 0.292±0.412 | 20.62 | 0.957±0.184 | 0.153±0.210 |
| 1.09 ±0.51 | 20.75 | 1.35 ±0.24 | 0.548±0.260 |
| 2.24 ±0.64 | 20.58 | 1.95 ±0.31 | 1.15 ±0.33 |
| 0.797±0.484 | 20.54 | 1.22 ±0.23 | 0.415±0.248 |
| 2.77 ±0.67 | 20.40 | 1.99 ±0.33 | 1.19 ±0.35 |
| 6.71 ±1.06 | 20.31 | 4.29 ±0.54 | 3.49 ±0.55 |
| 15.55 ±1.54 | 20.14 | 8.95 ±0.81 | 8.15 ±0.81 |

7-day peak periods in Experiment 2

| Minus control (±S.E.) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±S.E.) | Minus control (±S.E.) |
|---|---|---|---|
| — | 20.24 | 0.395±0.132 | — |
| 3.04±0.77 | 20.08 | 1.99 ±0.38 | 1.60±0.41 |
| 11.66±1.44 | 20.17 | 6.48 ±0.74 | 6.08±0.75 |
| — | 20.71 | 0.785±0.196 | — |
| — | 20.50 | 0.713±0.191 | — |
| 10.40±1.56 | 20.23 | 6.21 ±0.79 | 5.43±0.81 |
| 16.07±2.23 | 19.85 | 9.26 ±1.18 | 8.55±1.19 |

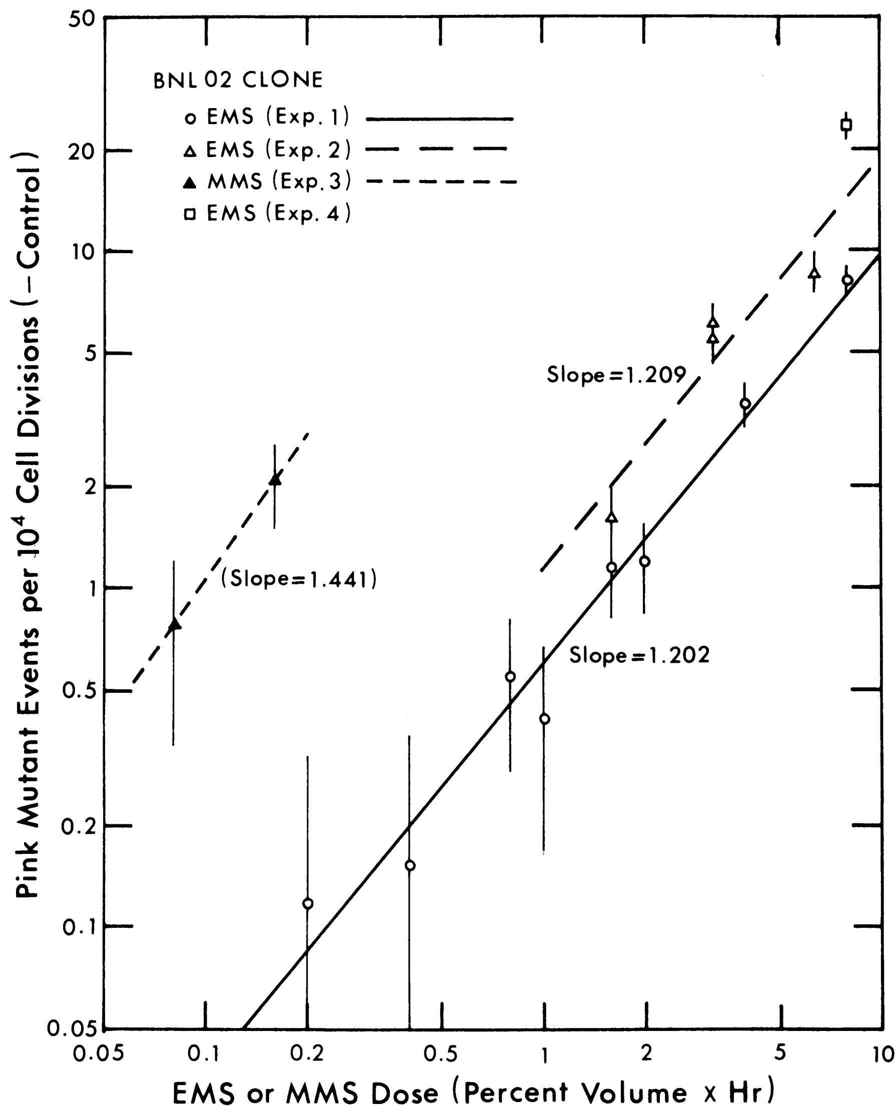


Fig. 1. The relationships between the number of pink mutant events per $10^4$ hair-cell divisions and EMS or MMS dose in %·hr on log-log graph.

control data shown in this table are those pooled for the whole scoring period of 19 days. It is evident in this table that the treatments with 0.5% EMS solution were much more effective in inducing pink mutations than those with 0.1% EMS solution. It is also seen that the longer the duration of EMS exposure the higher were the pink mutation frequencies, although the mutation frequencies obtained for the two lowest EMS doses (2- and 4-hr treatments at 0.1%) were not significantly higher than those in the control. When the EMS-induced mutation

frequencies (minus controls) pooled for the 7-day peak periods were plotted against EMS dose in %·hr on log-log graphs, the best-fit regression lines with the slopes of 1.199 and 1.202 were obtained for the number of pink mutant events per $10^3$ hairs and per $10^4$ hair-cell divisions, respectively. The dose-response relationship in case of the number of pink mutant events per $10^4$ hair-cell divisions are shown in Fig. 1.

The results of Experiment 2 are presented in Table 2. In this experiment, two controls treated with water for 8 and 16 hr were prepared, and thus the control data shown in this table are those collected in the periods corresponding to the 7-day peak periods in the EMS-treated lots (the 16-hr water-treated control data are shown for two different periods, because the peak periods after 16-hr treatments with EMS at 0.2 and 0.4% were different). The induced mutation frequencies observed in this experiment were apparently higher than those in Experiment 1, but the slopes of the best-fit dose-response regression lines on log-log graphs were very similar to those in Experiment 1, being 1.201 and 1.209 for the mutation frequencies per $10^3$ hairs and per $10^4$ hair-cell divisions, respectively (see Fig. 1 for the latter).

The mutation frequencies observed in Experiment 3 are shown in Table 3. Although only two MMS doses were applied at much lower concentrations (0.005 and 0.01% for 16 hr) than EMS, the mutation frequencies after the MMS treatment at 0.005% were significantly higher (at the 5% level) than the frequencies in the control. The MMS-induced mutation frequencies in the two lots roughly corresponded to those with 2.4- and 5.6-hr treatments with 0.5% EMS in Experiment 1, respectively (see Fig. 1).

The data obtained in Experiment 4 are shown in Table 4. The appearance of the peaks of mutation frequencies was delayed in this experiment, probably due to a retardation of flower development by growing the materials as cuttings besides the effect of the EMS treatment. The mutation frequencies obtained from the EMS-treated cuttings (minus controls) were about 2.7 to 2.9 times higher than those obtained with the same EMS dose in Experiment 1 (see Table 1 and Fig. 1).

## 4. DISCUSSION

The present study reconfirmed the effectiveness of the method of treatment of *Tradescantia* inflorescences with aqueous solutions of alkylating agents, the method first used by Ichikawa and Takahashi (1978), in inducing somatic mutations in the stamen hairs. The mutation frequencies observed were greater with higher EMS concentration and with longer EMS treatments in each of Experiments 1 and 2 (Tables 1 and 2). The mutation frequencies also exhibited a clear relationship with the dose of EMS in %·hr (Fig. 1). Clear responses to MMS solutions were also observed in Experiment 3 (Table 3 and Fig. 1).

The mutation frequencies determined in Experiment 1 were generally higher

Table 3. MMS-induced somatic mutation frequencies in the stamen hairs pooled for

| MMS concentration (%) | Duration treated (hr) | Peak period (days after treatment) | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs ($\pm$S.E.) |
|---|---|---|---|---|---|
| 0 | 16 | (8–14) | 9,119 | 9 | 0.987$\pm$0.329 |
| 0.005 | 16 | 8–14 | 4,522 | 11 | 2.43 $\pm$0.73 |
| 0.01 | 16 | 8–14 | 4,515 | 22 | 4.87 $\pm$1.04 |

Table 4. EMS-induced somatic mutation frequencies in the stamen hairs pooled for axillary cuttings

| EMS concentration (%) | Duration treated (hr) | Peak period (days after treatment) | No. of hairs observed | No. of pink mutant events | No. of pink mutant events /$10^3$ hairs ($\pm$S.E.) |
|---|---|---|---|---|---|
| 0 | 16 | (13–19) | 3,457 | 2 | 0.579$\pm$0.409 |
| 0.5 | 16 | 13–19 | 2,900 | 123 | 42.41 $\pm$3.74 |

than those reported earlier (Ichikawa and Takahashi, 1978). Further higher mutation frequencies were obtained in Experiment 2, being nearly two times higher than in Experiment 1 (Fig. 1). It should be noted that the environmental conditions in the earlier study were fairly different from those in Experiments 1 and 2 in the present study, and that the conditions in Experiments 1 and 2 were not identical.

However, when the mutation frequencies (minus controls) pooled for 7-day peak periods were plotted against EMS dose in %·hr on log-log graphs, very similar slopes of the best-fit dose-response regression lines were obtained in both Experiments 1 and 2 (Fig. 1). Namely, they were 1.199 and 1.202 for the number of pink mutant events per $10^3$ hairs and per $10^4$ hair-cell divisions, respectively, in Experiment 1, and the corresponding values in Experiment 2 were 1.201 and 1.209, respectively. These values are somewhat smaller than those (1.374 and 1.309) obtained earlier (Ichikawa and Takahashi, 1978).

Other earlier studies on *Tradescantia* stamen hairs exposed to EMS but in gaseous form (as vapors) for a fixed duration of 6 hr revealed that the slopes of increasing pink mutations with EMS concentration in ppm on log-log graphs were about 2.2 to 2.4 (Sparrow and Schairer, 1974; Sparrow et al., 1974; Nauman et al., 1976). The present values of the slopes after treating with aqueous solutions of EMS are much smaller than those values from the gaseous EMS treatments, reconfirming the different mutagenic responses to EMS after different treating methods. It seems interesting that the present values of slopes are rather closer to the values of about 1.3 to 1.5 obtained for gaseous DBE (Sparrow and Schairer, 1974; Sparrow et al., 1974; Nauman et al., 1976, 1979).

7-day peak period in Experiment 3

| Minus control (±S.E.) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±S.E.) | Minus control (±S.E.) |
|---|---|---|---|
| — | 20.12 | 0.516±0.172 | — |
| 1.45±0.80 | 19.98 | 1.28 ±0.39 | 0.766±0.423 |
| 3.89±1.09 | 19.79 | 2.59 ±0.55 | 2.08 ±0.58 |

7-day peak period in Experiment 4 which was conducted using

| Minus control (±S.E.) | Average no. of cells /hair | No. of pink mutant events /$10^4$ cell divisions (±S.E.) | Minus control (±S.E.) |
|---|---|---|---|
| — | 19.60 | 0.311±0.220 | — |
| 41.84±3.76 | 18.81 | 23.81 ±2.14 | 23.50±2.16 |

As discussed earlier (Ichikawa and Takahashi, 1978), the slopes larger than 1 seem to be the result of involving two-break deletions in the mutational events. Since a slope of 2 on a log-log graph indicates the occurrence of solely two-hit events (no single-hit events), such slopes larger than 2 as reported by Sparrow's group might be the result of insufficient penetration of gaseous EMS at lower concentrations. Nauman et al. (1976) stated that the use of EMS in gaseous state had generated difficulties in accurate dosimetry (in the floral tissues) because of its tendency to condense on the walls of the exposure chamber and on foliage. Although sufficient penetration of gaseous DBE even at low concentrations was confirmed by Nauman et al. (1979) using tritiated DBE, no direct confirmation has been made for gaseous EMS. Schairer and Sautkulis (1982) reported that incorporation of gaseous EMS was considerably affected by whether a vacuum injection technique was used or not.

The dose-response relationships for EMS reported earlier in some other organisms are also inconsistent. Yamashita et al. (1972) reported that chlorophyll mutations in barley were induced roughly as a function of square of EMS dose, while it can be found from the data published by Brock (1976) that morphological mutants in *Arabidopsis thaliana* increased with increasing EMS dose with a slope of about 1.1, if plotted on a log-log graph. The data of reverse mutations in EMS-treated *Neurospora crassa* (heat-shocked prior to the EMS treatments) reported recently by Tanaka et al. (1989) show that the slope of the dose-response curve on a log-log graph, if drawn, is nearly 1.4. The frequencies of EMS-induced somatic reversions in the UZ strain (thought to be unstable due to a transposon) of *Drosophila melanogaster* reported by Ryo et al. (1985) show a

slope between 1.7 and 1.8, while those reported by Fujikawa and Kondo (1986) indicate a slope of about 1.0, both if plotted on log-log graphs.

The mutation frequencies obtained from the EMS-treated cuttings in Experiment 4 were much higher than those observed with the same EMS dose but using potted plants in Experiment 1 (Tables 1 and 4 and Fig. 1). Although it is of course necessary to be reexamined, the result may suggest a possibility of enhancing the sensitivity of the stamen-hair system to alkylating agents by cultivating cuttings.

A much higher mutagenic efficiency of MMS than that of EMS was found in Experiment 3. Although only two MMS doses were tested, the mutation frequencies observed after 0.08 and 0.16%·hr MMS doses were comparable to those with about 1.2 and 2.8%·hr EMS doses in Experiment 1, respectively, or with about 0.7 and 1.7%·hr EMS doses in Experiment 2, respectively (Fig. 1), suggesting that MMS had a mutagenic efficiency ten or more times higher than that of EMS. It is impossible to determine the slopes of the dose-response curves on log-log graphs because of only two MMS doses having been applied, but a simply calculated slope between the two points for the number of pink mutant events per $10^4$ hair-cell divisions is indicated in Fig. 1.

In other organisms, a five to ten times higher mutagenic efficiency of MMS as compared with EMS has been reported in soybean (Vig et al., 1976), and a much greater difference in their mutagenic efficiencies has been demonstrated in *Neurospora crassa* (Tanaka et al., 1989). On the contrary, nearly comparable efficiencies of MMS and EMS have been reported for somatic reversions in *Drosophila melanogaster* (Fujikawa and Kondo, 1986). As for the dose-response relationship for MMS, a linear quadratic model has been found to fit best the induced frequencies of sex-linked recessive lethal mutations in *D. melanogaster* (Lee et al., 1989). That is, predominantly two-hit events occur with higher MMS doses, whereas single-hit events are predominant in a small-dose range.

The somatic pink mutation frequencies induced by the EMS and MMS treatments in the present study are roughly comparable to those induced by 4 to 300 mGy (0.4 to 30 rad) of acute 250-kVp X rays (Sparrow et al., 1972), 0.6 to 100R of chronic $^{60}$Co gamma rays (Ichikawa et al., 1981b), or 0.07 to 13 mGy (0.007 to 1.3 rad) of 0.43-MeV fast neutrons (Sparrow et al., 1972).

It has been demonstrated that somatic pink mutation frequency in the stamen hairs of *Tradescantia* increased linearly with increasing radiation dose not only in the range of smaller doses (Ichikawa, 1971, 1972; Sparrow et al., 1972; Ichikawa and Takahashi, 1977; Ichikawa et al., 1981b), but also at lower dose rates (Ichikawa, 1971, 1972; Ichikawa et al., 1978, 1981b). Such a linear relationship indicates that predominantly one-hit events occur at low radiation dose rates and with smaller radiation doses (Ichikawa et al., 1978; Ichikawa, 1981b). The mutagenic effects of EMS and MMS reconfirmed or confirmed in the present study are therefore somewhat different from those of ionizing radiations.

On the other hand, a synergistic effect of X rays and EMS in inducing mutations in *Tradescantia* stamen hairs has been reported by Cebulska-Wasilewska et al. (1981). A clear synergistic effect of X rays and MMS was also found in *Tradescantia* stamen hairs recently by Ichikawa et al. (in preparation; see Ichikawa, 1990). Thus, it seems likely that there is also a common mechanism in inducing mutations between ionizing radiations and alkylating agents.

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