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Flower production, stamen-hair growth, and spontaneous and induced somatic mutation frequencies in *Tradescantia* cuttings and shoots with roots cultivated with nutrient solutions

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ABSTRACT

For establishing more efficient blue/pink heterozygous *Tradescantia* testers of mutagenicity, the young inflorescence-bearing nodal and/or axillary cuttings of clones BNL 02, KU 27, KU 7 and KU 20 were cultivated with nutrient solutions, and the flower production, stamen-hair growth, and spontaneous and induced somatic pink mutation frequencies in the stamen hairs observed in the cuttings of each clone were compared with those in the potted plants of the same clone. The cuttings tended to show poorer flower productions, smaller flower sizes and decreases in the number of hairs, as compared with potted plants, but the spontaneous and X-ray-induced somatic mutation frequencies were comparable to those in potted plants. Using the BNL 02 cuttings, however, the mutagenic effect of nitrofurazone was detected for the first time in higher plants. On the other hand, the shoots with roots of clone BNL 4430 divided from the potted plants and cultivated with a nutrient solution exhibited excellent results in all aspects; i.e., the flower production, flower size, stamen-hair growth, and X-ray- and MMS-induced mutation frequencies being almost identical with those in the potted plants, and the spontaneous (background) mutation frequency being lower than that in the potted plants. The shoots with roots of clone BNL 4430 were thus judged to be the best *Tradescantia* tester of mutagenicity, requiring much smaller space than using the potted plants and supplying much larger samples much more constantly than the cuttings of other clones.

1. INTRODUCTION

The stamen-hair system of *Tradescantia* heterozygous for flower color has been established as one of the most suitable plant materials for detecting the genetic effects of ionizing radiations and various chemicals as reviewed earlier (Underbrink et al., 1973; Ichikawa, 1981b, 1992; Schairer and Sautkulis, 1982; Schairer et

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al., 1983), especially at such low levels to which the human population may be exposed (Ichikawa, 1992). Using this system, the mutagenic synergisms among different mutagens have been detected (Cebulska-Wasilewska et al., 1981; Ichikawa, 1992; Ichikawa et al., 1993; Shima and Ichikawa, 1994, 1995; Xiao and Ichikawa, 1995), and variation in spontaneous mutation frequency has also been studied (Sparrow and Sparrow, 1976; Takahashi and Ichikawa, 1976; Ichikawa, 1984; Imai et al., 1991). Several clones have been established as such testers of mutagenicity (Ichikawa, 1992).

In earlier studies with the *Tradescantia* stamen-hair system, young inflorescence-bearing potted plants or cuttings had been used (Ichikawa, 1992). Potted plants are suitable for better flower production, but their use requires large space, and it is difficult to obtain large sample sizes in some cases, e.g., for X-ray exposures at relatively short target distances, resulting in widely different doses for individual inflorescences of the same plants, thus often supplying only a single inflorescence per dose (Shima and Ichikawa, 1994). The use of young inflorescence-bearing nodal and axillary cuttings (Underbrink et al., 1973) has been able to minimize such difficulties, but the flower production per cutting has tended to remain low because of the absence of roots, or at least until fully rooted.

In the present study, the young inflorescence-bearing cuttings of four different clones were cultivated with three different nutrient solutions, and the flower production, stamen-hair growth, and spontaneous and induced mutation frequencies in the stamen hairs observed in these cuttings were compared with those in the potted plants. After obtaining unsatisfactory results with these cuttings for continuously supplying enough samples, use of the young inflorescence-bearing shoots with roots of another clone was examined, for establishing more efficient *Tradescantia* tester of mutagenicity.

2. MATERIALS AND METHODS

Clones used

Clones BNL 02, KU 27, KU 7 and KU 20 were used for examining cuttings, because their stems grow well and are strong enough for obtaining cuttings. On the other hand, clone BNL 4430 was used for testing shoots with roots, because many new shoots emerge constantly from the basal nodes. These five clones are all heterozygous for flower color (blue/pink; the blue color being dominant) (Ichikawa, 1992).

Clone BNL 02 is a diploid hybrid ($2n=12$) thought to be derived from a hybrid between *T. occidentalis* (Britt.) Smyth. and *T. ohiensis* Raf. (Mericle and Mericle, 1971, 1973), and has been frequently used in studies of somatic mutations as one of the most stable clones in terms of spontaneous mutation frequency (Sparrow and Sparrow, 1976; Ichikawa, 1984, 1992). Clone KU 27 is a diploid segregant from clone BNL 02 (Sanda-Kamigawara et al., 1991), and is as stable as the parental

clone but is more sensitive to alkylating agents (Sanda-Kamigawara et al., 1991; Ichikawa, 1992; Ichikawa et al., 1993). Clone BNL 4430 is a diploid hybrid between a blue-flowered *T. hirsutiflora* Bush and a pink-flowered *T. subacaulis* Bush (Emmerling-Thompson and Nawrocky, 1980), and has been also frequently used as one of the most excellent *Tradescantia* testers (Schairer and Sautkulis, 1982; Schairer et al., 1983; Ichikawa, 1992).

Clone KU 7 is a triploid clone ($3x=18$) of *T. ohiensis*, and its vigorous growth and sterility suit for use in outdoor *in situ* experiments (Ichikawa, 1981a, 1992). Clone KU 20 is also a triploid possessing a part of the characteristics of *T. ohiensis* (Ichikawa and Takahashi, 1977), and is highly mutable spontaneously especially at lower temperature (Takahashi and Ichikawa, 1976; Ichikawa, 1984, 1992; Imai et al., 1991).

Preparations and cultivations of cuttings

Young inflorescence-bearing nodal and axillary cuttings were taken from potted plants. The potted plants of clones BNL 02 and KU 27 had been grown in a growth chamber, and those of clones KU 7 and KU 20 outdoors. Nodal cuttings were obtained by cutting each main stem at about 3 mm below the node next lower to that bearing bracts. Axillary cuttings were obtained by pulling each axillary shoot downward carefully and cutting it off at its basal node. For each of the both types of cuttings, the leaf next lower to bracts was kept alive without removing it.

Immediately after making the cuttings, the cut ends of nodal cuttings and the basal nodes of axillary cuttings were immersed in either a 0.1% indolebutyric acid (IBA) solution or a 1% Menedael (MND; a commercial plant hormone activator containing Fe^{2+}; Menedael Chemical Laboratory) solution for 1 and 2 h, respectively, at 23 ± 0.5°C for promoting rooting. These cuttings were cultivated with either Knop, Hoagland's No. 2, or 1/1000 Hyponex (Hyponex Co., Inc.) solutions, in either a LPH-200-RD growth chamber (Nippon Medical and Chemical Instruments Co. Ltd.) or a growth room. In the LPH growth chamber, the temperature was gradually changed between 25 ± 0.5°C (at 2 pm) and 20 ± 0.5°C (at 2 am) with a sine curve, the humidity was kept at 55%, and the day length was 16 h with a light intensity of 6 klx from white fluorescent tubes. The environmental conditions in the growth room were 23 ± 0.5°C (constant), 50% humidity, and 16-h day length with a light intensity of 6 klx from Toshiba DR400/T(L) metal-halide sunlamps plus white fluorescent tubes.

Preparation and cultivation of shoots with roots

Young inflorescence-bearing shoots with roots of clone BNL 4430 were obtained by dividing the potted plants which had been grown in a growth chamber (Shima and Ichikawa, 1994). Older thick roots were cut back. The characteristics of this clone, i.e., many new shoots constantly emerging from the basal nodes one

after another and its short height favorable for early flowering, made it possible to prepare many young inflorescence-bearing shoots with roots at one time (Shima and Ichikawa, 1994). The shoots with roots were cultivated in a nutrient solution circulating (NSC) growth chamber (Kyoshin Riko Co.) designed for our requirements (Shima and Ichikawa, 1994). The environmental conditions in the NSC growth chamber were $22\pm0.5^{\circ}$C during the 16-h day with a light intensity of 7.5 klx from white fluorescent tubes, and $20\pm0.5^{\circ}$C at night. The nutrient solution used was a 1/2000 Hyponex solution.

Treatments with X rays and chemicals

X-ray treatments of the young inflorescences of the cuttings and the shoots with roots 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 $23\pm0.5^{\circ}$C. The exposures were measured simultaneously with thermoluminescence dosimeter (TLD) elements (National UD-170L) 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 methods used for treating the young inflorescences with two alkylating agents, methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS), 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). The treatments were conducted for 16 h. MMS was applied to the inflorescences of the shoots with roots, but EMS was applied to the inflorescences of potted plants, taking the cuttings with these inflorescences immediately after the EMS treatment.

Treatments of the nodal cuttings with nitrofurazone were carried out by dipping the cut ends into nitrofurazone solutions for 4 h at $23\pm0.5^{\circ}$C.

Scoring methods

The number of flowers opened per inflorescence was recorded daily at least for 3-week period enough to cover the peak period of induced mutation frequency in the stamen hairs (Ichikawa, 1992), but for much longer periods in some cases to know how long the flower production lasts. The number of hairs per stamen and the number of cells per hair were scored on each flower as measures of the stamen-hair growth, scoring the former on every stamen and the latter on 10 representative hairs each of two oppositely located stamens per flower, as in studies of mutation frequency (Ichikawa and Ishii, 1991; Ichikawa, 1992).

The methods used for determining pink mutation frequencies in the stamen hairs were identical to those described in detail elsewhere (Ichikawa, 1992). The mutation frequency was expressed as the number of pink mutant events (PMEs) per 10^4 hair-cell divisions (rather than that per 10^3 hairs), since the average

number of cells per hair differs between clones, and may differ after different mutagen treatments and also among the cuttings, shoots with roots, and potted plants. Induced mutation frequencies were determined by pooling the data for the 4-day peak period for each mutagen treatment as in earlier studies (Ichikawa et al., 1993; Shima and Ichikawa, 1994, 1995; Xiao and Ichikawa, 1995).

3. RESULTS

Flower productions

The flower productions of the nodal and/or axillary cuttings of clones KU 7 and KU 20 (scored for 30 and 51 days, respectively) cultivated with Hoagland's No. 2 solution are shown in Fig. 1, and those of the nodal and/or axillary cuttings of clones BNL 02 and KU 27 (scored for 21 days) cultivated with either Knop, Hoagland's No. 2 or Hyponex solutions in Fig. 2. The data for 21 days from the shoots with roots of clone BNL 4430 are also shown in Fig. 2. In these figures,

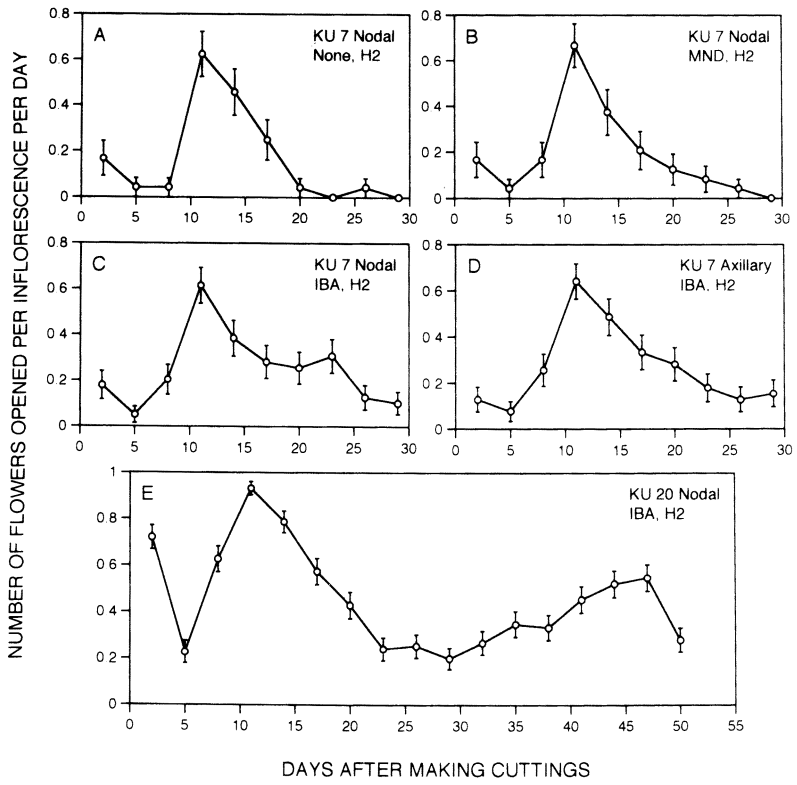

Fig. 1. Changes in flower production in the nodal and/or axillary cuttings of clones KU 7 and KU 20. Root-promoting agents and nutrient solutions used are shown, and the data are pooled for every 3 days (e.g., the data pooled for the first 3 days are plotted at day 2). Standard errors for the data plotted are also shown.

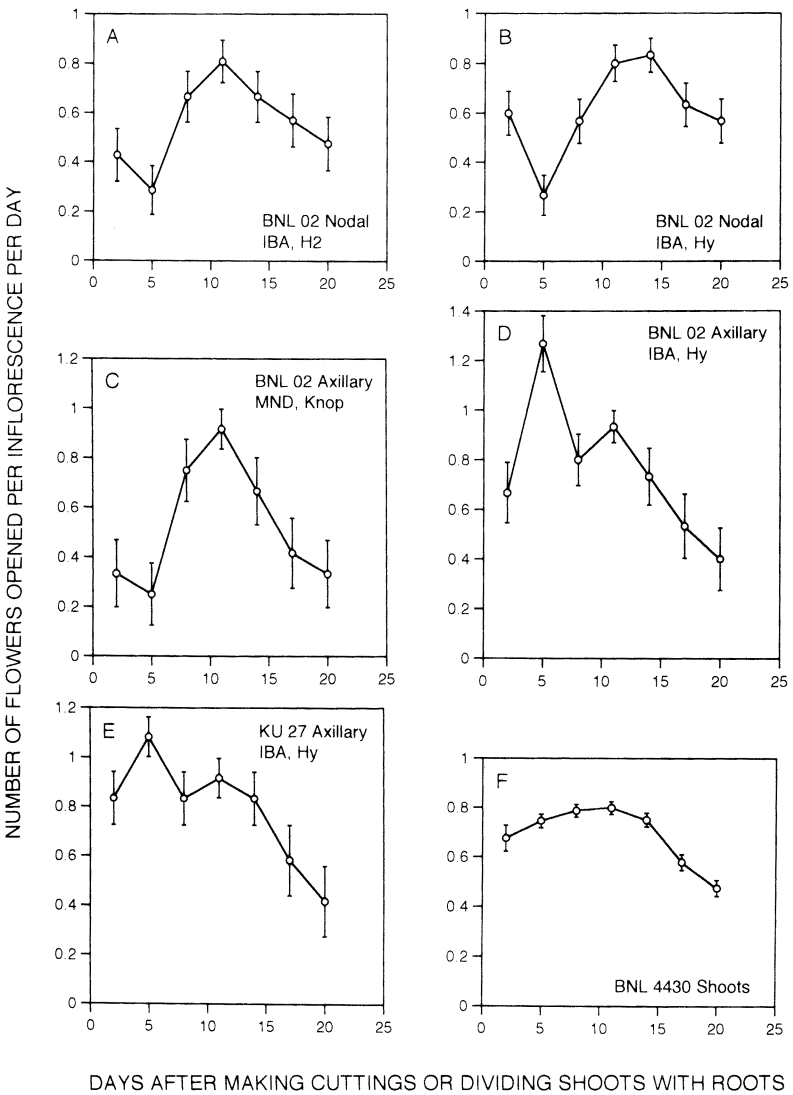

Fig. 2. Changes in flower production in the nodal and/or axillary cuttings of clones BNL 02 and KU 27 and in the shoots with roots of clone BNL 4430. Root-promoting agents and nutrient solutions used for the cuttings are shown, and data are pooled for every 3 days (e.g., the data pooled for the first 3 days are plotted at day 2). Standard errors for the data plotted are also shown.

the data are pooled for every 3 days.

As seen in Fig. 1, the both types of cuttings of clone KU 7 showed very poor flower productions for the first 6 or 9 days, but considerable recoveries in flower production were observed in the fourth 3-day period. The flower production then decreased rapidly when no treatment for promoting rooting was conducted (Fig. 1A), while it declined rather gradually when the cut ends or the basal nodes of the

cuttings were treated with IBA (Figs. 1C and D). The treatment with MND was less effective than the IBA treatment for promoting rooting.

The KU 20 nodal cuttings treated with IBA also showed a greatly reduced flower production in the second 3-day period, but the recovery in flower production was faster than in the KU 7 cuttings. The flower production then decreased gradually, but it showed a certain extent of recovery later after keeping a low level for some 3-day periods, the flower production lasting longer (Fig. 1E).

The flower production of the BNL 02 nodal cuttings treated with IBA also decreased for the first 6 days or in the second 3-day period, and was then restored to considerably high levels (Figs. 2A and B). A similar reduction in flower production for the first 6 days was also observed in the axillary cuttings of this clone treated with MND, but the recovery in flower production was faster (Fig. 2C) than in the nodal cuttings. However, the BNL 02 and KU 27 axillary cuttings treated with IBA showed no reductions in flower production in the earlier periods, showing, on the contrary, very high flower productions in the second 3-day period (Figs. 2D and E). The decrease in flower production in later periods was apparently faster in the axillary cuttings than in the nodal cuttings.

Reductions in flower size were also observed without exception in these cuttings of four clones. The flowers of the KU 7 nodal cuttings became particularly smaller, and also showed poor coloration, making it difficult to score accurately the pink mutations as well as the cell numbers in their stamen hairs.

On the other hand, the flower production of the shoots with roots of clone BNL 4430 was very stable for the first five 3-day periods (which can cover the peak period of an induced mutation frequency), as shown in Fig. 2F, although it declined gradually in the last two 3-day periods. The flowers of the shoots with roots were of normal size.

Stamen-hair growth

The stamen-hair growth was investigated for the cuttings of clones KU 20, BNL 02 and KU 27, and for the shoots with roots of clone BNL 4430. The numbers of hairs per stamen and the numbers of cells per hair counted in the KU 20 nodal cuttings and in the nodal and/or axillary cuttings of clones BNL 02 and KU 27 are plotted in Fig. 3, together with those in the shoots with roots of clone BNL 4430. The data of every 3 days are pooled also in this figure.

The number of hairs per stamen in the KU 20 nodal cuttings treated with IBA was kept at a similar level for the first four 3-day periods, and it then started to decrease gradually, and decreased sharply in later periods (Fig. 3A). The value in the last 3-day period was only about 60% of the values in the first four 3-day periods. On the other hand, no great change in the number of cells per hair was observed.

The number of hairs per stamen in the BNL 02 nodal and axillary cuttings treated with IBA or MND did not decrease for the first three 3-day periods, but it

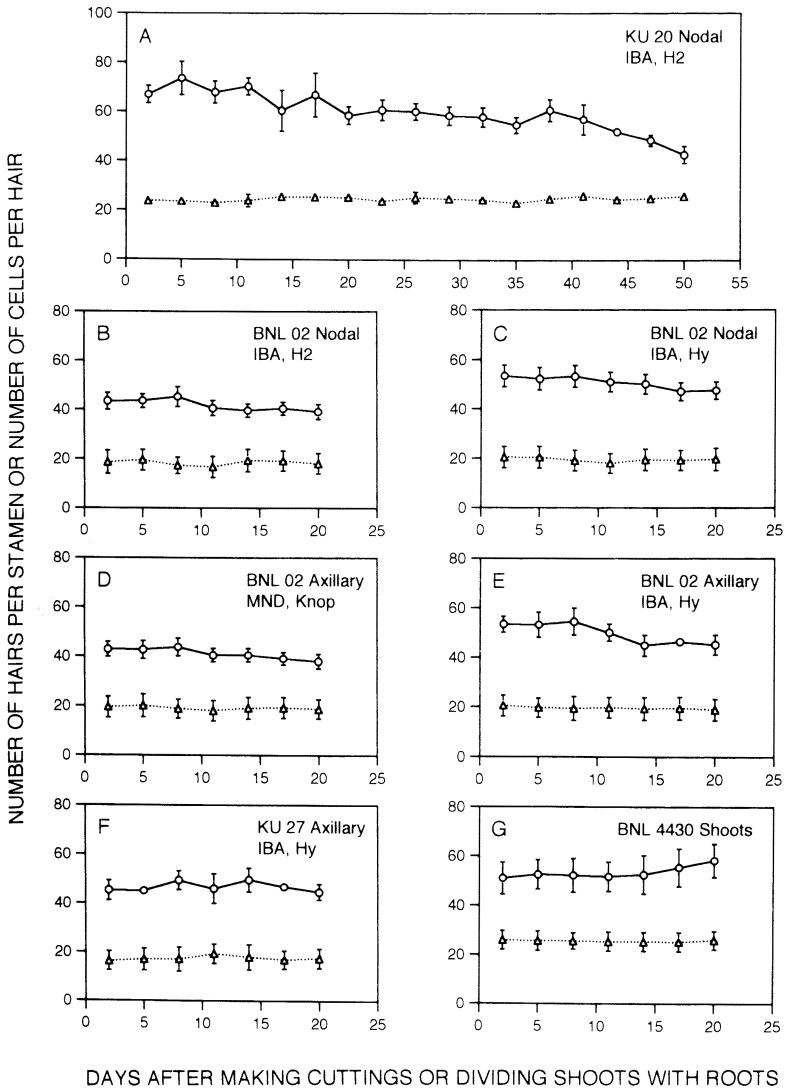

Fig. 3. Changes in the number of hairs per stamen (circle) and in the number of cells per hair (triangle) in the nodal and/or axillary cuttings of clones KU 20, BNL 02 and KU 27 and in the shoots with roots of clone BNL 4430. Root-promoting agents and nutrient solutions used for the cuttings are shown, and the data are pooled for every 3 days (e.g., the data pooled for the first 3 days are plotted at day 2). Standard deviations for the data plotted are also shown.

then declined gradually (Figs. 3B to E). However, the decreases were at most about 15%. No great changes in the number of cells per hair were observed also in the cuttings of this clone, although some decreases were seen in the nodal cuttings in the third and/or fourth 3-day periods. The KU 27 axillary cuttings treated with IBA showed no decrease at all in the number of hairs per stamen as

well as in the number of cells per hair (Fig. 3F).

On the other hand, the stamen-hair growth in the shoots with roots of clone BNL 4430 was very stable (Fig. 3G). Namely, the number of hairs per stamen was maintained at a constant level for the first five 3-day periods, even increasing in later periods, and the number of cells per hair was also kept at a fixed level throughout the scoring period.

Spontaneous mutation frequencies

Spontaneous pink mutation frequencies in stamen hairs were investigated for the nodal and/or axillary cuttings of clones KU 20, BNL 02 and KU 27 and for the shoots with roots of clone BNL 4430, and the results obtained are listed in Table 1. In this table, the data obtained during the whole scoring period are pooled for the BNL 02 and KU 27 cuttings and also for the BNL 4430 shoots with roots, but the data collected in the fifth through the last 3-day periods are pooled for the KU 20 cuttings, since the mutation frequencies of this temperature-sensitive mutable clone in the first four 3-day periods were considered to have been affected by the outdoor environmental conditions before the cuttings were prepared.

The spontaneous mutation frequency of about 8 PMEs per 10^4 hair-cell divisions obtained from the KU 20 nodal cuttings was very much higher than those in the cuttings of other clones. The spontaneous mutation frequencies in the BNL 02 nodal and axillary cuttings were mostly about 0.7 to 0.8 PMEs per 10^4 hair-cell divisions, with a lower value of about 0.4. The value determined in the KU 27 axillary cuttings was comparable to most of the values in the BNL 02 cuttings. The shoots with roots of clone BNL 4430 showed a value about two times higher than those in the BNL 02 and KU 27 cuttings.

Table 1. Spontaneous pink mutation frequencies in the stamen hairs determined in the nodal and/or axillary cuttings of clones KU 20, BNL 02 and KU 27 and in the shoots with roots of clone BNL 4430

Clone	Cuttings or shoots w. roots[1)]	Rooting agent	Cultured with[2)]	No. of hairs scored	No. of PMEs[3)]	Av. no. of cells /hair	No. of PMEs /10^4 cell divisions ± SE
KU 20	nd cut	IBA	H2	8,563	162	24.1	8.19 ±0.64
BNL 02	ax cut	MND	Knop	9,334	12	19.6	0.691±0.200
	nd cut	IBA	H2	5,502	7	18.4	0.731±0.276
	ax cut	IBA	Hy	10,593	8	19.6	0.406±0.144
	nd cut	IBA	Hy	23,305	34	19.4	0.793±0.136
KU 27	ax cut	IBA	Hy	7,363	9	17.5	0.741±0.247
BNL 4430	shoot	——	Hy	48,565	190	25.6	1.59 ±0.12

[1)] nd cut: nodal cuttings; ax cut: axillary cuttings; shoot: shoots with roots.

[2)] H2: Hoagland's No. 2; Hy: 1/1000 (for cuttings) or 1/2000 (for shoots with roots) Hyponex solution.

[3)] Pink mutant events.

Induced mutation frequencies

Induced mutation frequencies were studied using the nodal and/or axillary cuttings of clones BNL 02 and KU 27 and the shoots with roots of clone BNL 4430. The mutation frequencies induced by X rays, EMS, MMS and nitrofurazone are presented in Table 2, together with the doses applied.

When the X-ray-induced mutation frequencies obtained from the cuttings and the shoots with roots as listed in Table 2 are plotted against X-ray dose on a log-log graph, two clear dose-response relationships are obtained, one for the BNL 02 and KU 27 cuttings and the other for the BNL 4430 shoots with roots, as shown in Fig. 4.

The nitrofurazone-induced mutation frequencies in the BNL 02 nodal cuttings and MMS-induced mutation frequencies in the BNL 4430 shoots with roots also showed apparent relationships with the mutagen doses (Table 2).

Table 2. X-ray-, EMS-, MMS- and nitrofurazone-induced pink mutation frequencies in the stamen hairs of the nodal and/or axillary cuttings of clones BNL 02 and KU 27 and of the shoots with roots of clone BNL 4430

Mutagen / Clone	Cuttings or shoots w. roots[1]	Rooting agent	Cultivated with[2]	Dose	No. of hairs scored	No. of PMEs[3]	Av. no. cells /hair	No. of PMEs /10^4 cell divisions (− control) ± SE
X rays								
BNL 02	nd cut	IBA	Hy	716 mGy	1,314	119	18.3	51.6 ±4.8
	ax cut	IBA	Hy	520	1,167	74	19.5	33.9 ±4.1
				949	1,076	136	18.7	71.0 ±6.1
				1,320	975	198	17.7	121 ±9
KU 27	ax cut	IBA	Hy	520	1,135	65	18.7	31.6 ±4.0
				949	953	124	18.2	74.9 ±6.8
				1,320	942	170	17.3	110 ±8
BNL 4430	shoot	——	Hy	252	5,797	140	24.4	8.95±0.89
				442	5,666	224	23.9	16.0 ±1.2
				957	5,605	574	22.5	46.3 ±2.0
EMS								
BNL 02	ax cut	MND	Knop	0.5%-16 h	1,653	83	19.2	26.9 ±3.1
MMS								
BNL 4430	shoot	——	Hy	0.005%-4 h	4,276	177	21.8	18.6 ±1.5
				0.01%	5,092	348	16.4	43.1 ±2.4
Nitrofurazone								
BNL 02	nd cut	IBA	H2	2.5 mM-4 h	1,325	12	16.9	4.97±1.67
				5.0 mM	1,076	21	16.7	11.7 ±2.7

[1] nd cut: nodal cuttings; ax cut: axillary cuttings; shoot: shoots with roots.

[2] Hy: 1/1000 (for cuttings) or 1/2000 (for shoots with roots) Hyponex solution; H2: Hoagland's No. 2.

[3] Pink mutant events.

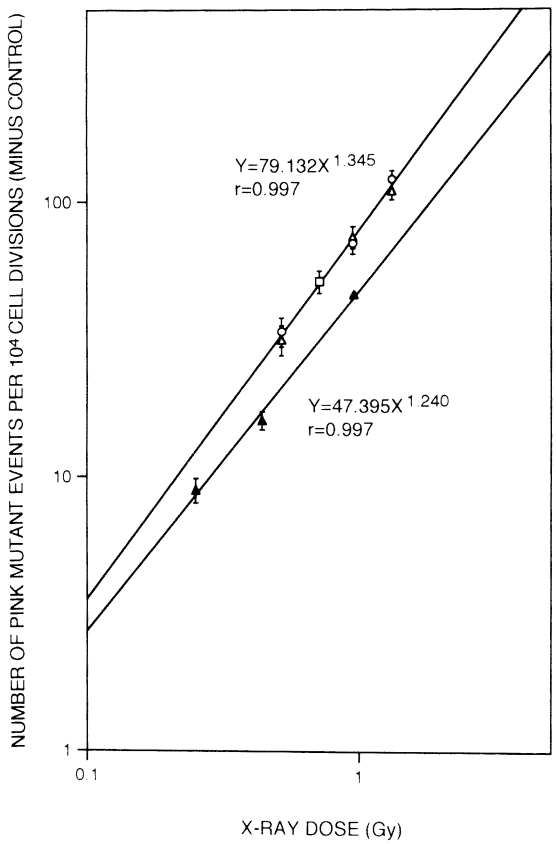

Fig. 4. The dose-response curves of somatic mutation frequency in the stamen hairs of the BNL 02 and KU 27 cuttings and of the BNL 4430 shoots with roots treated acutely with X rays. The best-fit lines determined by the least-squares method, the equations representing them, correlation coefficients, and standard errors for the points plotted (excepting those within marks drawn) are shown. Open circles and open square are the data for the BNL 02 axillary and nodal cuttings, respectively, open triangles for the KU 27 axillary cuttings, and solid triangles for the BNL 4430 shoots with roots.

4. DISCUSSION

Flower productions

Based on our earlier records, the number of flowers opening per inflorescence per day in the potted plants of clones BNL 02 and KU 27 is normally one, and occasionally two or zero, resulting in 1.12 and 1.09 flowers on the average, respectively. The potted plants of clones KU 7 and KU 20 produce more flowers, the average numbers of flowers opening per inflorescence per day being 1.22 and 1.36, respectively.

However, the flower productions observed in the cuttings of four clones examined were mostly poor especially in earlier and later scoring periods (Figs. 1

and 2). Relatively good flower productions were observed only in the axillary cuttings of clones KU 27 (Fig. 2E) and BNL 02 (Fig. 2D) treated with IBA, but they were still poorer than in the potted plants. Among the three nutrient solutions used, the 1/1000 Hyponex solution was judged to be better than others.

On the other hand, the flower production observed in the shoots with roots of clone BNL 4430 was very stable at least for the first five 3-day periods (Fig. 2F), although it declined later. The maximum flower production in the shoots with roots was 0.80 flowers per inflorescence per day. It may look as if this value means a poorer flower production than in the KU 27, BNL 02 and KU 20 cuttings, but it should be noted that, even in the potted plants of clone BNL 4430, only 0.83 flowers open per inflorescence per day on the average, because their inflorescences are composed of smaller numbers of flower buds than those of the above three clones.

At least some extents of reduction in flower size occurred in the cuttings examined. The reduction was most conspicuous in the KU 7 nodal cuttings being accompanied by a poor coloration, and showing that this clone is unsuited for using as cuttings. However, the flowers of the BNL 4430 shoots with roots maintained their normal size.

Stamen-hair growth

Decreases in the number of hairs per stamen occurred in the nodal and axillary cuttings, except for the KU 27 axillary cuttings (Fig. 3). The decreases by the seventh 3-day period were at most 15% in the KU 20 and BNL 02 cuttings, but the hair number decreased much more greatly (about 40%) when it was scored for a longer period in the KU 20 nodal cuttings (Fig. 3A). The average numbers of hairs per stamen in the potted plants of clones BNL 02, KU 27 and KU 20 have been described to be 51.5, 48.9 and 82.5, respectively (Ichikawa, 1992). Comparing with these figures in the potted plants, the hair numbers observed in the BNL 02 and KU 20 nodal cuttings (treated with IBA and cultivated with Hoagland's No. 2) and the BNL 02 axillary cuttings (treated with MND and cultivated with Knop) were obviously smaller (Figs. 3A, B and D). The hair numbers in the BNL 02 nodal and axillary cuttings (treated with IBA and cultivated with Hyponex) in earlier scoring periods (Figs. 3C and E) and those in the KU 27 axillary cuttings (also treated with IBA and cultivated with Hyponex) in whole scoring period (Fig. 3F) were comparable to those in the respective potted plants. The 1/1000 Hyponex solution exhibited much better results than other nutrient solutions also in the number of hairs.

On the other hand, the number of hairs per stamen in the shoots with roots of clone BNL 4430 did not decrease at all, and it showed even increases in later periods (Fig. 3G). The hair numbers observed in the shoots with roots were all comparable to 53.2 hairs per stamen on the average in the potted plants (Ichikawa, 1992).

Little or no great changes in the number of cells per hairs were observed in the cuttings (Fig. 3). However, the numbers scored in the BNL 02 and KU 27 cuttings were slightly or somewhat smaller than the values of average cell number per hair of 21.1 and 21.4 in the respective potted plants (Ichikawa, 1992). The cell numbers per hair in the KU 20 cuttings were nearly comparable to 25.0 cells per hair on the average in the potted plants (Ichikawa, 1992). In the shoots with roots of clone BNL 4430, the cell numbers per hair in whole scoring period rather exceeded the average cell number per hair of 24.4 in the potted plants (Ichikawa, 1992).

These results show that the stamen-hair growth in the cuttings is impaired mostly by the reductions in the number of hairs per stamen, and the reductions in the number of cells per hair are rather negligible. It became also clear that the shoots with roots of clone BNL 4430 continue to maintain the full stamen-hair growth.

Spontaneous mutation frequencies

The spontaneous mutation frequency in the KU 20 nodal cuttings cultivated in the LPH growth chamber, 8.19 ± 0.64 PMEs per 10^4 hair-cell divisions (Table 1), was very close to the value of 8.39 ± 0.48 determined earlier for the potted plants of this mutable clone grown in the Sherer CEL 38-15 growth chamber (Ichikawa, 1994).

The spontaneous mutation frequencies in the BNL 02 nodal and axillary cuttings cultivated in either the LPH growth chamber or the growth room, 0.406 ± 0.144 to 0.793 ± 0.136 PMEs per 10^4 hair-cell divisions (Table 1), were comparable to the values of 0.395 ± 0.132 to 0.804 ± 0.101 (Ichikawa et al., 1990), 0.530 ± 0.142 to 0.697 ± 0.201 (Ichikawa et al., 1991), 0.851 ± 0.142 (Ichikawa, 1992) and 0.515 ± 0.042 (Sanda-Kamigawara et al., 1995) obtained earlier from the potted plants grown under similar environmental conditions. The frequency in the KU 27 axillary cuttings cultivated in the growth room, 0.741 ± 0.247 PMEs per 10^4 hair-cell divisions (Table 1), was also comparable to the values of 0.803 ± 0.138 (Ichikawa, 1992), 0.565 ± 0.163 to 0.996 ± 0.352 (Ichikawa et al., 1993) and 0.553 ± 0.021 (Sanda-Kamigawara et al., 1995) determined earlier in the potted plants grown in the Sherer growth chamber.

The spontaneous mutation frequency determined in the shoots with roots of clone BNL 4430 cultivated in the NSC growth chamber, 1.59 ± 0.12 PMEs per 10^4 hair-cell divisions (Table 1), was somewhat lower than the values of 2.01 ± 0.21 (Ichikawa, 1992), 1.68 ± 0.22, 1.70 ± 0.27 (Ichikawa et al., 1993) and 1.71 ± 0.06 (Shima and Ichikawa, 1994) in the potted plants grown in the Sherer growth chamber. The present value is closer to the earlier value of 1.49 ± 0.03 in the shoots with roots cultivated in the NSC growth chamber, which was significantly lower than the above value of 1.71 ± 0.06 in the potted plants (Shima and Ichikawa, 1994). The fluctuations of the frequency in the shoots with roots in the

NSC growth chamber have been reported to be 1.16 ± 0.14 to 1.82 ± 0.18 (Shima and Ichikawa, 1994), 1.26 ± 0.13 to 2.09 ± 0.17 (Shima and Ichikawa, 1995) and 1.24 ± 0.17 to 1.92 ± 0.20 PMEs per 10^4 hair-cell divisions (Xiao and Ichikawa, 1995).

These results show that the spontaneous mutation frequencies in the cuttings are essentially identical with those in the potted plants, but that the spontaneous mutation frequency in the shoots with roots of clone BNL 4430 tends to be lower than that in the potted plants. The lower background (control) level of mutation frequency in the shoots with roots is favorable for detecting the genetic effects of low-level mutagens (Shima and Ichikawa, 1994).

Induced mutation frequencies

The X-ray-induced mutation frequencies of 33.9 ± 4.1 to 121 ± 9 and 31.6 ± 4.0 to 110 ± 8 PMEs per 10^4 hair-cell divisions in the BNL 02 and KU 27 cuttings, respectively (Table 2), had a clear relationship with X-ray doses as shown in Fig. 4. That is, the mutation frequency was found to increase with increasing X-ray doses with a slope of 1.345 on the log-log graph, when the best-fit regression line was computed, putting the data from the both clones together. These X-ray-induced mutation frequencies were very close to those reported earlier for the KU 27 and/or BNL 02 potted plants (Sanda-Kamigawara et al., 1991; Ichikawa et al., 1993), the slope value also nearly agreeing with earlier values for these potted plants, 1.237 (Sanda-Kamigawara et al., 1991) and 1.274 (Ichikawa et al., 1993).

The X-ray-induced mutation frequencies of 8.95 ± 0.89 to 46.3 ± 2.0 PMEs per 10^4 hair-cell divisions in the shoots with roots of clone BNL 4430 (Table 2) also showed a good correlation with X-ray doses, increasing with a slope of 1.240 on the log-log graph (Fig. 4). These X-ray-induced mutation frequencies were also very comparable to those in the potted plants of this clone reported earlier (Ichikawa et al., 1993). The slope value of the regression line is somewhat larger than 1.112 reported earlier for the potted plants (Ichikawa et al., 1993), but is very close to 1.252 reported earlier for the shoots with roots of this clone (Shima and Ichikawa, 1994).

The induced mutation frequency of 26.9 ± 3.1 PMEs per 10^4 hair-cell divisions in the BNL 02 axillary cuttings after treating with 0.5% EMS for 16 h (Table 2) was nearly three times higher than those (recalculated for 4-day peak periods) obtained earlier from the potted plants after treating with the same EMS dose in % x hr (Ichikawa and Takahashi, 1978; Ichikawa et al., 1990). This result may suggest a possibility of enhancing the sensitivity to EMS by cultivating cuttings (Ichikawa et al., 1990), and might be related to poor absorption of water (or Knop solution) by the MND-treated cuttings after the EMS treatment and to the resultant reduction in repair functions.

The MMS-induced mutation frequencies of 18.6 ± 1.5 and 43.1 ± 2.4 PMEs per 10^4 hair-cell divisions obtained in the shoots with roots of clone BNL 4430 (Table

2) were comparable to those reported earlier in the potted plants (Ichikawa et al., 1993) and in the shoots with roots (Shima and Ichikawa, 1994).

The mutagenic effects of nitrofurazone were tested for the first time in *Tradescantia* stamen hairs. By dipping the cut ends of the BNL 02 nodal cuttings into 2.5 and 5.0 mM nitrofurazone solutions for 4 h, mutation frequencies of 4.97 ± 1.67 and 11.7 ± 2.7 PMEs per 10^4 hair-cell divisions were obtained (Table 2). As a compound closely related to nitrofurylacrylamide (a strong food preservative known as AF-2 authorized in Japan in 1965 and prohibited to use in 1974), nitrofurazone has been reported to be mutagenic in *Neurospora crassa* (Ong, 1977), and also in Chinese hamster cells treated together with S9 mix (Matsuoka et al., 1979). The present confirmation of the mutagenicity of nitrofurazone adds a new evidence in higher plants.

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