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EVALUATION OF GENETIC ALTERATIONS INDUCED BY RADON GAS USING THE MICRONUCLEUS TEST (*Tradescantia* sp. CLONE KU-20)

Armando L. Bruschi[1], Heliana de Azevedo[1], José F. Macacini[1],Cláudio V. Roque[1]
[1] Comissão Nacional de Energia Nuclear - CNEN
Laboratório de Poços de Caldas - LAPOC
Rodovia Poços/Andradas, km 13
37701-970 Poços de Caldas, MG
abruschi@cnen.gov.br
hazevedo@cnen.gov.br
jfmacacini@cnen.gov.br
cvroque@cnen.gov.br

ABSTRACT

The first observations over the existence of radon gas (Rn), initially known as "thorium emanation", were carried out between the end of 19th and beginning of 20th centuries. A result of uranium-238 (U^{238}) radioactive decay, radon is a tasteless, odorless and colorless gas under room temperature, with a 3.825-day half life and particle α emission in its decay, and as final product of its disintegration, the stable lead-206 isotope (Pb^{206}). Being it is the gas with the highest density known, closed and poor ventilated environments are favorable to its accumulation, with its inhalation being the highest health risk. The use of vegetal bioindicators has shown to be excellent on the monitoring of air quality and on mutagenic potential of various pollutants contained in the atmosphere. Within this context, the objective of this study was to evaluate the micronucleus test application potential utilizing the *Tradescantia* sp. clone KU-20, in order to evaluate genetic alterations induced by radon gas. Stems of *Tradescantia* sp. clone KU-20, previously immerse in Hoagland solution, were introduced in a radon detection equipment's calibration chamber (Alphaguard), containing radium salt. Afterwards, the accommodated stems were exposed to radon gas (the average radon concentration was 7.639 KBq/m^3) for 24 hours. The results demonstrated an increase on micronucleus formation (39.23 $\pm$ 2.143 MCN/100 tetrads) in stems exposed in relation to the negative control (18.00 $\pm$ 1.396 MCN/100 tetrads). The difference between the values indicated a significant increase on micronucleus frequency in the inflorescences subjected to radon gas. The presented results demonstrated the micronucleus test application potential using *Tradescantia* clone KU-20 to evaluate genetic effects induced by radon gas.

1. INTRODUCTION

Among several forms of radiation to which we are exposed, radon (Rn) and its disintegration products are considered the most important source of natural radiation to human exposure [1]. Radon, the highest density gas known, emits an α (alpha) particle in its decay and, due to this particle's high ionization, it becomes dangerous when inhaled. One of the resulting products of the radioactive decay of uranium-238 (U^{238}), radon – with a 3.825 day half-life – is the only gaseous, radiologically important element present in the air, which, together with its short half-life, chemically active daughters (Po^{218} e Po^{214}), is deposited in the lungs when inhaled [2].

In practice, ideally, mammals should be utilized in order to study radiation effects, and the results could be extrapolated to human beings. However, such studies are difficult to perform

and require a very large sample in order to obtain consistent, statistically significant results of an increase on mutations in relation to the natural frequency of their occurrence [3].

The interest in the use of superior plants on the detection of environmental agents with mutagenic potential has been growing. Experiments with different vegetables have been described, since 1913, when the *Vicia faba* (onion) was used for experiments with radiation [3].

Since the early studies of genetic activity of chemicals and physical agents, several species and clones of *Tradescantia* have been used as experimental organisms, in face of a series of favorable genetic characteristics. Presenting only six pairs of large and easily observable chromosomes, cells from almost all parts of the plant, from the tip of the root to the developing pollinic tube, provide excellent material for cytogenetic studies [4].

With the constant use of *Tradescantia* in genetic studies, some assays were selected as indicators on the evaluation of genetic toxicity, and among these, the assay based on the formation of micronuclei (Trad-MCN) resulting from the chromosomal breakage on the pollen generating meiotic cells, demonstrated great sensitivity, versatility and reliability on the generated results.

The clone KU-20 is a sterile triploid (2n=18) of unknown origin. However, it presents part of the characteristics of the *Tradescantia ohiensis* and it seems to be a hybrid between this and an unknown species [5;6]. This clone is highly mutable, especially in low temperatures, presenting a spontaneous mutation frequency that is 23 times greater than in the temperature range between 18 and 28º C [7].

The objective of the current study was to evaluate the micronucleus test application potential utilizing the *Tradescantia* sp. clone KU-20, in order to evaluate genetic alterations induced by radon gas.

2. MATERIALS AND METHODS

The method's principle is the absorption of radon and its disintegration products by anthers of young inflorescences exposed to an atmosphere controlled by radon gas, provoking chromosomal breakage and the subsequent attempt at DNA enzymatic repair, resulting in the appearance of micronuclei in the cellular cytoplasm. Stems with inflorescences from *Tradescantia* clone KU-20 (E^{Rn}) were inserted in a radon detection equipment's calibration chamber (Alphaguard), containing radium salt (7.639 kBq/m^3). The total period of testing was 72 hours divided into three stages of 24 hours (adaptation, exposure, recovery) with stems immersed in a Hoagland nutrient solution [8].

The recovery time is required for meiosis to proceed from the initial stage of prophase I, when the pollen grain generating cells are susceptible to genetic alterations, to the young tetrads stage of meiosis II, when the chromosomal breakages are visualized.

For the negative control (Ct-), a group of stems were immersed in a Hoaglang nutrient solution for a period of 72 hours in an environment with average background of 0.05 kBq/m^3.

After the exposure, the stems were fixed in a 95% ethanol solution and glacial acetic acid in a 3:1 ratio for 48 hours and then preserved under refrigeration in 70% ethanol.

According to protocol established by Ma [9], the appropriate bud was selected for opening and its anthers were macerated on a microscope slide in an aceto-carmine staining solution [10]. After placing the coverslip, the slide was heated in a hot plate (80°C) to fixate the dye and the coverslip was gently pressed in order to flatten the tetrads. The recognition and counting of micronuclei were analyzed under optical microscopy with magnification of 400X. Then, 300 tetrads were randomly counted in every slide to a total of ten slides for each treatment, amounting to a population of 3000 tetrads. The micronuclei frequency (MCN) was expressed in MCN/100 tetrads unit and it was calculated by dividing the number of micronuclei by the total number of observed tetrads.

Figure 1 shows the schematic representation of the micronuclei methodology, from the choice of buds to preparation of slides and observation in microscopy.

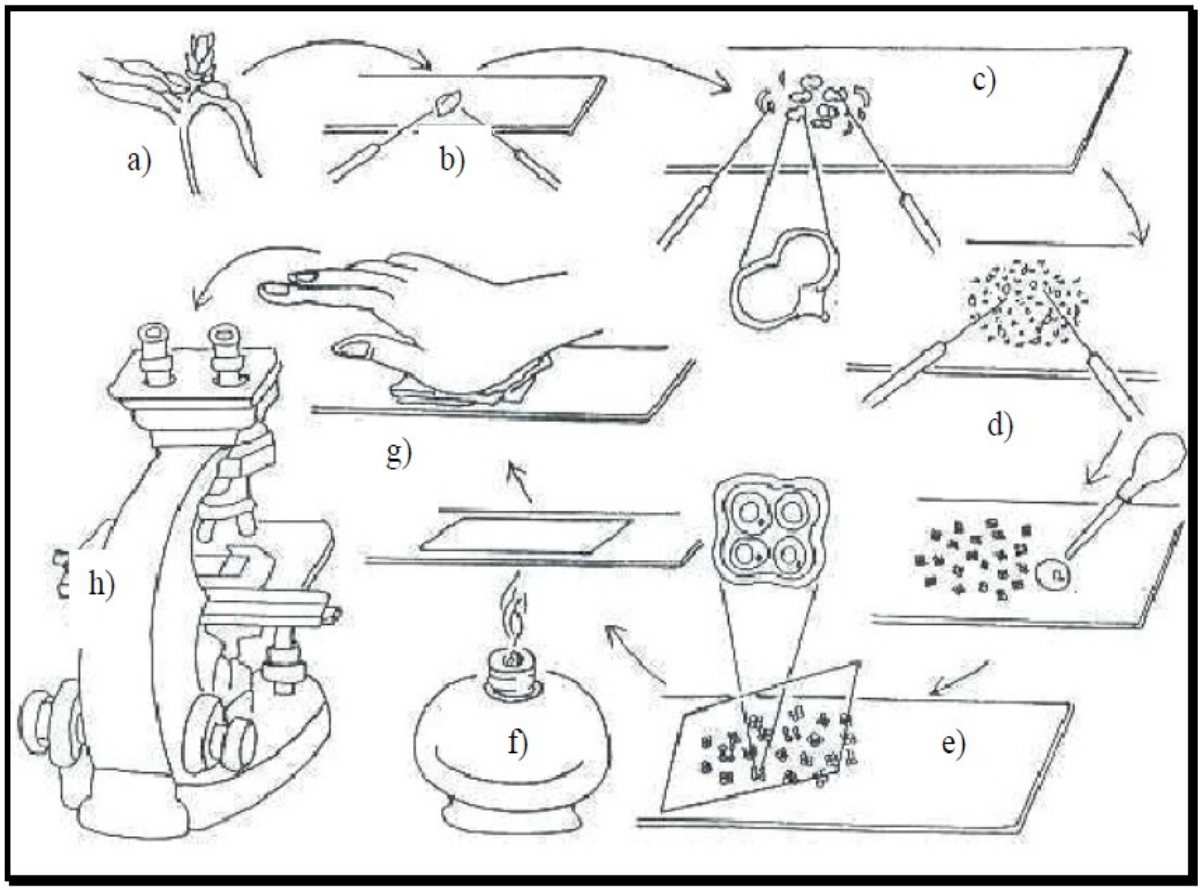

Figure 1. Schematic representation of micronuclei methodology: a) and b) selection of appropriate buds; c) bud opening and disposal of debris; d) exposure of pollen cells and staining with aceto-carmine dye; e) coverslip placement over the cells; f) slide heating for dye fixation; g) gentle pressure on the coverslip for tetrads flattening and h) recognition and counting of micronuclei under optical microscopy.

3. RESULTS AND DISCUSSION

Table 1 presents the micronuclei frequency and standard deviation of the negative control groups (Ct-) and radon exposed groups (Ex^{Rn}) (MCN/100 tetrads) and the average concentrations of Rn^{222} (kBq/m^3) obtained during the exposure period (24 hours).

Table 1. Micronuclei frequency and radon concentrations.

Treatment	Micronucleus frequency (MCN/100 tétrades ± sd*)	Average concentration of Ra^{222} (kBq/m^3)
Ct-	**18.00 ± 1.396***	**0.050**
Ex^{Rn}	**39.23 ± 2.143***	**7.639**

* sd = standard deviation significance level of 5% ($P<0.05$).

Figure 2 presents the micronuclei frequency obtained in Ct- and Ex^{Rn} groups and the standard deviation of each treatment.

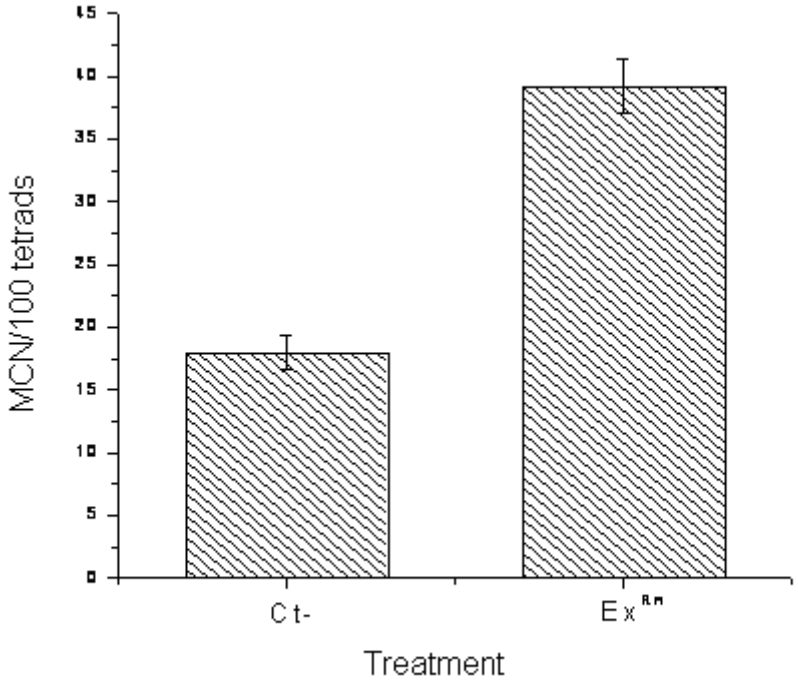

Figure 2. Micronuclei frequency between Ct- and Ex^{RN} groups

The results of the bioassays demonstrated that exposure for a period of 24 hours to radon gas and its decay products is sufficient to increase significantly the genetic alterations in inflorescences of the clone KU-20.

The inflorescences exposed to radon gas presented micronuclei frequency 2.18 times greater than the micronuclei frequency on the negative control, indicating the efficiency of the micronucleus test application and the sensitivity of this clone on the recognition of this type of environmental contamination.

The results obtained in this work are consistent with Villalobos-Pietrini et al. [11] who asserted that the bioassays, where different radon concentrations (0.85; 12.10; 36.50 and 98.16 kBq/m^3) are used, obtained a positive response concerning the genetic effects observed in the *Tradescantia* clone BNL-4430. Thus, the *Tradescantia* responses can translate into an indicative of genetic risk to human population when exposed to mutagenic agents. According to Sparrow [12], the results can be inferred in a way to predict the effects on human health and, according to results found in this work, these facts suggest that the verification of the mutagenesis occurrences on the response of the *Tradescantia* can be useful on risk prevention for humans and as a consequence, this bioindicator can be used as an alert to the ecosystem and to human populations.

The bioassays with the *Tradescantia* can establish important information about environmental mutagenesis, being one more aid instrument, indicated for environmental monitoring, on the prevention of possible accidents on the environment. The micronucleus test on *Tradescantia* cells is advantageous in terms of easy analysis and low uncertainty level [13]. And as the susceptibility to mutational damage is greater than of the human body [12], The Trad-MCN method, applied as a contamination indicator and associated to the use of biomonitoring techniques, becomes an essential ally in the Man-Technology relationship.

Although these results may not be directly extrapolated to the human population, they are indicative that if a substance does not bring harm to the bioindicator, which is generally more sensitive, it will not bring any harm to man either.

4. ACKNOWLEDGMENTS

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