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Higher Plants as a Warning to Ionizing Radiation:Tradescantia

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1. Introdution

Since the 19th century, with industrial and urban development and the consequent increase in emissions from industrial activities and vehicular emissions have been observed air pollution effects on living organisms. Thus, since the beginning of the twentieth century, have been carried out several studies that include studies of the effect of pollution on plants (Chies,1983). Some features observed in these surveys are genotoxic effects, observation of falling leaves, analysis of pigments associated with photosynthetic apparatus, deposition and accumulation of chemicals in the leaves, structural and ultrastructural and effects on reproductive organs.

The need for the scientific community to understand what are the environmental agents that cause genetic damage in humans has also been since the beginning of last century and, with this concern, began to enhance the studies on the processes that cause mutations in cells. To meet these challenges, then began to be developed several bioassays Toxicogenetics, from the simplest to the most sophisticated (Ribeiro *et al.*,2003)

Each year, the amount of radioactive waste from research institutions, hospitals and nuclear power plants in Brazil and around the world is growing, and so the need to store this waste grows too. Waste storage induces questions for society concerning the amount of radiation exposure to man and the environment in the neighborhoods of waste deposit sites. In Brazil, the organ responsible for inspecting the deposits of nuclear waste is the National Commission for Nuclear Energy (Comissão Nacional de Energia Nuclear- CNEN). The stored nuclear waste can be of low or medium activity; the material is previously compacted and maintained in steel drums. They can be stored in initial, intermediary or permanent deposits. The permanent deposits are protected by thick concrete walls and may house the materials for short or midterm intervals of time. There is, in Brazil, only one permanent deposit for waste of small to medium activity where part of the material resulting from the cesium-137 accident in Goiânia (1987) is stored. The construction of other prominent deposits is under consideration. However, selection for the location of these deposits depends on a technical analysis that includes details of different levels of data and information. There is also a need to comply with the laws nr 4.118/62 and 10.308/01 respectively and the regulations NE-6.05 – Management of Radioactive Waste in Radioactive Installations (Gerência de Rejeitos Radioativos em Instalações Radiativas), NE-6.06 – Selection and Choice of Locations for Deposits of Radioactive Waste (Seleção e Escolha de

Locais para Depósitos de Rejeitos Radioativos), NN-6.09 – Criteria of Acceptance for the Deposits of Low and Medium Levels of Radioactive Waste (Critérios de Aceitação para Deposição de Rejeitos Radioativos de Baixos e Médios Níveis de Radiação) and NE-3.01- Basic Directives for Radiological Protection (Diretrizes Básicas de Proteção Radiológica).

1.1 Effect in cell

A mutation is defined as a change in DNA sequence, which leads to a heritable change in gene function. Agents that change the sequence of DNA (mutagens) are "toxic" to the gene and are then called "genotoxic" (Ribeiro *et al*.,2003).

In order to assess and prevent the presence of genotoxic agents in the environment is necessary to use sensitive indicators to detect the action of these compounds. There are plants that are considered ideal for the study of mutagenesis, both in laboratory and in situ monitoring, thus acting as bioindicators. Among the tests with the bioindicators, the micronucleus test with Tradescantia spp. (Trad-MCN) is considered the most sensitive to genotoxic agents (Ennever *et al*.1998; Ribeiro *et al*.,2003; Saldiva *et al*.,2002). The genus Tradescantia has been used experimentally since the first studies related to gene activity with the action of compounds and chemical agents. The choice is due to a series of favorable genetic characteristics, among which stands out the fact that the cells of almost all parts of the plant provides excellent material for cytogenetic studies (Ma, 1979; Grant,1998).

The influence of chemical and physical agents (especially radiation) on the frequency of mutations has been extensively studied through analysis of changes observed in Tradescantia (Carvalho,2005). Among the features that allow the detection of agents that affect the stability of the genome in Tradescantia, some were selected as indicators in bioassays for evaluating genetic toxicity: testing the pollen tube mitosis and cell color change to pink in hair stem (Trad-SH) (Rodrigues and Campos, 2006). The evaluation of genotoxicity in Tradescantia can also be made by detecting fragments or segments of DNA induced by genotoxic agents in the air, soil and water (Carvalho, 2005), was developed as a cytogenetic test that is based on the micronucleus (Trad-MCN). Thus, the Trad-MCN test is based on the formation of micronuclei, which are a result of chromosome fragmentation, visualized in the tetrad stage of stem cells grain (Ma, 1979; Rodrigues, 1999a-b). Micronuclei are counted and the frequency in which they occur indicate the toxicity of the environment.

The micronucleus test in Tradescantia (Trad-MCN) has long been used in environmental monitoring, which is due to its effectiveness in detecting chromosomal damage, the simplicity with which it is executed and its low financial cost of its methodology. All these properties qualify it as an excellent tool for this type of monitoring (Zengh and Qingqiang, 1999). Moreover, studies over the years about the genetics and development of Tradescantia offer strong support for its use as bio-indicator for genetic toxicity testing environment (Ma, 1982). Micronuclei in stem cells of pollen grains are easily observable and tests with Tradescantia have proved valuable in studies of genotoxic agents (Rodrigues *et al*.,1997;1996; Ma and Grant, 1982).

Researchers at the International Program on Plant Bioassays with the University of Illinois (USA), were the first devised and used the micronucleus assay (Trad-MCN) in tetrads to assess the genotoxicity of certain agents. The plant used was a hybrid clone 4430 (T. Bush hirsutiflora x subacaulis T. Bush), a comparative study with tests of mutations of stem hairs (Trad-SH) to assess the effects of 1,2 - dibromoethane, a substance known to mutagenic, on the chromosomes of cells in meiosis(Ma, 1979). The basis for the development of this test was the fact that the biggest problem in the quantitative assessment of chromosomal

fragmentation was the loss of chromosomes in metaphase and his image appeared blurred in the preparations of the cells in meiosis. However, if the agent is applied at the beginning of Prophase chromosome and continues for a period of recovery, acentric fragments of chromosomes become micronuclei at the tetrad stage of meiosis, easily identified on light microscopy (Carvalho, 2005).

The use of the Trad-MCN test for monitoring environmental genotoxic agents was proposed after studies involving agents pro-mutagens (benzo-α-pyrene) in polluted cities (Ma, 1981;1982:1983;1990). In 1983, TH Ma established the protocol for the bioassay Trad-MCN [17]. The Trad-MCN with the clone 4430 was also performed to study the fitogenotoxicidade substance 2,4 - and 2,6-dinitrotoluene, intermediate in the production of toluene, explosives, propellants, among others. The tests showed positive genotoxicity of these two substances, but a greater genotoxicity of 2,4-DNT, compared to 2,6-DNT (Gong *et al*., 2003).

At the University of Metz-Bridoux, France, studies Tradescantia exposed to 4430 ^{137}Cs indicated genotoxicity of low doses of gamma and beta radiation emitted by this radionuclide (Minouflet *et al*., 2005). This study inspired this work in an attempt to verify the mutational effects on the response of *Tradescantia clone* 4430 (tentative) and *Tradescantia pallida* exposure to a ^{137} Cs radiation source.

2. Materials and methods

2.1 Biotesting I

The purpose of this study was to work with Tradescantia clone 4430, but this had some limitations when applied to tests that required a longer period of exposure to the source. The high temperature, humidity and variations in light intensity, here in Rio de Janeiro (Brazil) will affect the system, which favors the emergence of parasites and insects caused inhibition of flowering. These factors limit its use to a short period of time in a biomonitoring in regions of hot climates and is highly favored regions where they observed a mild climate. Although studies to establish positive results for its application in the case of ionizing radiation (Ichikawa, 1991;1992; Villalobos-Pietrini *et al*.,1999; Suyama *et al*., 2002), there was no success in implementing this kind for long periods , as was the aim of this work. Then a new species of the same family, Comelinacea, that better fit the environmental conditions in Brazil was introduced. *Tradescantia pallida* (Rose) Hunt. variety *purpurea* Boom is a small ornamental plant from the family Comelinacea the characteristics of which make it useful for experiments involving genetic damage to cells especially those originating from exposure in a genotoxic environment.

To develop and experiment biosensors, there is a need to ensure that it meets the environmental conditions where it will be used. Hence, the choice of *T. pallida* resulted from its good adaptation to the adverse climatic conditions in the various regions around the country. This plant can be found in many streets and gardens of the cities all over the country. It is a tetraploid species that has notable resistance to both parasites and insects. It blooms all year round and needs little care and attention to grow.

T. pallida allows us to obtain response curves of biological damage *versus* dosage, based on the micronuclei methodological system developed by T.H. Ma for *Tradescantia* clone 4430 and *Vicia faba*, in 1992. This methodology has been widely used by various groups of researchers to evaluate the damaging effects of genotoxic agents and to obtain a prognosis for human health.

In this work, we evaluated the responses obtained as *Tradescantia pallida*, when exposed to a radiation source, ^{137}Cs, low activity, in order to further long-term applications. The trial established the standard for short environmental conditions of Brazil, and thereby justifies the use of plants as biosensor for environmental testing of mutagenesis at low doses of gamma radiation.

2.2 Biotesting II

Tradescantia *pallida* (Rose) Hunt. variety *purpurea* Boom is a small ornamental plant from the family Comelinacea the characteristics of which make it useful for experiments involving genetic damage to cells especially those originating from exposure in a genotoxic environment.

To develop and experiment biosensors, there is a need to ensure that it meets the environmental conditions where it will be used. Hence, the choice of *T. pallida* resulted from its good adaptation to the adverse climatic conditions in the various regions around the country. This plant can be found in many streets and gardens of the cities all over the country. It is a tetraploid species that has notable resistance to both parasites and insects. It blooms all year round and needs little care and attention to grow.

T. pallida allows us to obtain response curves of biological damage *versus* dosage, based on the micronuclei methodological system developed by T.H. Ma for *Tradescantia* clone 4430 and *Vicia faba* (Ma, 1982; 1994). This methodology has been widely used by various groups of researchers to evaluate the damaging effects of genotoxic agents and to obtain a prognosis for human health.

3. Experimental procedure

3.1 Experimental procedure I

We analyzed four groups containing vessels of Tradescantia pallida. The first group, control, and three groups, which varied the time of exposure and hence the dose absorbed by the system, including: 24 h, 36h and 48 h, exposed to the same place and the same conditions, the background rate, 0.01 mGy. Each group, 30 samples were analyzed.

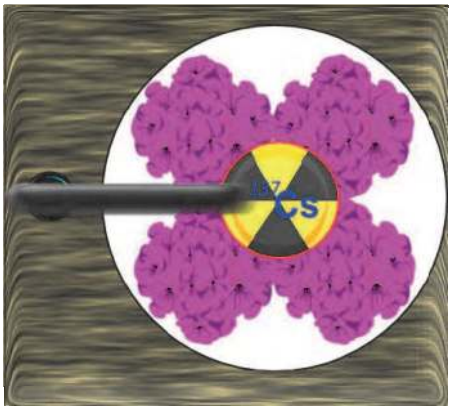
The radiometry was measured at each venue in three different distance of 50 cm, 100cm and 200cm from the source, using a MRA GP500 monitor, model 7237/03.44. Once the locations had been selected, vases containing *T. pallida* were placed, in such a way that twenty samples were exposed in each group, over an interval of 24 h, 36h and 48 h. After being exposed, the samples were placed into water, for at least six to eight hours. This is enough time for the meiosis process to continue and for the mother cells of the pollen grains to reach their tetrad phase. When the tetrad phase is reached, it is possible to see the micronucleus. In the final stage, the tetrads are fixed, in a solution of acetic acid and alcohol (1:3, v/v), in agreement with the protocol published by T.H. Ma in 1979 and cited by Saldiva *et al*., 2002.

To prepare the slides, once the inflorescences are chosen, they are mashed and treated with a drop of carmine (contrasting agent), to observe the different stages of the tetrads. The slide is squeezed slightly to visualize the tetrads under the microscope, on the same plane. The preparation is heated over a Bunsen burner at 80°C; the residuals are removed and the slides sealed with enamel. Three hundred tetrads per slide were counted, and by way of a table the number of micronuclei/slide was determined. In each selected group, 30 samples were analyzed, totaling 9000 cells per group, that were labeled as pertaining to the control

group (Co), group A, group B and group C respectively, in accordance with the levels of dosage according to the distance the source.

Figure 1 shows the experimental scheme in which the samples were exposed to ^{137}Cs source, the distance of 50cm, 100cm and 200cm. For a period of 24h, 36h and 48h.

SUPERIOR VISION (source the 50cm,100cm and 200cm of height)

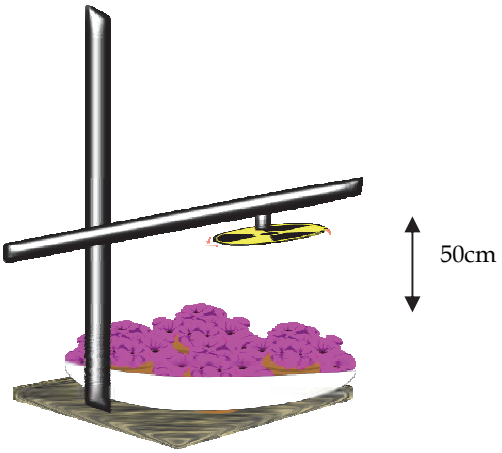


Source of Cs^{137}

Round dish with 5 pots each

Connecting rod for support of the source

LATERAL VISION



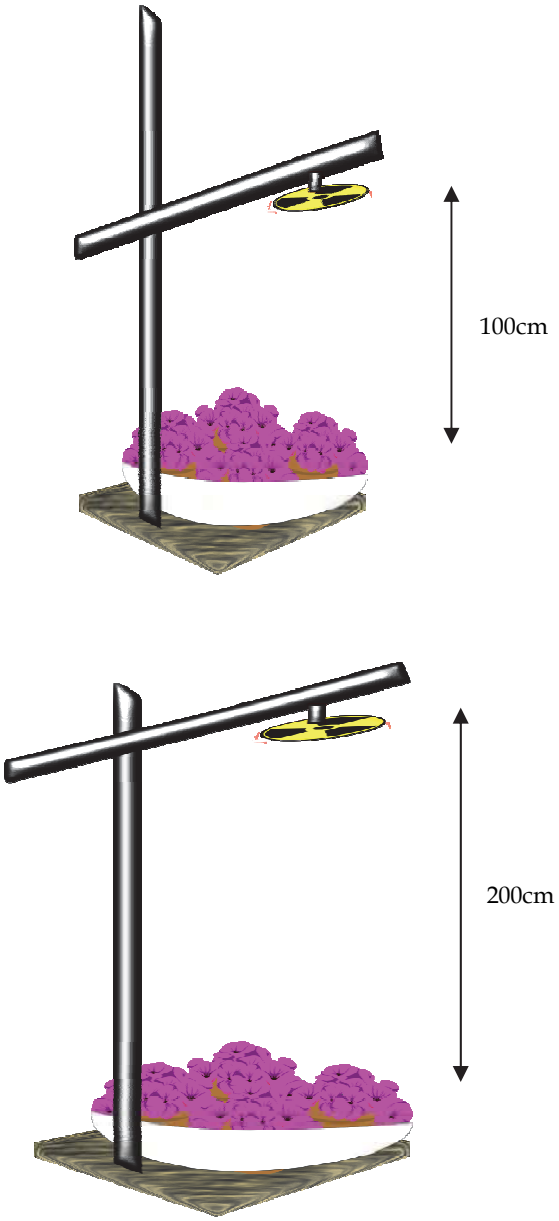


Fig. 1. Scheme of experimental exposure groups the source of ^{137}Cs, and the distances between the source and the biomarkers, 50 cm, 100cm and 200cm

3.1.1 Statistical analysis

To analyze the data the SPSS 9.0 for Windows program for statistic treatment was used (SPSS, 1999). The parameter variance was determined, in order to compare the counts in relation to the three groups from each region, to a level of significance of 0.05, the test t-Student was also used when comparing the samples (two in every two groups), in compliance with the protocol from T.H. Ma (1983).

3.2 Experimental procedure II

In this research, we have chosen four regions of merit around Brazil, because they contain nuclear waste deposits and because of their peculiar characteristics:

3.2.1 The Radioactive Waste Deposit at the Institute of Nuclear Engineering (IEN), located in the city of Rio de Janeiro: this deposit is considered of intermediate level. Some of the waste is stored for future use; others are removed to a permanent deposit.

3.2.2 The Radioactive Waste Deposit at the Nuclear Power Plant in Angra dos Reis (UNA) - located on the coastline of the state of Rio de Janeiro: it is considered to be an initial deposit; it contains richer active waste of low and medium activity. This deposit is under the custody of the Eletronuclear Corporation, and is supervised by CNEN.

3.2.3 The Radioactive Waste Deposit at the Institute for Nuclear Energy Research (IPEN) - located in the city of São Paulo: considered of intermediate level, however, it has a huge store of waste.

3.2.4 The Radioactive Waste Deposit at Abadia de Goiás (ABADIA): this is the only permanent waste deposit in Brazil, for small and medium activity.

Radiometric readings were carried out at the surroundings of each of these deposits using a MRA GP500 monitor, model 7237/03.44. At each waste deposit, three locations were selected cordance with the levels of dose rate: (1) CW (Control Waste deposit site) location where the dose rate was close to the dose rate measured at the garden where T. *pallida* was cultivated referred to as CG (Control Garden), (2) NE (nearby the entrance door of the waste deposit) and DE (along the waste deposit, but 1m distant of its entrance door).

Once the locations had been selected, vases containing T.*pallida* were placed, in such a way that 10 samples were exposed in each location, over an internal of 24h. After being exposed, the samples were placed into water, for at least 6-8hThis is time enough for the meiosis process to continue and for the mother cells of the pollen grains to reach their tetrad phase. When the tetrad phase is reached, it is possible to see the micronucleus. In the final stage, the tetrads are fixed, in a solution of acetic acid and alcohol (1:3, v/v), in agreement with the protocol published by T.H. Ma (1982).

To prepare the slides for microscope observation, chosen inflorescences are mashed and treated with a drop of carmine (contrasting agent), to observe the different stages of the tetrads. Then, the preparation is heated over a Bunsen burner at 80°C; the residuals are removed and the slides sealed with enamel. Three hundred tetrads per slide were counted, and by way of a table the number of micronuclei/slide was determined. In each radioactive waste deposit of each selected group, 100 samples were analyzed, totaling 30000 cells that were labeled as pertaining to the control negative group (Co), groups CW, NE and DE, respectively.

3.2.1 Statistical analysis

To analyze the data the SPSS 9.0 for Windows program for statistic treatment was used (SPSS, 1999). The parameter variance was determined, in order to compare the counts in relation to the three groups from each region, to a level of significance of 0.05, the test t-Student was also used when comparing the samples (two in every two groups), in compliance with the protocol from T.H. Ma (1983).

4. Results

4.1 Results and commentaries of experimental procedure I

As previously mentioned *Tradescantia* clone 4430, had some limitations when applied to tests that require a longer period of exposure to the environment. In hot climate cities such as Rio de Janeiro, was not successful in implementing this kind, and some plants died or not getting the bloom necessary.

With *Tradescantia pallida*, were analyzed 30 samples of each group and analyzed 9.000 cells per day for each of the groups, totaling 98.000 cells analyzed at the end of the experiment. Every count was compared with a control group (Co) of plants from the location of cultivation where the dosage rate measured was 0.26 μGy/h.

Table 1 presents a sample of the dosage rates resulting from each groups. The vessels with the inflorescences of *T. pallida* were placed at the site of exposure at a fixed distance of 50, 100 and 200 cm, respectively, for groups A, B and C (Table.1). The activity of the source of Cs-137 was 121KBq (2009).

Groups	Time exposure (h)	Taxa Dose	Total tetrads analyzed (cell)
*Co**	----------	0.26 μGy/h	36000
Grup A (50 cm)	24 / 36/ 48	4.15 μGy/h	36000
Grup B (100cm)	24 / 36/ 48	9.58 mGy/h	36000
Grup C (200cm)	24 / 36/ 48	2.13 mGy/h	36000

*Co is the control group from the cultivated location.

Table 1. Exposure data of Biosensor

Equations (1) and (2) show the relation between the dose and time. The dose is directly proportional to the time of exposure, so, for the control group, it is expected that there will be an increase in micronucleus frequency of cells of pollen grains of *T. pallida*. When comparing the groups A, B and C, it is expected that there is an increase in frequency of micronuclei, proportional to the increase of exposure time.

$$dX/dt = \Gamma A / d^2 \quad (1)$$

$$dD = 0.869\ dX\ (\text{in ar}) \quad (2)$$

where:

dX = the exposure rate,

dD = the dose rate,

Γ = a constant related to the specific radiation (tabulated values) of ^{137}Cs

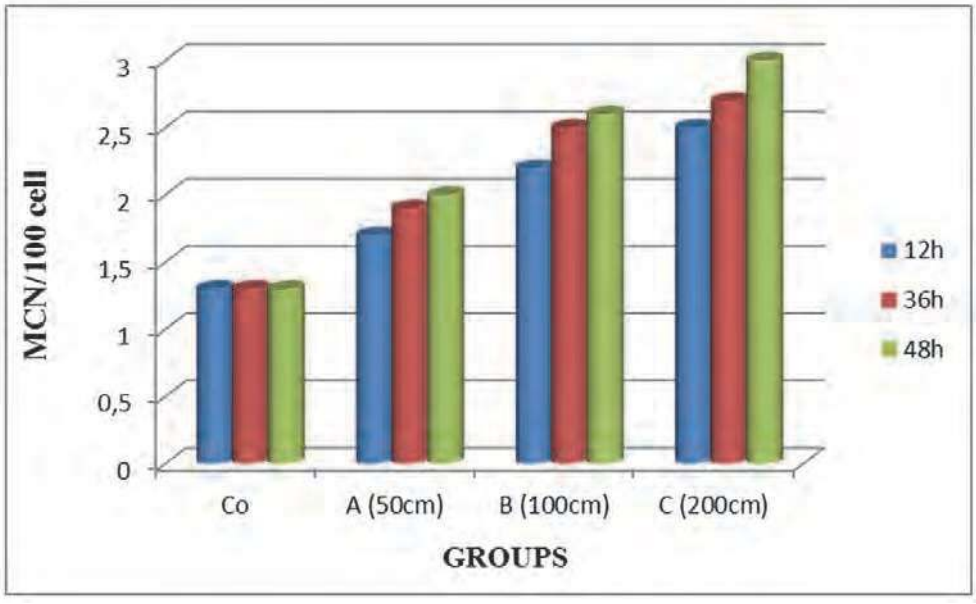
d= distance from the source to the biosensor.

Table 2 and the graph in Figure 2 represents the number of micronuclei per hundred cells analyzed, the results of the exposure of the biosensor that relates the dosage rates to the mutational effects observed in each group. The graphic in question showed a slight growth even that load dose rates.

Temps / Groups	*Micronuclei per 100 cell*		
	24 h	36h	48h
Co	1.3	1.3	1.3
A	1.7	1.9	2.0
B	2.2	2.5	2.6
C	2.5	2.7	3.0

Note: Co is the control group from the cultivated location

Table 2. Represents data obtained from exposure of the biosensor, per group analyzed in relation to distance and time of exposure.



Note: Co is the control group from the cultivated location

Fig. 2. MCN/100 cell for groups Co, A, B and C

By statistical analysis it was observed that in the control group, Co, the wrapping other groups had a significant increase when exposed to a source of ^{137}Cs. When comparing the groups A and B and A and C, we also observed a significant difference between them, both

for the exposed 24, 36 and 48 h. However, when comparing groups B and C, especially for times of 36 and 48 h, there were no significant differences between them. This suggests that there may have been an adaptation of the biosensor stress he underwent, or that this triggered a device to respond to stress. From the results, after comparison, we observed an increased frequency of micronuclei with a significant difference ($p<0.05$). Was also a linear relationship between absorbed dose and the response of *Tradescantia pallida*, although this has been a small increase in mutational frequency.
These results can be compared with those obtained in the literature. Villalobos-Pietrini *et al*. (1999) found a significant result, compared with the control containing the Tradescantia clone 4430, which exposed of a 800mGy with a source of Co-60 and found a response 17 MCN / 100. Suyama *et al.* (2002) reported research results that are consistent with the curve of the present study, positive response of the TRAD_MCN, for T.*pallida* when compared with the study had been validated previously for *Tradescantia* clone 4430 by Ma.

4.2 Results and commentaries of experimental procedure II

A total of 12000 cells were analyzed for each waste deposit. Every count was compared with the control group (CG) from the location of cultivation where the dose rate measured was 0.26μGy/h. Table 3 show the dose rates at each location for groups in each waste deposit.
Table 4 presents the number of micronuclei per hundred cells (MCN/100) analyzed for each group. The results tend to indicate higher micronuclei frequency per tetrads at the location of higher dose rates.

Location (Abbreviation)	Waste deposit sites Dose rates (μGy/h) IEN (Institute of Nuclear Engeneering)	UNA (Nuclear Power Plant at Angra dos Reis)	IPEN (Institute for Nuclear Energy Research)	ABADIA (Abadia of Goias)
Control garden (CG)	0.26	0.26	0.26	0.26
Control waste site (CW)	0.44	0.35	0.44	0.26
Nearby the entrance (NE)	21.9	25.4	30.0	2.20
Distant of the entrance (DE)	35.1	46.5	137	3.10

CG: negative control (garden where T.*pallida* was cultivated); CW: positive control (location of the waste deposit site where the dose rate was close to the dose rate measured at the garden); NE: location nearby the entrance door of the waste deposit; DE: location 1m distant of the entrance door of the waste deposit.

Table 3. Dose rates (μGy/h) at each location for each waste deposit site

First of all, to verify the influence suffered by the biosensors during transportation to the location of exposure, the frequencies MCN/100 tetrads were compared on the control points of the cultivation, CG (cultivation garden) with the frequencies at the positive control groups CW (radioactive waste deposit sites). From this comparison no significant difference was found ($p>0.05$), which leads one to conclude that the biosensor did not suffer any damages from stress during transportation.

Location (Abbreviation)	Waste deposit sites (MCN/100) tetrads IEN (Institute of Nuclear Engeneering)	UNA (Nuclear Power Plant at Angra dos Reis	IPEN (Institute for Nuclear Energy Research)	ABADIA (Abadia of Goias)
Control garden (CG)	1.26	1.26	1.26	1.26
Control waste site (CW)	1.37	1.73	1.43	1.20
Nearby the entrance (NE)	1.60	2.00	2.57	1.33
Distant of the entrance (DE)	1.93	2.27	5.90	1.47

Table 4. Number of micronuclei per hundred cells (MCN/100) for each location in the neighborhoods of deposits of radioactive waste as a function of dose rate

On comparing the NE and DE groups to the control group (CG), different responses could be observed. Thus, no significant difference was observed for the NE groups at IEN an ABADIA deposits. On the contrary, for groups NE at UNA and IPEN deposits (intermediate level) showed a significant increase ($p<0.05$) in mutational frequency. For group DE, a great a difference was found at the deposits of UNA, IEN and IPEN; only the deposit at ABADIA showed no significant increase when compared with the control (cultivation location).
Recent studies, using the species *Tradescantia pallida*, compare its sensitivity to the effects of exposure to radiation with those from genotoxic agents (Celebruska_Wasilewska, 1992; Ichikawa, 1991;1992 Gomes *et al*.,2001). The mutagenesis scale shown in figure 2 is coherent with that obtained by Suyama et al Suyama *et al*.(2002) when the studying a methodology of biomonitoring tests with *Tradescantia*, when exposed to x-rays. Villalobos-Pietrini *et al.*(1999) used this method with the biosensor when comparing the *Tradescantia* clone 4430, having registered and increase in the mutational frequency from 7 MCN/100 to 17MCN/100, when the plants were submitted to a dose of 0.8 Gy from a source of ^{60}Co. Recent studies have shown that the sensitivity of *Tradescantia* to the effects of radiation serve as a way of connecting gamma radiation dosage rates to which it was submitted to the mutational frequency from low dose rates , using the micronuclei a methodology (Santos leal et al., 2005;2008)

5. Conclusion

In the study of radiobiology, the system based on cells of Tradescantia *pallida* (Trad-MCN), is being considered. The sensitivity of the Tradescantia micronucleus has been used widely and has demonstrated the relation between radiation dose and frequency mutational obsevarded at low doses, looking through these studies contribute to the vexed question of the effects of low doses and their consequences for human health (Ma *et al*.,1994; Gomes *et al*., 2002; Santos *et al*.,2005; Santos *et al*.,2008).

This system carries the advantage of observing meaningful data in a short period of time, being able to meditate effects on human health and to prevent possible accidents, when adopted as periodical monitoring.

The biosensor T. *pallida* exhibits a noticeable quantity of cell alteration in the short time following radiation exposure. Hence, the effects caused on the environment might be anticipated, and by extension on the human being, as a result of its occupation exposition level. The use of method is recommended, therefore into the environment acclimatization, and may be used, in addition, in the prevention of radiological accidents.

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Note

1 SPSS 9.0 for Windows (SPSS Inc., Chicago, IL,1999) http://www.spss.com.br/spss.

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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 16 different countries. The book consists of 24 chapters written by 76 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

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