

# Dicentric chromosome bridges in root tips and micronuclei in pollen tetrads induced by X rays and maleic hydrazide in *Tradescantia* clone BNL 4430

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Inductions of dicentric chromosome bridges in root-tip cells and of micronuclei in pollen tetrads by X rays and maleic hydrazide (MH) were studied in *Tradescantia* clone BNL 4430, in order to compare the accuracies of these endpoints for mutagenicity testings with that of somatic pink mutations in the stamen hairs of the same clone, the most accurate endpoint established in higher plant testers. The frequency of dicentric bridges in root-tip cells increased with X-ray doses with a slope of 1.249 on a log-log graph, the slope value being unexpectedly small (much smaller than the theoretical value of 2 for two-break events). The dose-response curve on a log-log graph for MH-induced dicentric bridges had a somewhat smaller slope of 1.188, and the bridges observed were predominantly of chromatid type, reflecting the nature of MH acting at the S period of cell cycle. As for X-ray- and MH-induced micronuclei in pollen tetrads, on the other hand, the dose-response curves could not be determined, excepting that for X-ray-induced micronuclei in pollen tetrads at earlier stage. Namely, the data obtained from pollen tetrads at later stage were not consistent with X-ray and MH doses, and those from pollen tetrads at earlier stage were also inconsistent with MH doses. These results were contrary to our expectation that the frequency of micronuclei would be more reliable in pollen tetrads at later stage than in those at earlier stage at least in clone BNL 4430, a hybrid clone. The micronucleus assay in pollen tetrads was also found to be unsuited for determining the effects of MH. Therefore, the assay of somatic mutations in *Tradescantia* stamen hairs is concluded to be the most accurate and most reliable mutagenicity test system with a high degree of reappearance.

## INTRODUCTION

Higher plant mutagenicity assay systems including *Tradescantia* stamen-hair system have been described as ideal materials, especially for uses in developing countries, because of their advantages such as ease to handle and lower costs (Ichikawa, 1976; Plewa, 1985; Grant, 1994). Among them, *Tradescantia* stamen-hair system to assay somatic pink mutations has been established as one of the most suitable test systems to detect the genetic effects of environmental mutagens at low levels, as reviewed earlier (Underbrink et al., 1973; Ichikawa, 1981b, 1992; Schairer and Sautkulis, 1982; Ma et al., 1994a). The use of *Tradescantia* pollen tetrads to assay micronuclei resulting from chromosomal breaks has also been reported to be efficient (Ma, 1981, 1982, 1983; Grant, 1994; Ma et al., 1994b). Direct assays of chromosomal aberrations in root-tip cells, e.g.,

dicentric chromosome bridges easily detected at anaphase (Ichikawa et al., 1965; Ichikawa, 1981b), have also long been common ways of detecting mutagenicity, using various higher plants including *Tradescantia* having large chromosomes.

Using the stamen-hair system of *Tradescantia* clone BNL 4430, we have recently found that maleic hydrazide (MH) interacts with X rays both synergistically and antagonistically in inducing mutations (Xiao and Ichikawa, 1995). MH, which is applied in agriculture mainly as a herbicide, 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; Velemínsky and Gichner, 1988). The enzyme involved in the activation of MH in *Tradescantia* floral tissues has been shown to be peroxidase (Xiao and Ichikawa, 1996), and antagonistic effects often observed by delivering X rays after MH treatments (Xiao and Ichikawa, 1995) were considered to be due to suppression of the activation of MH by X rays delivered during the activation pe-

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riod (Xiao and Ichikawa, 1996). In contrast to this, clear synergistic effects were detected by treating with X rays before MH treatments (Xiao and Ichikawa, 1995).

In parallel with such studies on the mutagenic interactions of MH and X rays in the stamen hairs, experiments to compare the accuracies and reliabilities of assays of dicentric chromosome bridges in root-tips and of micronuclei in pollen tetrads with those of the stamen-hair system have been carried out, using the same clone and choosing also X rays and MH as mutagenic agents. The results obtained from these experiments are reported here.

## MATERIALS AND METHODS

**Materials used.** Clone BNL 4430 used in the present study is a diploid hybrid ( $2n = 12$ ) between a blue-flowered *Tradescantia 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 stamen hairs as reviewed earlier (Schairer and Sautkulis, 1982; Ichikawa, 1992) and also for assaying micronuclei in pollen tetrads and root-tip cells (Ma et al., 1994b). This clone has been shown to be more sensitive to alkylating agents than other clones, in spite of its radiosensitivity comparable to others (Sparrow et al., 1974; Nauman et al., 1976; Ichikawa et al., 1993).

For studying dicentric chromosome bridges in root-tip cells, young shoots with roots (not yet bearing inflorescences) divided from potted plants were used. Their older thick roots were cut back. For studying micronuclei in pollen tetrads, on the other hand, young inflorescence-bearing shoots with roots were used, as in studies of somatic mutations in stamen hairs (Shima and Ichikawa, 1994, 1995, 1997; Ichikawa et al., 1995; Xiao and Ichikawa, 1995).

**Cultivating conditions.** The shoots with roots for studying dicentric bridges in root-tip cells were cultivated with a 1/2000 Hyponex solution in a growth room. The nutrient solution was always aerated. The environmental conditions in the growth room were  $23.0 \pm 0.5^\circ\text{C}$  (constant), 50% humidity, and a 16-h day length with a light intensity of 6 klx from three Toshiba (Tokyo) DR400/T(L) metal-halide sunlamps plus two 40-W white fluorescent tubes.

The young inflorescence-bearing shoots with roots for studying micronuclei in pollen tetrads were cultivated in a nutrient solution circulating (NSC) growth chamber (Kyosin Riko Co., Yono, Saitama) designed for our requirements (Shima and Ichikawa, 1994). The environmental conditions in the NSC growth chamber were  $22.0 \pm 0.5^\circ\text{C}$  during the 16-h day with a light intensity of 7.5 klx from two 40-W white fluorescent tubes, and  $20.0 \pm 0.5^\circ\text{C}$  at night. The nutrient solution used was a 1/2000 Hyponex solution.

**X-ray treatments.** Young roots and young inflorescences

were treated with X rays using a Hitachi (Tokyo) MBR-1505R X-ray generator. The young roots were covered with moist tissue papers during the treatments. The treatments were conducted acutely at 150 kVp and 4 mA with a 0.5 mm Al + 0.1 mm Cu filter at  $23.0 \pm 0.5^\circ\text{C}$ . The target distance was 450 mm. The exposure data were obtained simultaneously with National (Osaka) UD-170L thermoluminescence dosimeter (TLD) elements attached to the roots or inflorescences, and with a National UD-502B thermoluminescence reader. The exposure data obtained in R were converted into absorbed doses in Gy with a converting factor of  $9.57 \times 10^{-3}$  (i.e.,  $1 \text{ R} = 9.57 \text{ mGy}$ ). The doses applied were 0.297 to 2.45 Gy and 0.0727 to 2.11 Gy for roots and inflorescences, respectively (at dose rates of 266 to 305 mGy/min).

**Treatments with MH.** Young roots were treated with 0.25 to 1 mM MH (CAS no. 123-33-1; Wako Pure Chem. Ind. Ltd., Osaka) dissolved in phosphate buffer (pH 7.0), immersing them directly into freshly prepared MH solutions for 4 h at  $23.0 \pm 0.5^\circ\text{C}$ , and being aerated. Controls were treated with the phosphate buffer. Immediately after the MH treatments, roots were washed with tap water for 1 min.

Young inflorescences just before initiating flowering were treated with freshly prepared 0.5 to 10 mM MH dissolved in phosphate buffer, for 4 h at  $23.0 \pm 0.5^\circ\text{C}$ . The treating methods were identical to those used for somatic mutation inductions by MH (Xiao and Ichikawa, 1995), which had originally been developed for treating young inflorescences with aqueous chemical mutagens (Ichikawa and Takahashi, 1978). Controls were treated with the phosphate buffer. Immediately after the MH treatments, inflorescences were washed with tap water for 2 min.

**Scoring dicentric bridges.** Root tips collected 16 to 22 h after X-ray treatments and 18 to 24 h after completing MH treatments were immediately fixed with freshly prepared Farmer's fluid (98% ethanol : acetic acid = 3 : 1). Preparations were made by the Feulgen smear method using Schiff agent. Scorings of dicentric chromosome bridges were made on the cells at anaphase (with no overlapping between chromosomes moving toward both poles), recording the numbers of anaphase cells observed and of dicentric bridges detected. The frequency of dicentric bridges was expressed as the number of bridges per  $10^2$  anaphase cells. Mitotic index was also scored on each preparation as an indicator of cell proliferation in each root tip treated with X rays or MH.

**Scoring micronuclei.** Young inflorescences collected 24 to 66 h after X-ray treatments and 24 to 43 h after completing MH treatments were immediately fixed with freshly prepared Farmer's fluid. Pollen tetrads taken out from anthers were stained with aceto-carmin. Scorings of

Table 1. The frequencies of X-ray-induced dicentric chromosome bridges in the root-tip cells of clone BNL 4430

X-ray dose (Gy)	Post-treatment period (h)	Number of anaphase cells observed	Number of bridges scored	Number of bridges/10 <sup>2</sup> cells ( $\pm$ SE)	Remark	Mitotic index (%) ( $\pm$ SE) <sup>a</sup>
0	16	46 033	16	0.035 $\pm$ 0.009		13.2 $\pm$ 0.2
0.300	16	10 359	149	1.44 $\pm$ 0.12	highest	8.49 $\pm$ 0.37
0.297	18	3 501	40	1.14 $\pm$ 0.18		10.2 $\pm$ 0.4
0.479	16	5 996	141	2.35 $\pm$ 0.20	highest	10.2 $\pm$ 0.4
0.565	18	3 320	105	3.16 $\pm$ 0.30		13.5 $\pm$ 0.5
	20	5 438	123	2.26 $\pm$ 0.20		12.5 $\pm$ 0.5
1.19	16	7 446	435	5.84 $\pm$ 0.27	highest <sup>b</sup>	8.34 $\pm$ 0.35
1.25	18	7 772	624	8.03 $\pm$ 0.31		16.4 $\pm$ 0.5
1.18	18	4 041	316	7.82 $\pm$ 0.42		12.8 $\pm$ 0.5
	20	3 105	234	7.54 $\pm$ 0.47		14.7 $\pm$ 0.5
2.45	16	7 096	979	13.8 $\pm$ 0.4	highest	3.49 $\pm$ 0.24
2.24	18	2 247	301	13.4 $\pm$ 0.7		7.11 $\pm$ 0.25
	20	2 589	450	17.4 $\pm$ 0.7		11.0 $\pm$ 0.4
2.31	22	1 688	211	12.5 $\pm$ 0.8		6.33 $\pm$ 0.35

<sup>a</sup> Scored on more than 5000 meristematic cells.<sup>b</sup> Highest value per Gy.

micronuclei were made mainly on pollen tetrads at later stage with condensed smaller nuclei, since micronuclei were more easily detected being less frequently concealed by nuclei at this stage (see Fig. 3B), as compared with pollen tetrads at earlier stage with larger nuclei not yet condensed (see Fig. 3A). The numbers of pollen tetrads observed and of micronuclei detected were recorded, and the frequency of micronuclei was expressed as the number of micronuclei per 10<sup>2</sup> pollen tetrad cells. Micronuclei were also scored in pollen tetrads at earlier stage for comparison.

## RESULTS

**X-ray-induced dicentric bridges.** The frequencies of dicentric chromosome bridges observed after treatments with about 0.30 to 2.33 Gy of X rays are presented in Table 1, together with the frequency in the control and mitotic indexes. As seen in this table, the highest frequencies were observed later with higher doses, i.e., 16 to 20 h after treatments. The mitotic indexes also showed more suppressions and delays of cell proliferation with higher doses. When these highest frequencies of bridges (induced by 0.300, 0.565, 1.18, and 2.24 Gy; see Table 1) after subtracting the control frequency are plotted against X-ray dose on a log-log graph, a dose-response curve as shown in Figure 1 is obtained. The slope value of 1.249 of the curve indicates that dicentric bridges increased curvilinearly with X-ray dose.

**MH-induced dicentric bridges.** The frequencies of di-

centric bridges observed after treatments with 0.25 to 1 mM MH are presented in Table 2, together with the frequency in the control and mitotic indexes. The frequencies observed were very much lower than those induced by

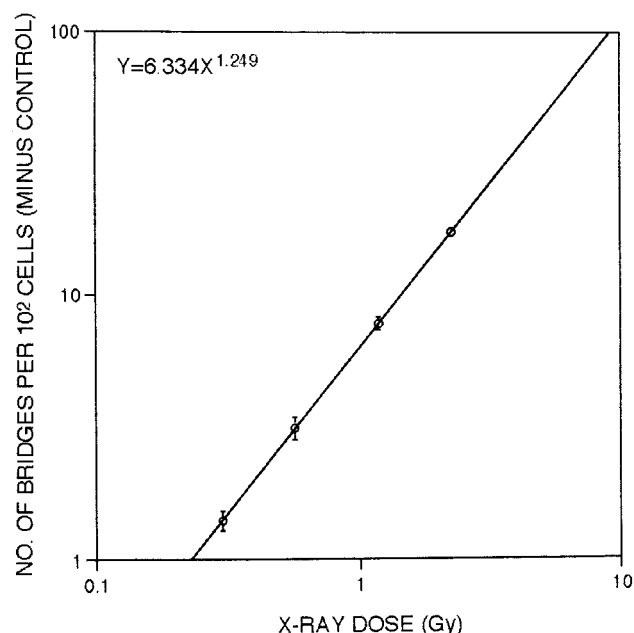


Fig. 1. The dose-response curve of dicentric chromosome bridges in the root-tip cells of clone BNL 4430 obtained by treating young roots acutely with 0.300 to 2.24 Gy X rays. The best-fit regression line determined by the least squares method and standard errors for the points plotted are shown.

Table 2. The frequencies of MH-induced dicentric chromosome bridges in the root-tip cells of clone BNL 4430

MH dose (mM)	Post-treatment period (h) <sup>a</sup>	Number of anaphase cells observed	Number of bridges scored	Number of bridges/10 <sup>2</sup> cells ( $\pm$ SE)	Remark	Mitotic index (%) ( $\pm$ SE) <sup>b</sup>
0	18	23 393	11	0.047 $\pm$ 0.014		12.0 $\pm$ 0.2
0.25	18	9 598	28	0.292 $\pm$ 0.055	highest	8.72 $\pm$ 0.39
	20	12 722	42	0.330 $\pm$ 0.051		9.13 $\pm$ 0.41
0.5	18	5 012	18	0.359 $\pm$ 0.084	highest	5.87 $\pm$ 0.23
	20	2 693	22	0.817 $\pm$ 0.173		6.16 $\pm$ 0.34
1	18	309	3	0.971 $\pm$ 0.558	highest	1 MI $\geq$ 0 <sup>c</sup>
	20	5 999	91	1.52 $\pm$ 0.16		7.13 $\pm$ 0.36
	22	3 617	42	1.16 $\pm$ 0.18		8.20 $\pm$ 0.39
	24	3 373	34	1.01 $\pm$ 0.17		10.8 $\pm$ 0.4

<sup>a</sup> After completing 4-h MH treatments.<sup>b</sup> Scored on more than 5000 meristematic cells.<sup>c</sup> Mitotic index was very low, thus no exact scoring was made.

X rays, being at most 1.52 bridges per 10<sup>2</sup> cells roughly comparable to that induced by about 0.3 Gy (see Table 1), and more suppressions of cell proliferation were caused by MH than by X rays. However, the highest frequencies were observed 20 h after completing MH treatments at any concentrations, as seen in this table. When these highest frequencies of bridges after subtracting the control frequency are plotted against MH concentration on a log-log graph, a dose-response relationship as shown in Figure 2 is obtained. The slope of 1.188 calculated for the best-fit regression line is somewhat smaller than that for X rays. The dicentric bridges observed were predominantly of chromatid type, indicating that they were induced by MH during the S to G<sub>2</sub> periods of cell cycle.

**X-ray-induced micronuclei.** The pollen tetrads (with micronuclei) at earlier and later stages are shown in Figure 3. At earlier stage, the nuclei are large, chromosome structure being still observed, and it is also true for micronuclei. It is often difficult at this stage to judge if a micronucleus observed is the result of broken chromosome(s) or of lagging one(s), especially in this hybrid clone, and micronuclei are often concealed by large nuclei (Fig. 3A). At later stage, the nuclei are condensed and much smaller as compared with the enlarged cell size, and micronuclei are also condensed and much smaller. Therefore, micronuclei are more easily detected being less frequently concealed by nuclei (Fig. 3B).

The frequencies of micronuclei after X-ray treatments with 0.574, 0.593, and 2.11 Gy were scored in about 2600 to 9900 pollen tetrads at later stage for each fixation time, but no dose-related responses were observed 24 to 39 h after treatments. Namely, the highest frequencies with these three doses were  $2.96 \pm 0.12$ ,  $4.22 \pm 0.15$ , and  $3.46 \pm 0.18$

micronuclei per 10<sup>2</sup> pollen tetrad cells, respectively. The control frequencies were  $1.93 \pm 0.07$  and  $1.95 \pm 0.08$  micronuclei per 10<sup>2</sup> pollen tetrad cells. A greatly increased frequency was detected 66 h after treatment with 0.574 Gy ( $67.6 \pm 0.4$  micronuclei per 10<sup>2</sup> pollen tetrad cells), but the highest frequency obtained 63 h after 0.391 Gy treatment was very much lower ( $9.21 \pm 0.48$  micronuclei per 10<sup>2</sup> pollen tetrad cells). Therefore, it was impossible to obtain a dose-response curve.

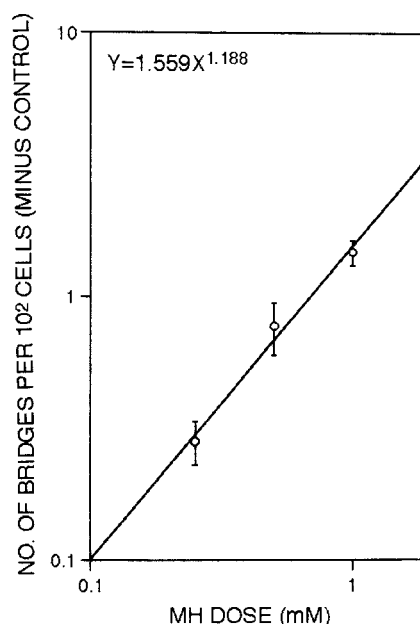


Fig. 2. The dose-response curve of dicentric chromosome bridges in the root-tip cells of clone BNL 4430 obtained by treating young roots with 0.25 to 1 mM MH for 4 h. The best-fit regression line determined by the least squares method and standard errors for the points plotted are shown.

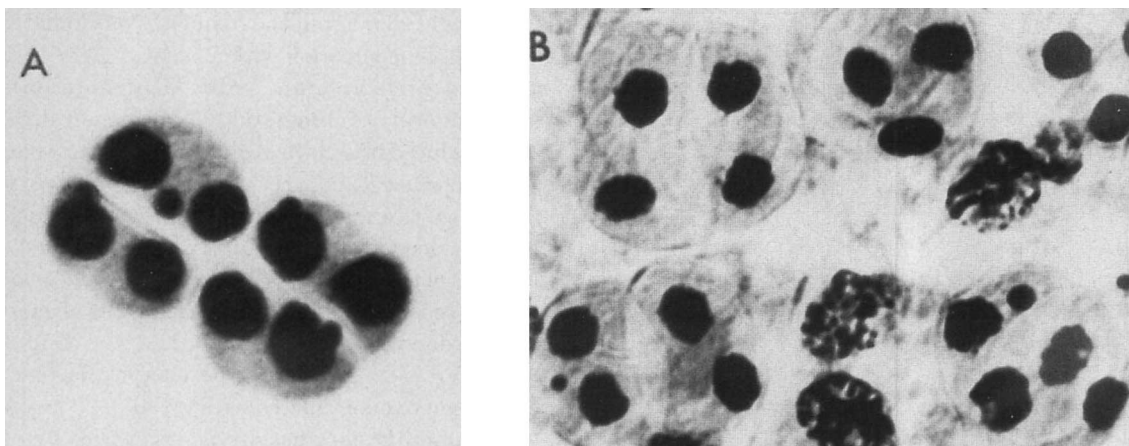


Fig. 3. The pollen tetrads of clone BNL 4430. (A), Those at earlier stage with relatively large nuclei and micronuclei; two and one of two micronuclei in the right and left tetrads, respectively, being overlapped by nuclei. (B), Those at later stage with relatively small nuclei and micronuclei; two micronuclei seen in two lower tetrads (one micronucleus each) not being overlapped by nuclei.

The frequencies of micronuclei were also examined in pollen tetrads at earlier stage for comparison. The data obtained 24 h after X-ray treatments with 0.0727 to 0.431 Gy are presented in Table 3. Plotting these frequencies after subtracting each control frequency against X-ray dose on a log-log graph, a dose-response curve with a slope of 1.330 as shown in Figure 4 is obtained.

**MH-induced micronuclei.** The highest frequencies of micronuclei in pollen tetrads at later stage observed 39 h after completing 0.5, 1, and 2 mM MH treatments were all low ( $4.10 \pm 0.12$ ,  $3.43 \pm 0.12$ , and  $2.94 \pm 0.08$  micronuclei per  $10^2$  pollen tetrad cells, respectively), being at most about 2.3 times the control frequency ( $1.77 \pm 0.08$  micronuclei per  $10^2$  pollen tetrad cells), and the data obtained were inconsistent with MH concentrations, although scorings were made on samples larger than those in Table 3.

The effects of MH in inducing micronuclei were also examined in pollen tetrads at earlier stage for comparison. However, data obtained were also inconsistent with MH concentrations. Namely, while the frequency of micronu-

clei 24 h after completing 1.25 mM MH treatment was  $2.05 \pm 0.09$  micronuclei per  $10^2$  pollen tetrad cells, those at 2.5 mM varied greatly between  $2.31 \pm 0.09$  and  $16.3 \pm 0.2$  micronuclei per  $10^2$  pollen tetrad cells in three experiments repeated. Moreover, while the frequencies at 5 mM were  $7.33 \pm 0.16$  and  $7.90 \pm 0.15$  micronuclei per  $10^2$  pollen tetrad cells in two experiments, those at 10 mM were  $3.26 \pm 0.10$  and  $13.4 \pm 0.3$  micronuclei per  $10^2$  pollen tetrad cells also in two experiments. Therefore, no dose-response relationship could be determined.

## DISCUSSION

**Dicentric bridges in root-tip cells.** Dicentric chromosomes are produced through chromosomal exchanges occurred between two different breaks of chromosomes (Ichikawa, 1981b). Dicentric bridge is formed at anaphase when two centromeres of a dicentric chromosome moved toward opposite poles, and the probability of forming bridge at anaphase for a dicentric chromosome is 50% (Ikushima and Ichikawa, 1967). There are two types of bridges, i.e.,

Table 3. The frequencies of X-ray-induced micronuclei in the pollen tetrads (at earlier stage) of clone BNL 4430

X-ray dose (Gy)	Post-treatment period (h)	Number of tetrads observed	Number of micronuclei scored	Number of micronuclei / $10^2$ cells ( $\pm$ SE)
0		7,399	519	$1.75 \pm 0.08$
0.0727	24	5,656	911	$4.03 \pm 0.13$
0.122	24	6,314	1,626	$6.44 \pm 0.15$
0		11,878	786	$1.65 \pm 0.06$
0.255	24	7,031	3,493	$12.4 \pm 0.2$
0.431	24	5,003	5,655	$28.3 \pm 0.3$
0		10,338	553	$1.34 \pm 0.06$
0.419	24	6,176	5,854	$23.7 \pm 0.3$



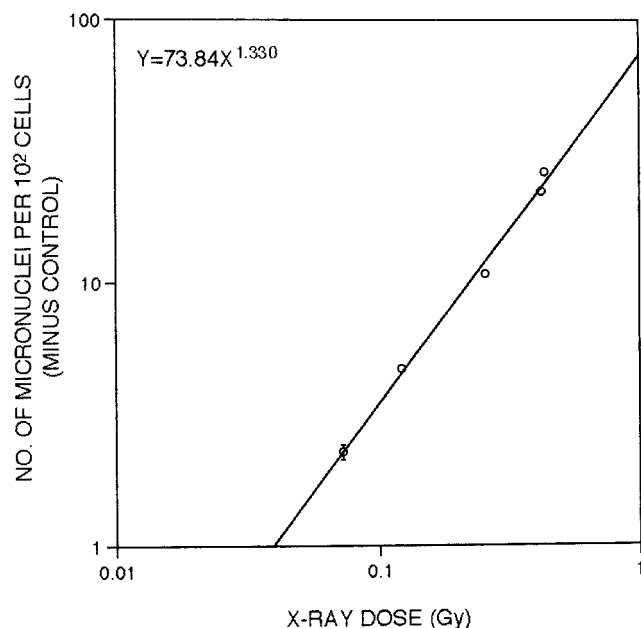


Fig. 4. The dose-response curve of micronuclei in the pollen tetrads (at earlier stage) of clone BNL 4430 obtained by treating young inflorescences acutely with 0.0727 to 0.431 Gy X rays. The best-fit regression line determined by the least squares method and standard errors for the points plotted are shown.

of chromosome and chromatid types resulting from two breaks and an exchange before DNA replication (occurred at the  $G_1$  period) and those after DNA replication (occurred during the S to  $G_2$  periods), respectively (Ichikawa, 1981b).

In the present study, the frequency of X-ray-induced dicentric bridges in the root-tip cells of *Tradescantia* clone BNL 4430 was found to increase with X-ray dose with a slope of 1.249 on the log-log graph shown in Figure 1. The slope value is unexpectedly small, being much smaller than 2 expected theoretically for two-break aberrations (Ichikawa et al., 1965). The frequency of X ray-induced dicentric bridges in barley root tips was found earlier to increase nearly as the function of square of X-ray dose, and the dose-response curve had a slope of 1.76 if drawn on a log-log graph (Ichikawa et al., 1965). However, there were also contradictory reports that radiation-induced dicentric bridges observed at anaphase unexpectedly increased approximately linearly with the dose, in spite of the fact that two-break chromosomal aberrations scored at metaphase increased as the square of the dose (Wolff and Luippold, 1957; Conger, 1965). As for such phenomena, Conger (1965) made assumptions that many of the dicentric chromosomes produced might have ruptured at radiation-induced 'weak spots' by a tension imposed upon them at anaphase, and that the 'weak spots' would have been induced as a linear function of radiation dose. The present result seems to be related to such earlier reports.

On the other hand, the frequencies of dicentric bridges induced by MH (Table 2) were very much lower than those

by X rays (Table 1), but the frequency was found to increase with MH concentration with a slope of 1.188 on the log-log graph shown in Figure 2. Also, MH-induced bridges were predominantly of chromatid type, obviously differing from X ray-induced bridges, a minor but significant part of which was of chromosome type. Davies (1963) reported earlier that more chromatid- than chromosome-type aberrations were induced by radiation in the terminal and subterminal cells of the stamen hairs of *Tradescantia* clone BNL 02, and his report agreed well with the results of analysis of cell cycle by double labelings with  $^{14}\text{C}$  and  $^3\text{H}$ , i.e., 1.0-h  $G_1$ , 10.5-h S, 2.5-h  $G_2$ , and 3.0-h M periods in the root tips of *T. paludosa* (Wimber and Quastler, 1963). The induction of predominantly chromatid-type dicentrics by MH in the present study agrees with the results obtained earlier in *Vicia faba* treated with MH (Evans and Scott, 1964). Most of the MH-induced dicentric chromosomes are considered to be formed at the S period of cell cycle, since MH has been described to be an agent acting at the S period (Evans and Scott, 1964; Scott, 1968; Cortes et al., 1987). The slope value somewhat smaller than that for X rays may suggest that chromatid-type dicentrics were more easily ruptured at anaphase than chromosome-type dicentrics. The somewhat smaller slope value for MH may also be related to mitotic index, since 1 mM MH and about 0.3 Gy X-ray treatments, which induced dicentric bridges at comparable frequencies 18 to 20 h after completing the former and 16 to 18 h after the latter (Tables 1 and 2), yielded quite different suppressions of cell proliferation, i.e., the mitotic indexes after the former were obviously lower than those after the latter (Tables 1 and 2).

**Micronuclei in pollen tetrads.** Micronuclei are formed by acentric chromosomes resulting from chromosomal breaks, because such acentrics can not be oriented to move toward poles. Micronuclei are also formed by lagging chromosomes which often occur after various mutagen treatments and also during meiosis in untreated interspecific hybrids.

Earlier mutagenicity testings with micronuclei in pollen tetrads have been carried out using the inflorescences of *T. paludosa* or clone BNL 4430, which were fixed 24 to 30 h after mutagen treatments, i.e., in pollen tetrads at earlier stage (Ma, 1981, 1983, 1990; Ma et al., 1984, 1994b). In the present study using the hybrid clone BNL 4430, however, the frequency of micronuclei was examined mainly in pollen tetrads at later stage, considering that it is often difficult at earlier stage to judge if a micronucleus observed is the result of broken chromosome(s) or of lagging one(s), especially in this hybrid clone, and that micronuclei are often concealed by large nuclei at earlier stage (see Fig. 3A).

Contrary to our expectation, however, no dose-response curve for X ray-induced micronuclei could be determined in pollen tetrads at later stage, since the results obtained

were inconsistent. It was possible, on the other hand, to determine the dose-response curve with a slope of 1.330 on a log-log graph for X ray-induced micronuclei in pollen tetrads at earlier stage as shown in Figure 4. This slope value is larger than 1.171 for X ray-induced micronuclei in pollen tetrads at earlier stage calculated by us based on the data published by Ma et al. (1980). The present value is rather closer to 1.249 obtained for X ray-induced dicentric bridges (Fig. 1), and also to those determined earlier for X ray-induced somatic pink mutations in stamen hairs, i.e., 1.252 to 1.390 in clone BNL 4430 (Shima and Ichikawa, 1994, 1995, 1997; Xiao and Ichikawa, 1995) and 1.237 to 1.454 in other clones, KU 27 (Sanda-Kamigawara et al., 1991; Ichikawa et al., 1993), BNL 02 (Sanda-Kamigawara et al., 1991) and KU 9 (Sakuramoto and Ichikawa, 1996). The present slope value for X ray-induced micronuclei in pollen tetrads at earlier stage close to those for X ray-induced somatic mutations suggests that the micronuclei observed were mostly the results of single chromosomal breaks. The present results also show the difficulty of studying micronucleus frequencies in pollen tetrads at later stage, as compared with scoring them at earlier stage as extensively made by Ma's group (Ma, 1981, 1983, 1990; Ma et al., 1984, 1994b), although the reason remains unknown.

In pollen tetrads at neither later nor earlier stages, determining dose-response curve for MH-induced micronuclei was successful, the data obtained showing no clear relationships with MH concentrations. It has been reported that no dose-related responses to MH in pollen tetrads at earlier stage were observed in four experiments conducted in Canada, China, and USA, with only exception of obtaining a dose-related response in Mexico (Ma et al., 1994b), in spite of the facts that MH is well known to induce chromosomal aberrations in plant cells (Evans and Scott, 1964; Scott, 1968; Swietlinska and Zuk, 1974, 1978) and that the action of MH on chromosomes has been described to be very similar to those of alkylating agents (Swietlinska and Zuk, 1978). Although it has also been demonstrated recently that MH is activated into a mutagen by peroxidase in the floral tissues of clone BNL 4430 (Xiao and Ichikawa, 1996), the micronucleus assay seems unsuitable for testing the effects of MH, since the frequencies of micronuclei in untreated controls are too high (see Table 3) for determining accurately the effects of such a cytotoxic agent (Swietlinska and Zuk, 1978) which can not be applied at high concentrations.

**Excellence of stamen-hair system.** The assays of dicentric bridges in root-tip cells and of micronuclei in pollen tetrads by using clone BNL 4430 were found to have defects as described above, in comparison with the assay of somatic mutations in the stamen hairs of the same clone (Shima and Ichikawa, 1994, 1995, 1997; Xiao and Ichikawa, 1995). The both assay systems examined in the present study consumed longer times and more expenses for mak-

ing preparations and scorings on them. The assay of dicentric bridges at anaphase has a problem for detecting all the events actually occurred; a considerable part of dicentric chromosomes produced can not be observed at anaphase, i.e., 50% of them not forming bridges and many others rupturing at anaphase. The micronucleus assay also has problems; one is the very high spontaneous (background) frequencies of micronuclei in controls (see Table 3), another is that only one to at most a few flower buds in an inflorescence can supply useful data. This system also proved to be unsuited for detecting the effects of MH.

Compared with these assay systems, *Tradescantia* stamen-hair system is superior at any points related to these problems. As reviewed earlier, the primary asset of the stamen-hair system is its capability to detect all pink mutant cells easily without being concealed by other cells (by just placing stamens in liquid paraffin dropped on a slide glass) as well as the capability of scoring a large number of samples (7500 to 18,000 stamen-hair cells can be observed in a single flower) (Ichikawa, 1992). These assets have proven to be especially suitable for determining the genetic effects of low-level ionizing radiations (Ichikawa, 1971, 1981a, 1992; Sparrow et al., 1972; Ichikawa and Ishii, 1991; Ichikawa et al., 1996a) and chemicals (Schairer and Sautkulis, 1982; Schairer et al., 1983), for detecting the synergisms among several chemicals and X rays (Ichikawa, 1992; Ichikawa et al., 1993; Shima and Ichikawa, 1994, 1995, 1997; Xiao and Ichikawa, 1995), as well as for studying the variation of spontaneous mutation frequency (Sparrow and Sparrow, 1976; Takahashi and Ichikawa, 1976; Ichikawa, 1984, 1992; Ichikawa et al., 1995, 1996a, 1996b) at the order of  $10^{-4}$  pink mutant events per cell division (Ichikawa, 1992). Especially, newly developed use of young inflorescence-bearing shoots with roots of clone BNL 4430 cultivated in the NSC growth chamber (Shima and Ichikawa, 1994, 1995, 1997; Ichikawa et al., 1995; Xiao and Ichikawa, 1995) can supply a much larger number of samples per space than using potted plants or cuttings, assures a high degree of reappearance, and decreases significantly the spontaneous mutation frequency (Shima and Ichikawa, 1994; Ichikawa et al., 1995). The young inflorescence-bearing shoots with roots of clone KU 9 cultivated in another NSC facility have also exhibited a significantly lower spontaneous mutation frequency (Sakuramoto and Ichikawa, 1996). The lower background level and high degree of reappearance are two other important assets of the *Tradescantia* stamen-hair system, especially in comparison with the micronucleus assay.

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