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 occurring radiation in Brazilian radioactive waste deposits (waste deposits from the Mineral Treatment Unit/Brazilian Nuclear Industries (UTM/INB), from the Centro de Desenvolvimento de Tecnologia Nuclear (CDTN) and from the Instituto de Pesquisas Energéticas e Nucleares (IPEN). The mutagenesis was evaluated in environments presenting gamma radiation exposure rates ranging from 1.6 µR.min$^{-1}$ up to 3300.0 µR.min$^{-1}$. It was detected a significant increase in the mutation rate for pink Tradescantia stamen-hair only for the local presenting the highest exposition rate within UTM/INB which had a radiation exposition rate of 750 µR.min$^{-1}$. The Tradescantia plants exposed to the radioactive waste deposits from CDTN and IPEN presented an insufficient number of flowers for the statistical evaluation of mutagenicity.

1. INTRODUCTION

The employment of nuclear technologies has been finding resistance in its implementation related to the possible biological effects from ionizing radiation in the balance of the ecosystem and therefore over mankind. In this way, the use of a bioindicator of mutagenesis associated with the radiological environmental protection in nuclear installations could constitute in an important social-political tool.

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)[1].
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[2]. The sensitivity of Tradescantia to the genetic effects of radiation and chemical agents is widely known[3],[4]. Studies on the effects of very low radiation levels with the Trad-SHM assay involve a series of exposure situations, from absorbed radioisotopes, radiation-contaminated substrates[5],[6], and high level background radiation from monazite sand[7]. 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 radiation occurring in Brazilian radioactive waste deposits.

2. MATERIAlS AND METHODS

2.1. Exposure “in situ”

The mutagenesis evaluation was carried out in different environments, presenting gamma radiation exposure rates varying from 1.6 µR.min\(^{-1}\) to 3300 µR.min\(^{-1}\), as shown in Table 1. Groups of ten pots containing flowering Tradescantia plants (BNL clone 4430) were kept in their respective exposure sites for 24 hours. 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 µR.min\(^{-1}\). These Tradescantia stock plants maintained in the greenhouses were considered also as the reference to evaluate the spontaneous mutation frequency for clone BNL 4430. 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 exact 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.

<table>
<thead>
<tr>
<th>Exposure site (Abbreviation)</th>
<th>Radiation exposure rate (µR.min(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>Radioactive Waste Deposit (Outside Control)- CDTN</td>
<td>0.5</td>
</tr>
<tr>
<td>Greenhouse (GH)</td>
<td>1.6</td>
</tr>
<tr>
<td>Radioactive Waste Deposit - CDTN</td>
<td>5.0</td>
</tr>
<tr>
<td>Radioactive Waste Deposit (WDe) - UTM</td>
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<tr>
<td>Radioactive Waste Deposit (WDD) - UTM</td>
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</tr>
<tr>
<td>Radioactive Waste Deposit (WDE) - UTM</td>
<td>750.0</td>
</tr>
<tr>
<td>Radioactive Waste Deposit – CDTN and IPEN</td>
<td>1400.0</td>
</tr>
<tr>
<td>Radioactive Waste Deposit – CDTN and IPEN</td>
<td>3300.0</td>
</tr>
</tbody>
</table>
2.2. 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 et al., (1997)[4]. For each field experiment, twenty flowers were evaluated daily, being ten coming from exposed pots and other ten coming from control (greenhouse) pots. Mutation scoring was performed between the 7th and 13th days after exposure, in order to allow the exposed flower buds to open as mature flowers in which the stamen hairs can be observed (under X60 magnification). The number of stamen hairs per flower in each treatment group was estimated[8], 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]] \[9\] by ANOVA (p≤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≤0.05).

3. RESULTS AND DISCUSSION

The results obtained for plants exposed in different sites of UTM waste deposit showed that there was not a significant increase in the mutation rate for plants exposed in sites presenting radiation exposure rates of 200 μR.min\(^{-1}\) and 400 μR.min\(^{-1}\). It was detected a significant increase in the mutation rate for pink Tradescantia stamen-hair only for the local presenting the highest exposition rate within UTM which had a radiation exposure rate of 750 μR.min\(^{-1}\) (Figure 1).

For plants exposed within the interior of the radioactive waste deposit from CDTN it was observed a significant reduction in the number of flowers available through the analysis period (between the 7th and the 13th day of exposure) in relation to the control plants, as shown in Figure 2. Besides that, it was verified that there was a significant reduction in the number of flowers when the treatments of 5 μRh\(^{-1}\) and 3300 μRh\(^{-1}\), when compared for plants exposed within the waste deposit from CDTN. These results were not observed in control plants, exposed on the outside of the radioactive waste deposit, eliminating a possible effect of stress due to the transportation of plants between the cities of Poços de Caldas and Belo Horizonte. Differences in the luminosity inside and outside the radioactive reject deposits were discarded as a variable, since the exposition of plants was performed under similar conditions in UTM waste deposit (values of luminosity were determined but are not shown in here), and the effect of Tradescantia flowering reduction was not detected.
**Figure 1** – Radiation exposure rate and mutation events for greenhouse (GH) and waste deposit (WD). *: $p < 0.05$; full circles: radiation exposure rates; empty column: control plant; full column: exposure plant.

**Figure 2** – Exposure rate and flower number for CDTN waste deposit. *: $p < 0.05$
According to the results obtained, all Tradescantia flowers exposed within and outside the radioactive waste deposit from IP EN did not flourish, impeding the analysis of mutagenesis during the experiment. A significant reduction in the number of flowers for the experiments performed in the CDTN and IPEN indicated a presence of variables which are not controlled in the experimental environment considered and interfered in the physiology of the plants. In this way it was not possible to perform analysis of mutagenicity in the Tradescantia plants exposed to the radioactive waste deposits from CDTN and IPEN, since a reduction in the number of flowers during the experiment did not allow the evaluation of at least 10 flowers per day, per treatment, as described in the experimental protocol.

4. CONCLUSIONS

The significant reduction in the number of flowers for the experiments performed in the CDTN and IPEN indicated a presence of non controlled variables in the experimental environment considered which interfered in the physiology of the plants. Currently in our laboratory, evaluations of possible effects from the surrounding atmosphere are being performed within and outside these deposits of radioactive rejects in order to identify variables that could interfere in the flourishment of Tradescantia, as well as its spontaneous mutation rate. It is known that the spontaneous mutation rate of Tradescantia can be affected by several environmental factors such as light, temperature, nutritional status, and air impurities[10],[11]. Besides this, a recent study showed genetic effects in tétrades of Tradescantia induced by radon [12].

ACKNOWLEDGMENTS

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REFERENCES


