Brazilian Journal of Medical and Biological Research (2002) 35: 127-129
ISSN 0100-879X Short Communication

Pollen mother cells of *Tradescantia* clone 4430 and *Tradescantia pallida* var. *purpurea* are equally sensitive to the clastogenic effects of X-rays

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Abstract

The *Tradescantia* micronucleus test is a sensitive bioassay for mutagenesis that may be employed both under field and laboratory conditions. This test has been standardized mostly on the basis of the results obtained with clone 4430. However, this clone is not well adapted to tropical weather, frequently showing problems with growth and flowering. In addition, it is attacked by parasites and insects, a fact that limits its use in field studies aiming at the biomonitoring of air pollution. In the city of São Paulo, *Tradescantia pallida* (Rose) Hunt. var. *purpurea* Boom is widely distributed as an ornamental plant in gardens and along roadsides and streets, mostly because of its natural resistance and its easy propagation. In this report, we present dose-response curves indicating that the sensitivity of *T. pallida* and clone 4430 to X-radiation (1, 10, 25 and 50 cGy) is similar. The results confirm our previous suggestion that *T. pallida* represents a good alternative for *in situ* mutagenesis testing in tropical regions, especially biomonitoring studies in which the exposure conditions may not be fully controllable.

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Research supported by FAPESP, CNPq, PRONEX and LIM-HCFMUSP.

Received October 17, 2000
Accepted October 9, 2001

Key words
- *Tradescantia*
- Micronucleus
- Environmental mutagenesis
- Clastogenesis
- X-radiation
- Pollution biomonitoring

Plant bioassays are ideally suited to use in short-term studies of mutagenesis both for laboratory and *in situ* biomonitoring investigations (1-8). In addition, because of their low cost and high efficiency, they can be used for the screening of selected environments such as the vicinity of stationary sources of air pollution (2-4,7,8), traffic emissions (6-8), as well as soils, waters, and selected substances, requiring minimal procedures of chemical extraction and sample manipulation (4-6).

Because of these characteristics, plant mutagenesis bioassays play a special role in the investigation of the harmful consequences of pollution to human health, mainly in countries and regions experiencing a rapid increase in environmental load as a result of urbanization and industrialization. These regions require, as a precautionary measure for the environmental quality control and monitoring, rapid and cost effective evaluation of

the impact of residues derived from industrial and transportation processes.

Among the mutagenesis tests based on plants, the *Tradescantia* micronucleus bioassay is considered to be, perhaps, the most sensitive (2,5). Briefly, the test is based on the visualization of segments of chromosomes as small round and dark structures (micronuclei) in early tetrads of pollen mother cells. The micronuclei represent the consequence of defects occurring during chromosome meiotic replication of the pollen progenitor cells. After exposure to mutagenic agents, the frequency of micronucleus formation increases and is expressed numerically, allowing the quantification of the degree of chromosomal damage during meiosis.

Because of its high sensitivity, the *Tradescantia* micronucleus bioassay has been employed extensively by our group to evaluate the deleterious effects of air pollution in São Paulo and to obtain a prognosis for risks to human health (6,9). However, a limitation we have been experiencing in our experiments is the susceptibility of clone 4430 to the hardships of tropical weather (high temperature, humidity and rainfall), often manifesting as inhibition of growth and flowering. Furthermore, when kept for prolonged periods of time in the open environment, *Tradescantia* clone 4430 is often heavily attacked by parasites and insects, a fact limiting its use in medium- to long-term biomonitoring studies under "real world" conditions.

In order to develop an assay better suited to the environmental and institutional conditions commonly found in Brazil, we devoted our attention to a plant of the same family (Commelinaceae), *Tradescantia pallida* (Rose) Hunt. var. *purpurea* Boom, which is widely distributed in the streets and gardens of São Paulo city. This species is tetraploid, exhibits notable resistance to parasites and insects, and is propagated quite easily. It blooms all year and has very few growth requirements, actually being extensively grown as an ornamental in the city's gardens and squares.

On the basis of these considerations, we explored the feasibility of the use of *T. pallida* in pollution studies, with positive results (6-8). In the present study, we compared the dose-response relationships obtained when *Tradescantia* clone 4430 and *T. pallida* were exposed to radiation. We reasoned that such approach could be of interest to determine the relative sensitivity of the two plants in a well-defined model of environmental injury and thus help to justify the use of *T. pallida* as an indicator plant for biomonitoring studies of mutagenesis.

Young inflorescences of both clone 4430 and *T. pallida* were obtained from a plant nursery and kept in the laboratory for 24 h in Hoagland's solution before X-ray treatment. Plants were assigned at random to groups receiving different X-ray doses. X-Irradiation was applied at the doses of 1, 10, 25 and 50 cGy by means of a therapeutic X-ray device (Stabilipan-Siemens) operated at 250 kV, 15 mA, with a Thores 1 filter. After treatment, the inflorescences were allowed to recover for 24 h in Hoagland's solution and then fixed in 1:3 acetic acid/ethanol solution for 24 h.

Inflorescences were dissected and young anthers squashed in a solution of acetocarmine stain on a microslide (6). Only preparations containing early tetrads were considered. The number of micronuclei in 300 tetrads per slide was counted at 400X magnification, and the results were reported in terms of percentage (frequency of micronuclei). Micronuclei were counted on coded slides, with the code being broken only after the completion of the experiment.

Statistical analysis was done using the SPSS v9.0 statistical software, and the significance level was set at 5%. The significance of the results was assessed by means of a generalized linear model using dose and type of plant as factors.

The dose response curves are presented in Figure 1. A significant effect of X-ray dose was detected ($P<0.001$), but no differences between plant type ($P = 0.246$) or interactions between X-ray dose and type of plant ($P = 0.362$) were observed. The generalized linear model fitted well the data ($r^2 = 0.457$) with no evidence of significant deviation from normality of the residues. The values for the present study were quite similar to those reported in previous studies (1,2,6).

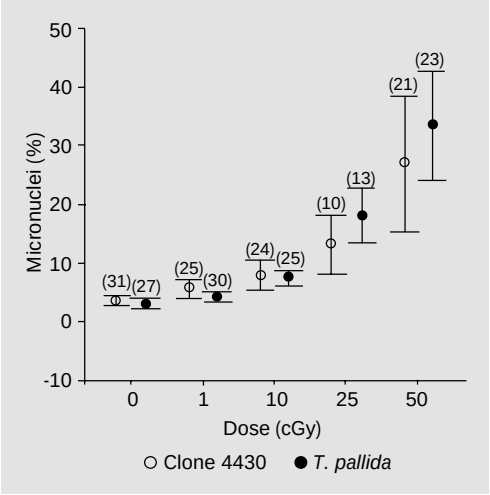

Figure 1. Radiosensitivity of *Tradescantia* clone 4430 and *T. pallida*. Data are reported as means ± SEM as percent micronuclei measured in early tetrads of *Tradescantia* clone 4430 (open circles) and *T. pallida* (filled circles), 24 h after treatment with increasing doses of X-rays. The number of individuals studied at each dose is given in parentheses.

Our results indicate that both species of *Tradescantia* are equally sensitive to X-ray treatment. Such findings reinforce the possibility of using *T. pallida* in studies of environmental mutagenicity. A detailed description of the methodology and review of the literature regarding plant mutagenesis bioassays have been recently published in Portuguese (9-11). We believe that the validity of the use of the more robust *T. pallida* as a suitable experimental model indicated by the present results may encourage other groups to adopt this simple method for the assessment of environmental genotoxicity, mainly when sophisticated conditions of plant cultivation are not available.

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