



Project Summary

***Tradescantia* MCN-In-Tetrad Mutagen Test for On-Site Monitoring and Further Validation**

Te-Hsiu Ma

Tradescantia paludosa (Spiderwort) clone #03, an extensively cultivated experimental plant for cytological studies, has been utilized exclusively for the present investigation. Year-round growth and reproduction of this plant can be maintained in the greenhouse with supplemented artificial light during the short-day season thus facilitating the uninterrupted supply. Taking the advantages of high degree of synchrony and differential sensitivity of the meiotic stages, a short-term bioassay was developed for testing environmental mutagens. This was done by treating the synchronized, sensitive, early prophase I meiotic chromosomes and observing the damaged chromosomes in the form of micronuclei in the synchronized early tetrad stage in order to attain the highest efficiency. This test is called *Tradescantia* Micronucleus (Trad-MCN) Bioassay and has been utilized to screen a group of chemicals in gaseous or liquid forms and monitor the air pollutants at some chosen sites. Of 16 chemicals tested, 8 had relatively higher micronucleus frequencies (0.01 level of significance) than their controls. Of 11 sites monitored, 3 sites showed higher micronucleus frequencies than their controls. According to these results, the Trad-MCN bioassay showed high efficiency and versatility. It is suitable

for preliminary screening of environmental pollutants and *in situ* monitoring.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction and Summary

Tradescantia Micronucleus Bioassay is a highly efficient, simple and inexpensive mutagen test. The extraordinary high efficiency is the result of exposure of the meiotic chromosomes at their sensitive and synchronized early prophase I stage, and scores the frequencies of chromosome damage in the forms of micronuclei (MCN) at the synchronized tetrad stage. The meiotic pollen mother cells can be obtained year round from a population of cultivated *Tradescantia* plants by maintaining a 16-hr photoperiod during the short day season (October 1 - April 1). Thus, the experiments can be carried out year round without interruption.

Three kinds of experiments can be carried out, namely, *in situ* air monitoring, screening of chemical mutagens of gaseous, and liquid forms. Beside the chemicals tested during the initial stage

of the development of this bioassay, including 1,2-dibromoethane, ethyl methanesulfonate, sodium azide, since 1976, results of *in situ* air monitoring and chemical tests during this grant period (March 1, 1979 to June 1, 1980) are reported here.

Tradescantia paludosa clone #03 was utilized exclusively throughout this study because of its profuse branching ability and greater productivity of inflorescences although clones #4430 and #02 can also be used in the Trad-MCN test. The overall scheme of treatment procedure includes: collection of plant cuttings which bear inflorescences of appropriate stage, and exposure of the inflorescences to gaseous agent in ambient air or in an enclosed chamber, or treatment of the plant cuttings in water solutions containing mutagen. Water insoluble agents were first dissolved in DMSO or ethanol before mixing with water. Generally, a 24-hr recovery (meiotic) time was allowed before fixation (acetoalcohol 1:3). Fixed inflorescences were stored in 70% ethanol and used to prepare microslides for scoring MCN frequencies at early tetrad stage (Figure 1).

Conclusions

Eleven sites were monitored using Trad-MCN bioassay. They included two industrial complexes, three public parking garages, two truck stops, one bus stop, one smoking office, one herbarium with fumes of paradichlorobenzene, and one farm animal house. Positive response (double or triple of background level, around five MCN/ 100 tetrads) were obtained from every kind of site except the office and farm animal house.

Four kinds of gases, including NO₂, SO₂, O₃, HN₃ were tested and NO₂ (1 ppm, 22 hr), SO₂ (5 ppm, 24 hr), HN₃ (136 ppm, 6 hr) yielded positive responses.

Results of nine water-soluble chemical tests are as follows: ascorbic acid (+, 0.1%), MnCl₂ (+, 10 mM), NaHSO₄ (+, 0.05 mM), niacin (-, 20 mM), NaNO₃ (-, 50 mM), propionic acid (+/-, 1%), lead acetate (+/-, 10 mM), saccharin (+, 5 - 50 mM), ZnCl₂ (+, 1 - 10 mM).

Results of tests on three known mutagens are as follows: benzo(a)pyrene (+, 0.05 mM, in 10% DMSO), cyclohexylamine (+, 0.25% - 0.5%),

maleic hydrazide (dose-response, 8.9 - 15.6 mM).

Dimethyl sulfoxide (DMSO) at gradient concentrations (5, 10, 20, 30, 40%) was tested, negative responses at 5 and 10%, but positive at 20% and up.

Spring Lake (Macomb city reservoir) water was tested and compared with tap water after disinfection treatment. In most cases, tap water gave relatively higher mutagenicity than the lake water.

Diesel exhaust fumes were tested at various concentrations (represented by hydrocarbons, 0.3, 0.6, 0.9, 1.8, 4.2, 6.3, 9.4, 13.2 ppm). A trend of dose-response was demonstrated. Diesel exhaust condensates at 100 - 200 ppm concentrations were tested. No positive response was revealed.

Recommendations

Based upon the results of this series of studies, Trad-MCN bioassay has been shown to be well-suited for *in situ* monitoring of air pollutants, and it is more efficient for testing water-soluble agents than for water-insoluble agents.

Tradescantia plant cuttings can be maintained in tap water or nutrient solution and sustain normal life processes under artificial light. Thus, it is a best-suited material for studies of gaseous agents in glass chambers. Further study of the automobile exhaust fumes in the dilution chambers should continue.

Comparative studies of the clastogenic effects between diesel and gasoline engine emissions should be carried out using this bioassay, since the difference in clastogenicity of these two kinds of emissions is not well known at the present time.

Trad-MCN bioassay is ideal for testing clastogenic effects of pesticides either in the laboratory or in the field in the real-world situation.

Application of this bioassay to common beverages, foodstuff, pills and non-prescription drugs, and industrial and farm run-off in the real world is highly recommended.

Tap water, after chlorination and other kinds of disinfection, gave relatively higher mutagenicity than lake water from last year's long study. Further studies with distilled water control and nutrient solution control should be continued.

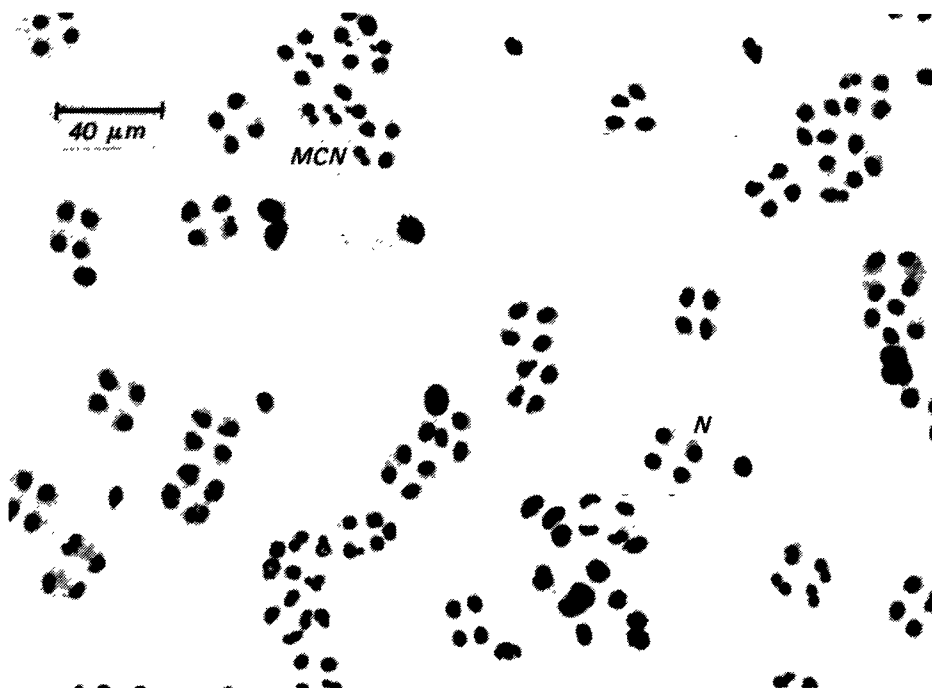


Figure 1. Photomicrograph of a population of tetrads of meiotic pollen mother cells showing normal tetrads (N) and tetrads containing micronuclei (MCN).

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The complete report, entitled "Tradescantia MCN-in-Tetrad Mutagen Test for On-Site Monitoring and Further Validation," (Order No. PB 81-168 700; Cost. \$6.50, subject to change) will be available only from:

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