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VAPORS OF METHYL ALCOHOL INDUCED SOMATIC MUTATIONS IN *Tradescantia* CLONE 02*

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ABSTRACT

Plants of *Tradescantia* clone 02 bearing young flower buds were exposed to different concentrations of methanol vapors (14×10^3 ppm to 175×10^3 ppm) in order to investigate the mutagenicity of methanol and, if positive, to determine the exposure-response relationship.

Pink mutant events in the stamen hairs were recorded and increases in the frequencies were observed between 7 and 16 days after treatments.

The mutation frequency produced by doses lower than 43×10^3 ppm was the same as that of the control, whereas higher exposures caused an exponential increase in the number of mutations.

The slope and amplitude of the exposure-response curve were compared with results obtained using the alkylating agent, ethyl methanesulfonate, the fumigant and gasoline additive, 2-dibromoethane, and x rays. The results of this comparison allow us to consider methanol as a "weak" mutagen.

To the best of our knowledge this is the first report on the mutagenic action of methanol in vapor form.

RESUMEN

Plantas del Clone 02 de *Tradescantia* fueron expuestas a vapores de metanol de diversas concentraciones (14×10^3 ppm a 175×10^3 ppm) para investigar la mutagenicidad del metanol y, si es positiva, determinar la relación exposición-respuesta.

Se registraron los eventos mutantes rosa y los aumentos en las frecuencias fueron observados entre los días 7 y 16 después de los tratamientos.

La frecuencia de mutación producida por dosis menores de 43×10^3 ppm fue similar a la de los testigos, mientras que exposiciones mayores causaron un aumento exponencial en el número de mutaciones.

La pendiente y la amplitud de la curva de exposición-respuesta fue comparada con los resultados obtenidos usando el agente alkilante. Etilmetanosulfonato, el fumigante y aditivo de gasolina, 1, 2 dibromoetano y los raxos X. Los resultados de esta comparación permiten considerar al metanol como un mutágeno débil.

Hasta lo que se sabe, ésta es la primera descripción de la acción mutagénica del metanol aplicado en forma de vapor.

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INTRODUCTION

An evergrowing number of substances are being released by man into his environment. It is highly probable that human beings and other organisms as well are being exposed with increasing frequency and at increasing levels to many kinds of weak and potent mutagens. By the time an increase in the rate of human mutation is detected, the genetic damage will have already occurred. Therefore, it is urgent to develop biological test systems sufficiently sensitive to monitor the genetic damage caused, especially that brought on by exposures to low concentrations of chemicals.

Much work has been done with the effects of radiation on the stamen hair system of *Tradescantia* (Ichikawa and Sparrow, 1967; Underbrink *et al.*, 1970; Sparrow *et al.*, 1972). Furthermore, this system has been shown to be sensitive to the gaseous forms of such chemical mutagens as ethyl methanesulfonate (EMS) and 1, 2-dibromoethane (DBE) (Sparrow *et al.*, 1974) and certain atmospheric oxidants (Sparrow and Schairer, personal communication).

Sparrow and Schairer (1971) have shown that this stamen hair system was sensitive enough to detect an accidental exposure to a gaseous chemical that produced a high mutagenic response.

These facts enable us to regard the system as effective in the detection of mutagenic activity of atmospheric oxidants or pollutants that are in the gaseous state.

MATERIALS AND METHODS

Clone 02 (or 0081) of *Tradescantia* was discovered by the late Dr. Arnold H. Sparrow in 1958. It was growing outdoors in Profesor W. V. Brown' collection at the University of Texas at Austin (Underbrink *et al.*, 1973).

This clone 02 is heterozygous for flower color. A color gene mutation results in the appearance of pink cells among the normally blue cells in the stamen hairs of the inflorescences exposed to mutagenic agents.

The plants were cultivated in an ordinary greenhouse in the Jardín Botánico Exterior of the Universidad Nacional Autónoma de México.

Plants bearing inflorescences were brought from the greenhouse to laboratory conditions at least a week before the theatment and were placed in beakers containing Hoagland's solution No. 2 (Conger, 1964) and the beakers were wrapped in aluminum foil to retard algal growth in the nutrient solution.

Plants in beakers with Hoagland's solution were placed into closed glass chambers of 9.5 liters. The methanol was placed in an open Petri dish in the chamber and vaporized. No special provision was made to circulate the gas within the chambers because methanol, is highly volatile. All of the liquid became vaporized in each exposure (0.75 to 3.0 ml). Control groups were enclosed in similar chambers with an amount of distilled water equal to that of the substance used for the exposure. The chambers were sealed with high vacum grease and submerged in a bath at 25°C. A period of exposure of six hours was selected in order

to compare meaningfully the effects produced by other substances used at other institutions.

After treatment the plants were placed in beakers with fresh Hoagland's solution aerated and maintained at laboratory temperature (25 ± 5°C) throughout the remainder of the experiment.

Pink mutant events were recorded daily in mature blue stamen hairs of blooming flowers of controls and of the inflorescences exposed to methanol. A single mutant cell or a row of contiguous mutant cells that sometimes involved the whole hair was scored as one mutant event. When the mutant cell or groups of mutant cells were separated by one or more normal blue cells, they were regarded as the result of two separate mutant events.

The number of mutant events per mature flower was recorded each day from day seven through day sixteen following treatment. The mean values of mutant events per 100 hairs per treatment for each day were tabulated.

RESULTS AND DISCUSSION

Based on preliminary results, days seven to sixteen were selected as peak days for pink mutant events. The mean values for these days were used as the mutation frequency induced by methanol.

TABLE I

FREQUENCIES OF PINK MUTATIONS INDUCED IN STAMEN HAIRS OF *Tradescantia* CLONE 02 BY 6-HR EXPOSURES TO METHANOL

Concentration ppm X 10_3	*Number of hairs scored*	*Average number of pink events/100 hairs (—Control)* ± *S.E (Days 7-16)*
43.76	46,672	0.1316 ± 0.0410
58.35	33,520	0.2117 ± 0.0761
87.53	11,950	0.9161 ± 0.0861
116.71	29,470	0.4182 ± 0.1680
145.89	22,385	0.5677 ± 0.2503
175.07	32,993	0.9679 ± 0.2367

Methanol exposures were performed in a range of concentrations from 14.59 x 10^3 parts per million (ppm) to 175.07 x 10^3 ppm. Doses below 43.76 x 10^3 ppm did not produce more the doubling of the spontaneous mutation rate and hence were considered as nondistinguishable from the control value of 0.091/100 hairs. Doses higher than 175.07 ppm caused physiological damage resulting in floral inhibition, gradual fading and death.

In order to compare the mutation frequency induced by methanol with those produced by other substances applied in gaseous form, the data presented in Table I were plotted on a log-log graph (Fig. 1).

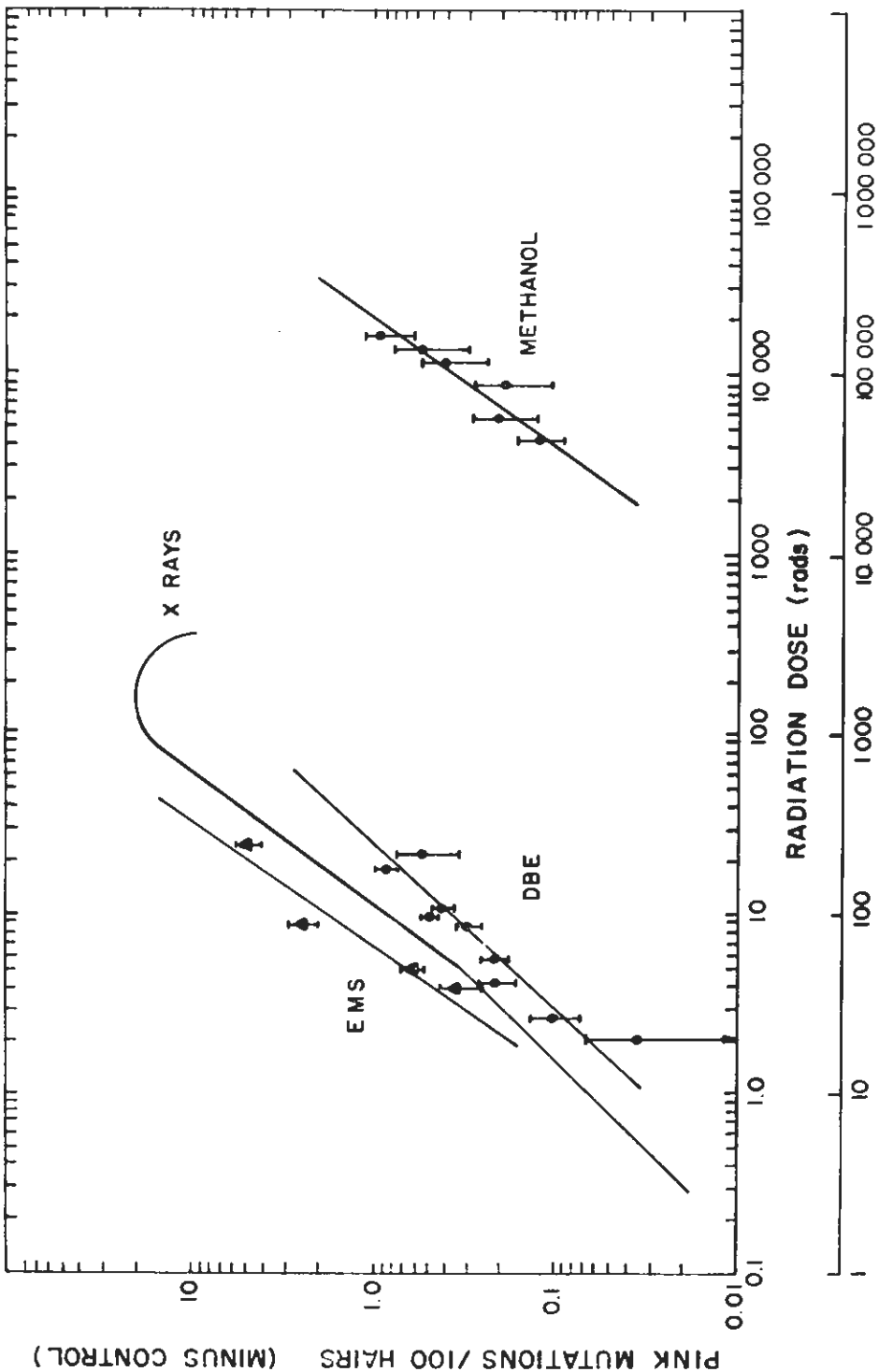

Fig. 1. Comparison of dose response curves for pink mutations induced by x rays and 6 hr. exposures to ethyl methanesulfonate (EMS ●), 1, 2-dibromoethane (DBE ▲) and methanol (●).

Data from Sparrow *et al.* (1974) on ethyl methanesulfonate and x rays and data from Nauman *et al.* (1976) on 1, 2-dibromoethane were used for comparison, where one rad of radiation was arbitrarily made equivalent to 100 ppm of gas (6 hr exposure) for convenience in plotting curves obtained from chemical an physical agents on the same set of axes (Fig. 1). Pink mutations were plotted along the ordinate axis versus dose (in rad or ppm) along the abscissa. The regression line for x rays below 5 rad has a slope of + 1. At higher doses the slopes is + 1.41 and at yet higher doses saturation of the response occurs. Anywhere above 5 rad, as the slope is greater than + 1, the response is no longer linear but quadratic.

The slope of the regression line due to EMS is + 2.41 when bases on three points. This is considerably higher than the slopes for the x-ray dose-response curve. However, when a fourth point regarded as the saturation point is included in the calculation, the slope of the regression line becomes + 1.43. The slope become parallel to the x-ray second component slope which, in both cases, could be due to a combination of deletions and point mutations (Mericle and Mericle, 1967; Nauman *et al.*, 1975, 1976).

The exposure-response curve of mutant events induced by gaseous DBE has a slope of 1.33 (Nauman *et al.*, 1976). However, when all the points including those regarded to be in the saturation level are taken into account, the slope is lower and equal to + 1.11.

The mutagenic effect of EMS on plants and animals has been demonstrated several times (Loveless, 1966) and the mechanism of action of this and other alkylating agents has been investigated (Wheeler, 1967; Price *et al.*, 1969; Osterman-Golkar *et al.*, 1970; Freese, 1971; Lawley, 1974).

In order to compare the slopes and amplitudes of the curves, as one rad was made equivalent to 10 ppm it could then be seen (Fig. 1) that the methanol slope (+ 1.32) was close to that of the x-ray regression line (+ 1.41) and the EMS curve (+1.43).

The amplitudes of the curves can also be seen in Fig. 1. Exposures of 170 x 10^3 ppm of methanol, 42 ppm of EMS and 160 ppm of DBE induced mutation frequencies equal to 8.5 rads of x rays. Thus methanol, like SO_2, NO_2, O_3 freon and vinyl chloride (Schairer and Nauman, personal communication), is one of the weak mutagens. The difference in activity between potent mutagens, like EMS and DBE, and weak mutagens could be the result of some activation mechanism or rate of penetration of the mutagen or a detoxification mechanism occuring at lower exposures that it is saturated at high exposures.

It has been shown that methanol (Gómez Arroyo and Villalobos-Pietrini, unpublished results) as well as ethanol (Michaelis and Rieger, 1968; Gómez Arroyo and Villalobos-Pietrini, unpublished results) induce chromosomal aberrations and lagging chromosomes in *Vicia faba* root tips. Methanol and ethanol also produce chlorophyll mutations in gametophytes of fern spores (Villalobos-Pietrini and Meneses, unpublished results). Although Ehrenberg (1973) found ethanol completely inactive in the barley test, Badr and Badr (1975) found it could induce dominant lethal mutations in mice.

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