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A N I N T E R N A T I O N A L J O U R N A L

Biological Response of *Tradescantia* Stamen-hairs to High Levels of Natural Radiation in the Poços de Caldas Plateau

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ABSTRACT

The objective of the present study was to apply a highly sensitive botanical test of mutagenicity (the Tradescantia *stamen-hair mutation bioassay) to assess* in situ *the biological responses induced by naturally occurring radiation in the Poços de Caldas Plateau. The mutagenesis was evaluated in environments presenting gamma radiation exposure rates ranging from 1.5 $\mu R.min^{-1}$ up to 100.0 $\mu R.min^{-1}$. The results consistently showed only borderline increases in mutation frequencies in plants exposed to areas with high radiation background, as compared to non-exposed plants. It was concluded that the levels of natural radiation prevalent in the Poços de Caldas Plateau were not sufficient to induce significant increases in mutation rate, even in the extremely sensitive* Tradescantia *stamen hair mutation bioassay and mutagenesis evaluation test could be a useful monitoring system for natural radiation exposure.*

Keywords: Mutagenesis, natural radiation, bioassay, *Tradescantia*

INTRODUCTION

The current policy of radiation protection is based on the hypothesis of linear dose-response from which all procedures and dose limits related to nuclear activities have been established. Although this can be considered a conservative and efficient policy in its protection function, some controversy exists regarding the applicability of the linearity hypothesis for the protection of the genome (Maugh II, 1978). The effects of ionizing radiation on the integrity of the genetic material have been studied since the discovery of radiation-induced mutations, both in animals (Muller, 1927) and plants (Stadler, 1928). Mechanisms of chromosome susceptibility to radiation damage were demonstrated in early studies using *Tradescantia* as an experimental subject (Sax, 1938). Cellular activity was shown to play an important role in susceptibility, and the greatest activity represented by meiotic replication coincided with greatest sensitivity to radiation (Sax, 1938; Sparrow and Singleton, 1953).

Normally less sensitive, somatic mutation evaluation offers valuable information for the assessment of naturally occurring radiation and its potential mutagenicity to exposed populations. Evaluations of radiation-induced mutations carried out on the stamen hairs of *Tradescantia* indicated that this could provide an excellent test system for

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in situ studies and that there was an approximate linear relationship between radiation exposure and frequency of mutant cells and mutation events (Nayar and Sparrow, 1967; Sparrow et al., 1972). This linear relationship has been contested by some investigators however, on the grounds of some alleged qualitative difference due to type of radiation exposure, discontinuity of the reaction according to site of radiation damage, or due to synergism between radiation and chemical compounds (environmental or metabolic) (Dennis and Dennis, 1988; Kirby-Smith and Daniels, 1953; Tano, 1987).

The departure from linearity in the dose-response relationship of radiation effects has important consequences regarding the uncertainty related to the different parameters used in the evaluation of potential radiation hazards. Mutagenesis induction is one particularly valuable radiation assessment parameter, and plants are especially adequate experimental subjects for mutagenesis evaluation, not only for their amenability to *in situ* exposure, but also due to the high sensitivity of some plant test systems, such as the *Tradescantia* stamen hair mutation assay (Trad-SHM) (Tano and Yamaguchi, 1985). The Trad-SHM assay is, thus, especially suited for the study of complex environmental situations, such as those found on the Poços de Caldas Plateau, which has been identified as amongst the most naturally radioactive locations on the Earth.

The Trad-SHM is a somatic mutation (mitotic) bioassay in which expression of the heterozygous dominant blue character of the stamen hair cell is prevented, resulting in the appearance of the recessive pink color (Emmerling-Thompson and Nawrocky, 1980; Underbrink et al., 1973). The sensitivity of *Tradescantia* to the genetic effects of radiation and chemical agents is widely known (Ichikawa, 1992; Rodrigues et al., 1997). Studies on the effects of very low radiation levels with the Trad-SHM assay involve a series of exposure situations, from absorbed radioisotopes (Tano and Yamaguchi, 1979), radiation-contaminated substrates (Cebulska-Wasilewska, 1992; Ichikawa and Ishii, 1991a), and high level background radiation from monazite sand (Nayar et al., 1970), to short-wave radiofrequencies emitted by antennae and the bombardment of cosmic rays occurring in orbital flight (Delone et al., 1986; Sparrow et al., 1968).

Regarding the radiation protection policy implications of low level radiation exposure and potential biological responses, Ichikawa (1981) conducted a large-scale long term assessment of mutation frequency around nuclear power plants in Japan. The Trad-SHM assay showed to be an adequate genotoxicity bioindicator, both in terms of detecting radiation exposure, as well as in terms of sorting out the confounding environmental factors that interfere with biological responses to radiation. In the present study, the Trad-SHM assay was used to assess the mutagenicity induced by the high levels of natural radiation occurring on the Poços de Caldas Plateau.

MATERIALS AND METHODS

Exposure "*in situ*"

The mutagenesis evaluation was carried out in different environments, presenting gamma radiation exposure rates varying from 1.50 $\mu R.min^{-1}$ to 100.0 $\mu R.min^{-1}$ as shown in Table 1.

Table 1 - Exposure sites of *Tradescantia* plants in the Poços de Caldas Plateau and radiation level determined for each site.

Exposure site (Abbreviation)	Radiation level ($\mu R.min^{-1}$)
Pit Mine (PM)	1.5
Greenhouse (GH)	1.6
Tailing Dam (TD)	6.0
Itataia Ore (Ita)	10.0
Morro do Ferro 1 (MF1)	21.0
Gallery of Morro do Ferro (Gal)	41.0
Morro do Ferro 2 (MF2)	50.0
Waste Deposit (WD)	100.0

Groups of ten pots containing flowering *Tradescantia* plants (clone 4430) were kept in their respective exposure sites for 24 h. In the mean time, for each exposed group, there was one control group kept in controlled-environment greenhouses presenting a radioactivity background of 1.6 $\mu R.min^{-1}$. These *Tradescantia* stock plants maintained in the greenhouses were considered also as the reference to evaluate the spontaneous mutation frequency for clone 4430 on the Poços de Caldas Plateau. In order to evaluate possible greenhouse effects, and as a means of ascertaining a more stable set of controls, two *Tradescantia* stock populations were kept in two separate greenhouse spaces (the greenhouse itself, and its

annex, set to the same environmental conditions). These plants were cultivated in 5-inch pots containing humus, supplemented with fertilizer each 15 days (nitrogen-phosphate-potassium), watered every other day and maintained clean and pest-free by manual scouting and pruning.
The radiation level of each of the exposure sites was determined at the same position where the plants were placed using a 1800 cc ionizing chamber and a radiation monitor controller, models Radcal 10x5 – 1800 and 9015, respectively. The measure was repeated 10 times for each exposure site.

Tradescantia bioassay

The Trad-SHM assay applied in the present experiments is a mutation (mitotic) assay in which expression of the heterozygous dominant blue character of the stamen hair cells is prevented, resulting in the appearance of the recessive pink color. Details of the experimental methods and a review of the results obtained with this bioassay are available in Rodrigues, (1999a) and Rodrigues (1999b); Rodrigues et al., (1997).
In order to check the sensitivity of our *Tradescantia* plants to radiation and to standardize the experimental procedures, pots containing mature plants were exposed in the laboratory to a gamma radiation source delivering from 200 up to 2,000 mGy. For each field experiment, twenty flowers were evaluated daily, ten coming from exposed pots and other ten coming from control (greenhouse) pots. Mutation scoring was performed between the 7^{th} and 13^{th} days after exposure in order to allow the exposed flower buds to open as mature flowers in which the stamen hairs could be observed (under X60 magnification). The number of stamen hairs per flower in each treatment group was estimated (Ichikawa and Ishii, 1991b), and the number of mutation events per 1000 hairs was determined. On average, over 3000 hairs were scored for each treatment day. Statistical comparisons were carried out on the transformed data (y=[sqrt X]+[sqrt[X+1], (Snedecor and Cochran, 1967)) by ANOVA ($p \leq 0.05$) for the days of largest mutation frequencies for all the treatments. Specific comparisons between each treatment and its specific control were carried out by unpaired t-Test ($p \leq 0.05$).

RESULTS

The *Tradescantia* plants employed in the present study showed to be sensitive to radiation exposure (Figure 1). The mutation frequencies obtained within the groups of plants maintained in the greenhouse (and its annex) did not show statistically significant differences ($p > 0.05$) throughout the complete period of evaluation (Figure 2B).

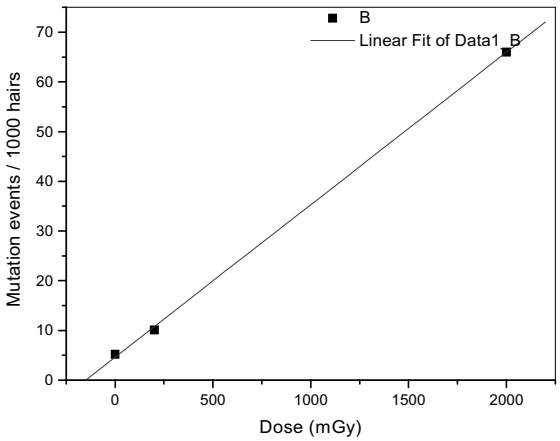

Figure 1 - Response of the Trad-SHM assay to exposure to a gamma radiation source. The value corresponding to zero radiation level is the average mutation frequency obtained in the greenhouse.

Likewise, no statistically significant differences were obtained between the plants maintained in the greenhouse and its annex (labeled Greenhouse 1 and 2 in Figure 2B). The higher mutation frequency occurred on day nine for these control plants placed in the greenhouse and its annex must be the result of some interference not accounted for in our experiments, but attest to the reproducibility of the Trad-SHM assay, since the two independent populations presented similar behavior.
The complete set of mutation frequencies observed for the days following exposure in all treatments, together with their corresponding controls kept in the greenhouse throughout the experimental period can be observed in Figure 2 (A to H). It should be noticed that by and large every series of data corresponding to the exposed group in each treatment showed at least one peak of maximum mutation frequency, whereas the data series corresponding to the control group in each treatment tended to maintain a lower and more stable mutation frequency throughout the scoring period.

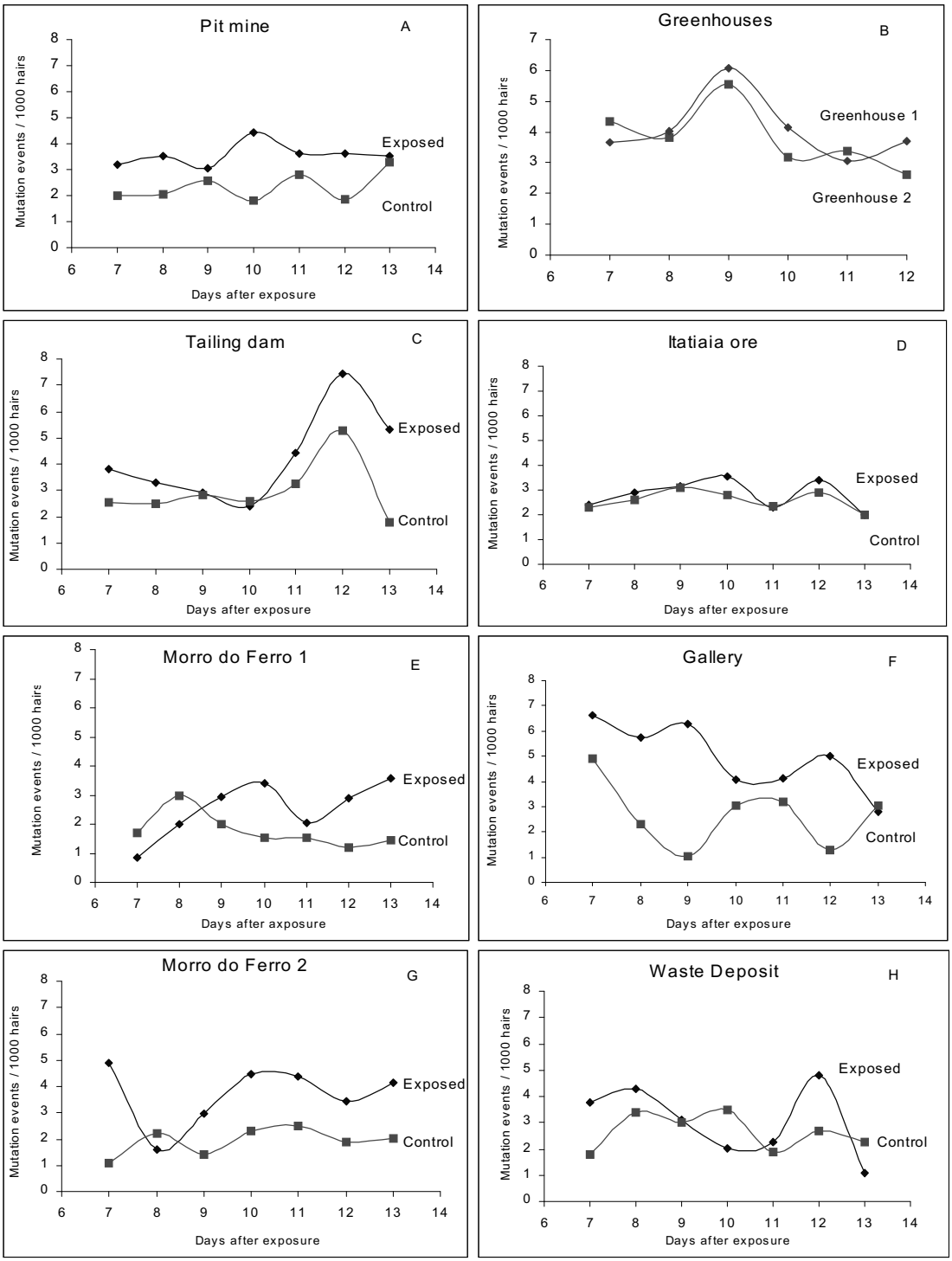

Figure 2 - Mutation frequencies for the Trad-SHM assay in different exposure sites of varying radiation levels in the Poços de Caldas Plateau. The data series for each exposure site represents days after exposure, and the day of maximum mutation frequency corresponds to the valid day for evaluation of radiation response. Each group of exposed plants is accompanied by its corresponding control group, which was kept in the greenhouse during the same period.

According to the Trad-SHM method (Rodrigues, 1999a), the set of daily data showing the highest mutation frequency during the period between the seventh and the twelfth day after exposure, represented the stamen hairs that were actively forming on the day of exposure and should be considered as valid for scoring. In other words, the day of maximum mutation frequency was the one in which the stamen hairs forming during exposure appeared in the opened flowers. It was from this particular day that the data were drawn for the comparisons of mutation frequency among the different exposure sites.

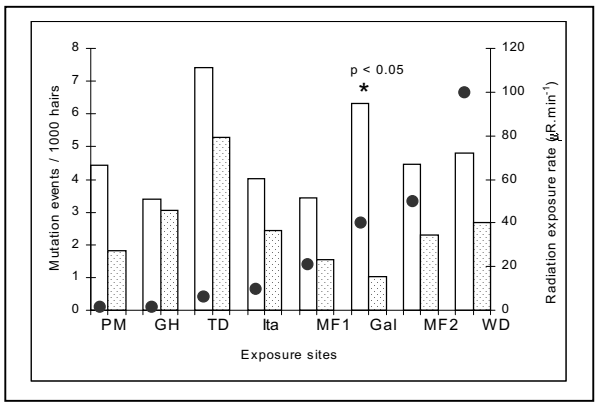

Figure 3 - Mutation frequency of the Trad-SHM assay after exposure to different sites of varying natural radiation levels on the Poços de Caldas Plateau. Each site is represented by the exposed plants (plain bars) and their corresponding controls (dotted bars), in addition to its gamma radiation exposure rate (full circles). The (*) symbol indicates statistical significance. The exposure sites are as follows: PM - pit mine; TD - tailing dam; Ita - Itataia ore; MF - Morro do Ferro (sites 1 and 2); Gal – Gallery of Morro do Ferro; WD - waste deposit; GH - greenhouse.

Figure 3 shows the mutation frequencies for all the exposure sites and their corresponding controlsalong with each site's natural gamma radiation exposure rate. The only exposure site for which a statistically significant increase in mutation frequency from the exposed plants relative to their control occurred was the Gallery of Morro do Ferro site. This result indicated that although an apparent increase in mutation frequency was evident for all *Tradescantia* plants exposed to sites with high levels of natural radiation on the Poços de Caldas Plateau, this increase was only borderline and not sufficient to induce a consistent biological response, even in this highly sensitive bioindicator of mutagenicity represented by the Trad-SHM assay.

DISCUSSION

Many studies have shown that a linear increase in mutation frequency occurred in Tradescantia stamen hairs exposed to increasing radiation doses (Ichikawa and Takahashi, 1977; Mericle and Mericle, 1965; Sparrow et al., 1972). The nonlinear relationship between gamma radiation exposure and mutation frequencies observed in the present study indicateed that other interfering factors might be having a role in the exposure sites on the Poços de Caldas Plateau. The spontaneous mutation rate of Tradescantia could be affected by several environmental factors such as light, temperature, nutritional status, and air impurities In the present study, however, even though the plants were exposed in situ to environments presenting not only different gamma radiation exposure rates, but also a whole set of different environmental conditions, only one site, the Gallery of the Morro do Ferro (gamma radiation exposure rate of $40 \mu R.min^{-1}$) showed a significant increase in mutation frequency relative to its corresponding control. The borderline response, showed for most of the exposure sites studied, indicates that the higher natural radiation levels occurring on the Poços de Caldas Plateau were not sufficient to induce significant increases in mutation frequency, even for a sensitive mutagenesis evaluation bioassay, such as the Trad - SHM.

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RESUMO

A presente política de proteção radiológica está baseada na hipótese de linearidade da relação dose-efeito, da qual derivam os procedimentos de proteção e o estabelecimento de limites aceitáveis de exposição. Embora tal política possa ser considerada conservadora e eficiente, há controvérsias quanto à aplicabilidade da hipótese de linearidade quando efeitos genéticos são

considerados, especialmente para exposições a doses reduzidas presentes em áreas com altos níveis de radiação natural, o que justifica estudos adicionais. O Planalto de Poços de Caldas é considerado um dos locais da Terra de maior radiatividade natural, e o objetivo do presente estudo foi aplicar um teste muito sensível de avaliação de mutagênese (o ensaio da mutação em pêlos estaminais de *Tradescantia*) para averiguar *in situ* as respostas biológicas induzidas pela radiação natural. A indução de mutagênese foi avaliada em ambientes apresentando taxas de exposição à radiação gamma variando de 1,5μR.min^{-1} a 100μR.min^{-1}.Os resultados indicam que a radiação presente na maioria dos ambientes estudados não foi suficiente para induzir aumentos significativos na taxa de mutação, mesmo neste bioensaio muito sensível.

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