Jpn. J. Genet. (1995) **70**, *pp.* 473–485

Mutagenic interactions between maleic hydrazide and X rays in the stamen hairs of *Tradescantia* clone BNL 4430

Ling Zhi XIAO and Sadao ICHIKAWA*

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

(*Received 13 March 1995*)

ABSTRACT

Mutagenic interactions between maleic hydrazide (MH; a promutagen known to be activated into a mutagen in plant cells) and X rays were studied in the stamen hairs of *Tradescantia* clone BNL 4430, a blue/pink heterozygote. The young inflorescence-bearing shoots with roots cultivated in the nutrient solution circulating growth chamber were used as tester plants. After determining dose-response curves for X rays and for MH, nine combined treatments with MH (0.5 and 1 mM) and X rays (292 to 1,240 mGy) were conducted, exposing to X rays either 20 or 44 h before, at the midpoint of, or 2 or 44 h after the MH treatments for 4 h. Clear synergistic effects in inducing somatic pink mutations were detected when X rays were given before the MH treatments. On the contrary, however, antagonistic effects were often observed when X-ray treatments were carried out during or after the MH treatments. The synergistic effects detected were thought to be the results of interactions between DNA strand breaks (and the resultant chromosomal breaks) induced by X rays and those by MH, whereas the antagonistic effects observed were presumed to have resulted from X-ray-caused inhibition of the activation of MH in the stamen-hair cells.

1. INTRODUCTION

Many different types of mutagens (mostly man-made) exist in our environment. Using various test systems, it has been revealed that many chemical agents have different degrees of mutagenicity and/or carcinogenicity, but most tests have dealt with the mutagenic or carcinogenic effects of individual agents. Under environmentally relevant conditions, however, various mutagens and carcinogens exist together. Since combinations of two or more different mutagens may result in unknown effects significantly modified from those expected from their additive effects, intensive studies on interactions between different mutagens are urgently needed. Surveys of synergisms between different mutagens are especially important, because the occurrence of synergism has implications for current risk evaluation of individual mutagens (Ichikawa, 1992; Ichikawa et al., 1993; Shima

* Corresponding author.

and Ichikawa, 1994, 1995). Namely, if a synergistic effect exists between any two (or more) mutagens, then simply summing the risks evaluated for the individual mutagens will result in an obvious underestimation of the real risk (Ichikawa, 1992).

Mutagenic synergisms among some monofunctional alkylating agents and X rays have clearly been demonstrated using *Tradescantia* stamen-hair system, i.e., between ethyl methanesulfonate (EMS) and X rays (Cebulska-Wasilewska et al., 1981; Shima and Ichikawa, 1994), methyl methanesulfonate (MMS) and X rays (Ichikawa, 1992; Ichikawa et al., 1993; Shima and Ichikawa, 1994), dimethyl sulfate (DMS) and X rays (Shima and Ichikawa, 1995) and EMS and MMS (Shima and Ichikawa, 1994). Differences in patterns of the synergisms have also been reported (Shima and Ichikawa, 1994, 1995).

In the present study, mutagenic interactions between maleic hydrazide (or maleic acid hydrazide; MH) and X rays were investigated in the stamen hairs of *Tradescantia* clone BNL 4430. MH has been extensively applied as a herbicide, fungicide, growth inhibitor and growth regulator in agriculture (Fishbein, 1972), and is a promutagen known to be activated into a mutagen in plant cells (Gichner et al., 1982; Plewa and Gentile, 1982; Heindorff et al., 1984; Veleminsky and Gichner, 1988). MH is also known to induce chromosomal aberrations in *Vicia faba* (Evans and Scott, 1964; Scott, 1968; Swietlinska and Zuk, 1974) and many other plants (Fishbein, 1972; Swietlinska and Zuk, 1978). Therefore, it was assumed that MH must act synergistically with X rays, since chromosomal breaks are also efficiently induced by X rays.

2. MATERIALS AND METHODS

Tester plants used

Young inflorescence-bearing shoots with roots of *Tradescantia* clone BNL 4430 as described by Shima and Ichikawa (1994) were used as the tester plants in the present study. This clone is a diploid hybrid ($2n=12$) between a blue-flowered *T. hirsutiflora* Bush and a pink-flowered *T. subacaulis* Bush (Emmerling-Thompson and Nawrocky, 1980), thus is a blue/pink heterozygote. This clone has been frequently used in studies of somatic mutations in the stamen hairs induced by ionizing radiations and various chemicals as reviewed earlier (Schairer and Sautkulis, 1982; Schairer et al., 1983; Ichikawa, 1992), and has been reported to be more sensitive to alkylating agents than other clones (Sparrow et al., 1974; Nauman et al., 1976; Ichikawa et al., 1993).

Growing conditions

The young inflorescence-bearing shoots with roots were cultivated in a nutrient solution circulating (NSC) growth chamber (Kyoshin Riko Co.) which was designed for our requirements (Shima and Ichikawa, 1994). The environmental

conditions in the NSC growth chamber were 22.0±0.5°C during the 16-h day with a light intensity of 7,500 lux from white fluorescent tubes, and 20.0±0.5°C at night. The nutrient solution used was 1/2,000 Hyponex solution.

X-ray treatments

X-ray treatments of young inflorescences were performed using a Hitachi MBR-1505R X-ray generator. The treatments were conducted acutely at 150 kVp and 4 mA with a 0.5 mm Al+0.1 mm Cu filter at 23.0±0.5°C, keeping the target distance at 45 cm. The exposures were measured simultaneously with thermoluminescence dosimeter (TLD) elements (National UD-170L) set at the same target distance as the inflorescences treated (six to ten elements per treatment), 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 14 different doses applied ranged between 257 and 1,170 mGy.

Treatments with MH

Young inflorescences just before initiating flowering were treated with 0.25 to 2.5 mM MH (CAS registry no. 123-33-1; Wako Pure Chemical Industries, Ltd) dissolved in phosphate buffer (pH 7.0), for 4 h at 23.0±0.5°C. The methods used for the treatments were essentially identical to those used in earlier studies (Ichikawa and Takahashi, 1978; Ichikawa et al., 1990, 1993; Sanda-Kamigawara et al., 1991; Shima and Ichikawa, 1994, 1995). Briefly, 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 freshly prepared MH solution; each inflorescence was carefully covered with the MH-soaked cotton, covered by a small polyethylene bag (27×40 mm), and sealed tightly. Control inflorescences were treated with the phosphate buffer. Immediately after the MH treatments, inflorescences were washed with tap water for 2 min.

Combined treatments with MH and X rays

Young inflorescences just before initiating flowering were treated with 0.5 or 1 mM MH for 4 h and with acute 292 to 1,240 mGy X rays in various combinations. Namely, the X-ray treatments were conducted either 20 or 44 h before starting, at the midpoint of (2 h after starting), or 2 or 44 h after completing the 4-h MH treatments.

Scoring mutations

The methods used for scoring pink mutations in the stamen hairs in the present study were identical to those described in detail elsewhere (Ichikawa, 1992). Briefly, the numbers of stamen hairs and of pink mutant events (PMEs) were scored on each of six stamens, and the numbers of cells per hair were also counted

on ten representative hairs each of two oppositely located stamens per flower (Ichikawa and Ishii, 1991) to estimate the average numbers of cells per hair for calculating mutation frequency per hair-cell division (Ichikawa, 1992). A PME represents the result of a single mutation (Ichikawa, 1992). Somatic mutation frequency was expressed as the number of PMEs per 10^4 hair-cell divisions, rather than that per 10^3 hairs, since the average number of cells per hair may differ after different mutagen treatments (Ichikawa et al., 1991, 1993; Shima and Ichikawa, 1994, 1995). The data were pooled for the 4-day peak period of mutation frequency for each treatment as in earlier studies (Ichikawa et al., 1993; Shima and Ichikawa, 1994, 1995).

Statistical examinations

Chi-square test was used for determining the occurrence of a synergistic or an antagonistic effect, examining whether or not an induced mutation frequency observed after a combined treatment with MH and X rays was significantly higher or lower than that expected from an additive effect of MH and X rays.

3. RESULTS

X-ray-induced mutation frequencies

The induced mutation frequencies per 10^4 hair-cell divisions obtained after treating with 14 different X-ray doses ranging from 257 to 1,170 mGy are plotted on a log-log graph as shown in Fig. 1. Each of the data plotted were obtained from 3,660 to 13,938 stamen hairs scored, and 10,907 to 22,364 stamen hairs were observed in each control for subtracting spontaneous mutation frequency. The best-fit line drawn, which was determined by the least square method, represents an equation, $y=50.368x^{1.390}$ (x in Gy unit and y is the number of PMEs per 10^4 hair-cell divisions), and this equation was used for calculating the mutation frequencies expected to be induced by X-ray doses applied for combined treatments with MH.

MH-induced mutation frequencies

The mutation frequencies per 10^4 hair-cell divisions induced by 0.25 to 2.5 mM MH treatments for 4 h are plotted against MH dose in Fig. 2. The mutagenic effect of MH at 0.5 mM was examined twice, and that at 1 mM were tested seven times. Each of the data plotted were obtained from 3,090 (for 2.5 mM) to 38,350 (for 0.25 mM) hairs scored, and 8,979 to 43,971 hairs were observed in each control. Although the data from 1 mM MH treatments are fluctuating, the value of slope of this regression line is 1.031, being obviously smaller than 1.390 for X rays (Fig. 1).

The treatment with 2.5 mM MH caused a great reduction in cell number per hair (down to 13.15 from 25.03 in the control), and it meant that the mutation

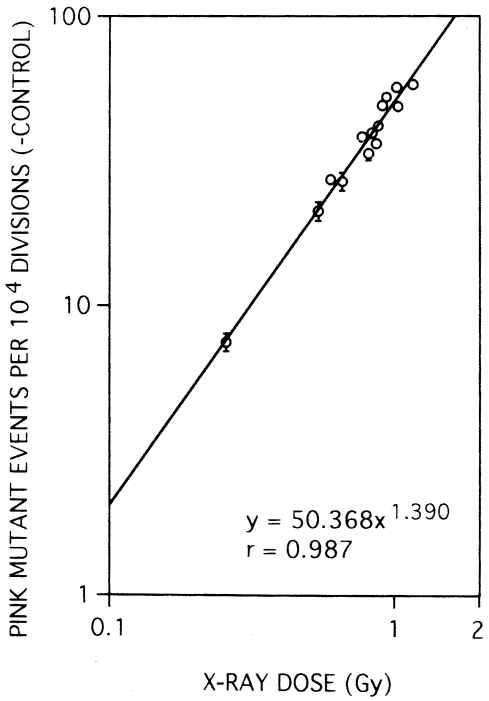

Fig. 1. The dose-response curve obtained in the stamen hairs of clone BNL 4430 by treating acutely with X rays. The best-fit line determined by the least-square method, the equation representing it, correlation coefficient, and standard errors for the points plotted (excepting those within open circles drawn) are shown.

frequency per 10^3 hairs with this MH dose was no longer reliable. Because of the cytotoxicity of MH at the highest concentration, it was determined to apply 0.5 or 1 mM MH in combined treatments with X rays.

Effects of combined treatments with MH and X rays

The data obtained after combined treatments exposing to X rays 20 or 44 h before starting 1 mM MH treatments for 4 h (Experiments 1 and 2) are presented in Table 1. The X-ray doses applied were 976 and 897 mGy in cases of applying 20 and 44 h before the MH treatments, respectively. Each of the expected mutation frequencies listed was obtained by adding the mutation frequency induced by MH alone (Table 1) and that expected to be induced by X rays alone, the latter being calculated using the above equation, $y=50.368x^{1.390}$ (see Fig. 1). The both combined treatments produced mutation frequencies which were significantly higher (at 0.1 and 2% levels, respectively) than the additive values, showing that X rays and MH acted synergistically when X rays were given before the MH treatments.

The data obtained by treating with 0.5 or 1 mM MH for 4 h and X-raying at the

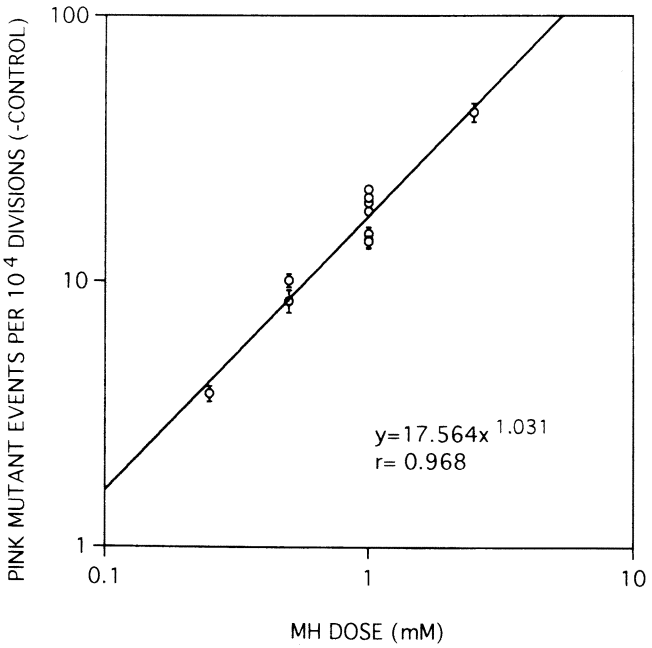

Fig. 2. The dose-response curve obtained in the stamen hairs of clone BNL 4430 by treating with MH for 4 h. The best-fit line determined by the least-square method, the equation representing it, correlation coefficient, and standard errors for the points plotted (excepting those within open circles drawn) are shown.

Table 1. Synergistic effects observed by exposing to X rays before starting 4-h MH treatments

Exp.	MH dose (mM)	X-ray dose (mGy)	Hrs before MH	No. of hairs scored	No. of PMEs[1]	Av. no. of cells /hair	No. of PMEs /10^4 cell divisions (-control)±SE	Expected[2]	P
1	0	0		17,778	53	25.08	1.24±0.17		
	1	0		12,249	485	21.20	18.4 ±0.9		
	1	976	20	8,281	1,031	16.71	78.0 ±2.5	66.6	<0.001
2	0	0		22,364	91	25.32	1.67±0.18		
	1	0		9,686	377	24.16	15.1 ±0.9		
	1	897	44	10,226	1,221	19.03	64.6 ±1.9	58.6	<0.02

[1] Pink mutant events.

[2] Expected number of PMEs per 10^4 cell divisions. Expected value for X rays was calculated using the equation, $y=50.368x^{1.390}$ (see Fig. 1), and that for MH from the same experiment.

midpoint of the MH treatments (Experiments 3 to 5) are listed in Table 2. The X-ray doses applied were 292 and 593 mGy during the 0.5 mM MH treatments and 1,240 mGy during the 1 mM MH treatment. The expected mutation frequencies

Table 2. Antagonistic or non-antagonistic effects observed by exposing to X rays during 4-h MH treatments

Exp.	MH dose (mM)	X-ray dose (mGy)	No. of hairs scored	No. of PMEs[1]	Av. no of cells /hair	No. of PMEs /10^4 cell divisions (-control)±SE	Expected[2]	*P*
3	0	0	21,029	71	26.08	1.35±0.16		
	0.5	0	15,756	436	25.15	10.1 ±0.6		
	0.5	292	15,959	632	24.70	15.4 ±0.7	19.3	<0.001
4	0	0	10,997	41	24.84	1.56±0.24		
	0.5	0	6,919	153	23.05	8.47±0.83		
	0.5	593	4,851	329	22.53	30.0 ±1.7	33.0	NS[3]
5	0	0	17,901	72	25.03	1.67±0.19		
	1	0	9,788	346	23.12	14.3 ±0.9		
	1	1,240	6,726	961	18.34	80.7 ±2.7	82.9	NS

[1] Pink mutant events.

[2] Expected number of PMEs per 10^4 cell divisions. Expected value for X rays was calculated using the equation, $y=50.368x^{1.390}$ (see Fig. 1), and that for MH from the same experiment.

[3] Not significantly different.

Table 3. Antagonistic or non-antagonistic effects observed by exposing to X rays after completing 4-h MH treatments

Exp.	MH dose (mM)	X-ray dose (mGy)	Hrs after MH	No. of hairs scored	No. of PMEs[1]	Av. no. of cells /hair	No. of PMEs /10^4 cell divisions (-control)±SE	Expected[2]	*P*
6	0	0		19,041	76	25.48	1.63±0.19		
	1	0		7,759	364	22.88	19.8 ±1.1		
	1	842	2	8,207	807	21.38	46.6 ±1.7	59.7	<0.001
7	0	0		18,340	87	26.09	1.89±0.20		
	1	0		10,650	579	23.57	22.2 ±1.0		
	1	1,160	44	10,005	1,386	19.68	72.3 ±2.0	84.5	<0.001
8	0	0		19,702	91	25.07	1.92±0.20		
	1	0		9,522	342	23.33	14.2 ±0.9		
	1	1,050	44	7,318	828	19.52	59.2 ±2.1	68.0	<0.01
9	0	0		22,254	75	26.48	1.32±0.15		
	1	0		8,703	420	22.89	20.7 ±1.1		
	1	756	44	7.080	827	20.90	57.4 ±2.0	55.0	NS[3]

[1] Pink mutant events.

[2] Expected number of PMEs per 10^4 cell divisions. Expected value fof X rays was calculated using the equation, $y=50.368x^{1.390}$ (see Fig. 1), and that for MH from the same experiment.

[3] Not significantly different.

were obtained by the same method as described above. Contrary to the above results, a highly significant (at 0.1% level) antagonistic effect was detected in Experiment 3 as seen in this table. No significant differences were detected, however, between the observed and expected mutation frequencies in Experiments 4 and 5.

Significantly antagonistic effects between MH and X rays were also detected after treating with 1 mM MH and exposing to 842 mGy X rays 2 h after (at 0.1% level) or to 1,160 and 1,050 mGy X rays 44 h after completing the MH treatments (at 0.1 and 1% levels, respectively), as shown in Table 3 (Experiments 6 to 8). However, a merely additive effect was observed after the combined treatment with 1 mM MH plus 756 mGy X rays 44 h after the MH treatments (Experiment 9).

4. DISCUSSION

Effects of single agents

The dose-response curve determined for X-ray-induced pink mutation frequency per 10^4 hair-cell divisions in the present study had a slope of 1.390 on a log-log graph (Fig. 1). This value of slope is somewhat larger than 1.252 (Shima and Ichikawa, 1994) and 1.314 (Shima and Ichikawa, 1995) for X rays determined earlier in the same clone, and also than 1.274 for X rays obtained in clone KU 27 (Ichikawa et al., 1993).

On the other hand, the dose-response curve determined for MH had a slope of 1.031 (Fig. 2). This value is apparently smaller than 1.390 for X rays (Fig. 1) and also than the above earlier values of 1.252 to 1.314 for X rays, and seems to indicate that the most of the MH-induced mutations were the results of so-called one-hit event. This value of slope is even smaller than 1.087 determined by Shima and Ichikawa (1995) for *N*-methyl-*N*-nitrosourea (MNU), a monofunctional alkylating agent, which has a relatively low Swain-scott substrate constant (s) of 0.42 (Veleminsky et al., 1970) and reacts to DNA via SN_1-type mechanism (producing much more O^6-alkylguanine than N-7-alkylguanine) that is thought to be important in inducing so-called point mutations.

MH has been confirmed to be a promutagen which is activated in plant cells and becomes mutagenic (Gichner et al., 1982; Plewa and Gentile, 1982; Heindorff et al., 1984; Veleminsky and Gichner, 1988). Much earlier, MH had been found to be a strong agent to break chromosomes, first in plant cells (Darlington and McLeish, 1951), and the induction of chromosomal aberrations by MH has been reported in *Vicia faba* (Evans and Scott, 1964; Scott, 1968; Swietlinska and Zuk, 1974) and other plants (Fishbein, 1972; Swietlinska and Zuk, 1978). MH was also reported to affect chromosomes predominantly at the S period of cell cycle (Evans and Scott, 1964; Scott, 1968), and the action of MH on chromosomes has been described to be very similar to those of alkylating agents (Swietlinska and Zuk,

1978).

However, the slope value of 1.031 of the dose-response curve for MH (Fig. 2) is obviously smaller than 1.283 and 1.293 for MMS and EMS, respectively, determined earlier in the same clone (Shima and Ichikawa, 1994), suggesting that MH has an action mechanism different from these alkylating agents. MMS and EMS are monofunctional alkylating agents having high or relatively high s values of 0.88 and 0.67, respectively (Ehrenberg et al., 1974), but MMS reacts to DNA via SN_2-type mechanism (much less alkylation of O^6 than N-7 of guanine), while EMS by a mixed SN_1/SN_2-type mechanism (Lawley, 1974). Therefore, the action mechanism of MH may resemble only alkylating agents with lower s values, since the dose-response curve for MNU ($s=0.42$) has been demonstrated to have a smaller slope of 1.087 (Shima and Ichikawa, 1995) as mentioned above (but see below for further discussion).

The peaks of X-ray-induced somatic pink mutation frequency were observed 10 or 11 days after treatments as expected. However, the appearance of the peaks of MH-induced mutation frequency was delayed for 2 or 3 days, the peaks being observed 12 to 14 days after treatments. The delayed appearance of the mutagenic effects of MH is considered to be related at least partly to the period required for the activation of this promutagen in the stamen-hair cells.

Synergistic and antagonistic effects between MH and X rays

Significantly synergistic effects were detected between MH and X rays when X rays were delivered acutely 20 or 44 h before starting the 4-h MH treatments (Table 1), as expected from our assumption (see Introduction). Contrary to the assumption, however, significantly antagonistic effects of MH with X rays were often observed when X-rayed acutely at the midpoint of or 2 or 44 h after completing the 4-h MH treatments (Tables 2 and 3). The occurrences of both synergism and antagonism between the same two mutagens in the same tester have scarcely been reported.

It has been reported that the frequency of chromosomal aberrations in *Vicia faba* root tips treated with 0.25 mM MH for 50 min and with 479 mGy X rays 10 min after the MH treatment was somewhat lower than that expected from the additive effects, but the difference observed was not statistically significant (Scott, 1968).

The mutagenic synergisms found between MH and X rays when exposed to X rays before the MH treatments (Experiments 1 and 2 in Table 1) are considered to be the results of interactions between DNA strand breaks (and the resultant chromosomal breaks) induced by X rays and those caused by MH, since the both agents commonly produce DNA strand breaks. The evident synergisms detected earlier among MMS, EMS, DMS and X rays (Ichikawa et al., 1993; Shima and Ichikawa, 1994, 1995) and the merely additive effects found between MNU and X rays (Shima and Ichikawa, 1995) appeared to show the importance of O^6-

alkylguanine productivity of alkylating agents (Shima and Ichikawa, 1995), since DMS also has a high s value of 0.86 (Ehrenberg et al., 1974). However, comparing the different patterns of the synergisms detected among these mutagens, it seemed most likely that DNA single-strand breaks resulted commonly from treatments with these alkylating agents and X rays, rather than O^6-alkylguanine productivity itself, were the major cause of the mutagenic synergisms (Shima and Ichikawa, 1995). The mutagenic synergisms detected recently between *N*-ethyl-*N*-nitrosourea (ENU) and X rays (Shima and Ichikawa, unpublished) support this consideration, since ENU has a much lower s value of 0.26 (Veleminsky et al., 1970).

It has been reported that L-cysteine, formamide, acetoamide, hydroquinone and catalase efficiently decreased MH-induced chromosomal aberrations (Heindorff and Rieger, 1984). Since all these agents are known to be radical scavengers (Heindorff and Rieger, 1984; Gichner et al., 1988), it is considered that the production of free radicals is a crucial step in the process of inducing chromosomal aberrations by MH (Gichner et al., 1988). It is of course well known that ionizing radiations yield their biological effects (including DNA strand breaks) largely through the production of free radicals. Therefore, the occurrences of synergism between MH and X rays are rather easily understood also in connection with the production of free radicals.

On the other hand, the antagonistic effects often observed between MH and X rays when X rays were given during or after the MH treatments (Experiments 3, 6, 7 and 8 in Tables 2 and 3) are presumed to have resulted from X-ray-caused inhibition of the activation of MH in the stamen-hair cells. The mechanism of the activation of MH in plant cells has not yet been fully understood, but the involvement of peroxidase has been thought very likely. As early as in 1950s, it had been found that the activity of peroxidase increased very much when MH existed, thus MH was considered to become the substrate of this enzyme (Andreae and Andreae, 1953; Kenten, 1955). Recently, it has been shown that peroxidase is important for activating some other promutagens into mutagens in plant cells (Stiborova and Anzenbacher, 1991; Plewa et al., 1991; Plewa and Wagner, 1993; Seo et al., 1993; Gichner et al., 1994), and some indirect evidences on the activation of MH by peroxidase have been presented (Heindorff and Rieger, 1984; Veleminsky and Gichner, 1988; Gichner et al., 1988). For example, Gichner et al. (1988) reported that the MH-induced somatic mutation frequency in the stamen hairs of clone BNL 4430 decreased remarkably by the pre-treatment with diethyldithiocarbamate (DEDTC), an agent suppressing peroxidase activity.

If MH is activated by peroxidase, it becomes easier to understand the antagonistic effects between MH and X rays in cases of X-raying during or after the MH treatments. Peroxidase catalyzes both peroxidative and oxidative reactions requiring hydrogen peroxide and oxygen, respectively. Early report of highly significant decrease of MH-induced chromosomal aberrations in *Vicia faba* under

anoxic conditions (Kihlman, 1956) indicates that oxygen is required for the activation of MH. On the other hand, an obvious oxygen enhancement effect on X-ray-induced mutation frequency in the stamen hairs of *Tradescantia* has been demonstrated (Underbrink et al., 1975). Namely, oxygen is required for the appearance of X-ray-induced genetic damages, and also highly probably for the activation of MH. It seems therefore likely that either the activation of MH or the effects of X rays is, or the both are, suppressed because of the competitive requirements of oxygen when X rays were given during or after the MH treatments (i.e., during the activating period of MH), often resulting in the antagonistic effects as observed in the present study.

The present study was supported in part by the Grant-in-Aid for Scientific Research (No. 05454607) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Andreae, W. A. and Andreae, S. R. (1953). Studies on indoleacetic acid metabolism, I. The effect of methyl umbelliferone, maleic hydrazide, and 2,4-D on indoleacetic acid oxidation. *Can. J. Bot.* **31**, 426–437.

Cebulska-Wasilewska, A., Leenhouts, H. P. and Chadwick, K. H. (1981). Synergism between EMS and X-rays for the induction of somatic mutations in *Tradescantia*. *Int. J. Radiat. Biol.* **40**, 163–173.

Darlington, C. D. and McLeish, J. (1951). Action of maleic hydrazide on the cell. *Nature* **167**, 407–408.

Ehrenberg, L., Osterman-Golkar, S., Singh, D. and Lundqvist, U. (1974). On the reaction kinetics and mutagenic activity of methylating and β-halogenoethylating gasoline additives. *Radiat. Bot.* **14**, 185–194.

Emmerling-Thompson, M. and Nawrocky, M. M. (1980). Genetic basis for using *Tradescantia* clone 4430 as an environmental monitor of mutagens. *J. Hered.* **71**, 261–265.

Evans, H. J. and Scott, D. (1964). Influence of DNA synthesis on the production of chromatid aberrations by X rays and maleic hydrazide in *Vicia faba*. *Genetics* **49**, 17–38.

Fishbein, L. (1972). Pesticidal, industrial, food additive, and drug mutagens. In: *Mutagenic Effects of Environmental Contaminants* (eds.: H. E. Sutton and M. I. Harris), pp. 129–170, Academic Press, New York.

Gichner, T., Veleminsky, J. and Pokorny, V. (1982). Somatic mutations induced by maleic hydrazide and its potassium and diethanolamine salts in the *Tradescantia* mutation assay. *Mutat. Res.* **103**, 289–293.

Gichner, T., Veleminsky, J. and Rieger, R. (1988). Antimutagenic effects of diethyldithiocarbamate towards maleic hydrazide- and *N*-nitrosodiethylamine-induced mutagenicity in the *Tradescantia* mutagenicity assay. *Biol. Plant. (Prague)* **30**, 14–19.

Gichner, T., Lopez, G. C., Wagner, E. D. and Plewa, M. J. (1994). Induction of somatic mutations in *Tradescantia* clone 4430 by three phenylenediamine isomers and the antimutagenic mechanisms of diethyldithiocabamate and ammonium meta-vanadate. *Mutat. Res.* **306**, 165–172.

Heindorff, K. and Rieger, R. (1984). Exogenous factors affecting yield and intrachromosomal distribution of maleic hydrazide-induced chromatid aberrations in *Vicia faba*. *Biol. Zent. Bl.* **103**, 9–23.

Heindorff, K., Rieger, R., Veleminsky, J. and Gichner, T. (1984). A comparative study of the

clastogenicity of maleic hydrazide and some of its putative degradation products. *Mutat. Res.* **140**, 123–126.

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

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

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

Ichikawa, S., Imai, T. and Nakano, A. (1991). Comparison of somatic mutation frequencies in the stamen hairs of one mutable and two stable clones of *Tradescantia* treated with small doses of gamma rays. *Jpn. J. Genet.* **66**, 513–525.

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

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

Kenten, R. H. (1955). The oxidation of indolyl-3-cetic acid by waxpod bean root sap and peroxidase systems. *Biochem. J.* **59**, 110–121.

Kihlman, B. A. (1956). Factors affecting the production of chromosome aberrations by chemicals. *J. Biophys. Biochem. Cytol.* **2**, 543–555.

Lawley, P. D. (1974). Some chemical aspects of dose-response relationships in alkylation mutagenesis. *Mutat. Res.* **23**, 283–295.

Nauman, C. H., Sparrow, A. H. and Schairer, L. A. (1976). Comparative effects of ionizing radiation and two chemical mutagens on somatic mutation induction in one mutable and two non-mutable clones of *Tradescantia*. *Mutat. Res.* **38**, 53–70.

Plewa, M. J. and Gentile, J. M. (1982). The activation of chemicals into mutagens by green plants. In: *Chemical Mutagens: Principles and Methods for their Detection*, Vol. 7 (eds.: F. J. de Serres and A. Hollaender), pp. 401–420, Plenum, New York.

Plewa, M. J. and Wagner, E. D. (1993). Activation of promutagens by green plants. *Annu. Rev. Genet.* **27**, 93–113.

Plewa, M. J., Smith, S. R. and Wagner, E. D. (1991). Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. *Mutat. Res.* **247**, 57–64.

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

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

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

Scott, D. (1968). The additive effect of X-rays and maleic hydrazide in inducing chromosomal aberrations at different stages of the mitotic cycle in *Vicia faba*. *Mutat. Res.* **5**, 65–92.

Seo, K.-Y., Riley, J., Cortez, D., Wagner, E. D. and Plewa, M. J. (1993). Characterization of stable high molecular weight mutagenic product(s) of plant-activated *m*-phenylenediamine. *Mutat. Res.*

299, 111–120.

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

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

Sparrow, A. H., Schairer, L. A. and Villalobos-Pietrini, R. (1974). Comparison of somatic mutation rates induced in *Tradescantia* by chemical and physical mutagens. *Mutat. Res.* **26**, 265–276.

Stiborova, M. and Anzenbacher, P. (1991). What are the principal enzymes oxidizing the xenobiotics in plants: Cytochromes P-450 or peroxidases? (a hypothesis). *Gen. Physiol. Biophys.* **10**, 209–216.

Swietlinska, Z. and Zuk, J. (1974). Effects of caffeine on chromosome damage induced by chemical mutagens and ionizing radiation in *Vicia faba* and *Secale cereale*. *Mutat. Res.* **26**, 89–97.

Swietlinska, Z. and Zuk, J. (1978). Cytotoxic effects of maleic hydrazide. *Mutat. Res.* **55**, 15–30.

Underbrink, A. G., Sparrow, A. H., Sautkulis, D. and Mills, R. E. (1975). Oxygen enhancement ratios (OERs) for somatic mutations in *Tradescantia* stamen hairs. *Radiat. Bot.* **15**, 161–168.

Veleminsky, J. and Gichner, T. (1988). Mutagenic activity of promutagens in plants: Indirect evidence of their activation. *Mutat. Res.* **197**, 221–242.

Veleminsky, J., Osterman-Golkar, S. and Ehrenberg, L. (1970). Reaction rates and biological action of *N*-methyl- and *N*-ethyl-*N*-nitrosourea. *Mutat. Res.* **10**, 169–174.