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EVALUATION OF DNA BY THE MICRONUCLEUS TEST OF THE SAMPLES *Allium cepa* GERMINATED IN THE PRESENCE OF URANIUM

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ABSTRACT

Although Brazil composes the seventh position in the world ranking of uranium reserves, the ore is still little explored in Brazil, considering its vast existing arsenal. On the one hand, despite nuclear energy has brought great benefits, technological and socio-economic development, it generates controversy about environmental contamination and risks to public health. Studies on this subject indicate that areas where uranium ore concentration is high, natural environmental radiation exposure levels are already higher than in other regions. The aim of this study is to observe the simple germination of the bio-indicator (*Allium cepa*), typically used to assess potential chromosomal aberrations, suffer any adverse effect caused by natural radiation uranium. The choice of this bio-indicator is based on its potential for evaluating the mutation caused by innumerous chemical compounds. Four treatments with three replicates were designed. In each treatment, 10 seeds of onion *A. cepa* without any pesticides were packed in the Petri dish lined with germination paper and room temperature (25°C) was kept until the root reaches approximately 1cm long and 0, 5 g of the U. As a result 3000 cells were counted during the interphase, and structural changes were observed in the chromosomes, elongated nuclei and loose chromosomes of the spindle fiber. Concluind that U possibly affected the cell cycle da Allium cepa.

1. INTRODUCTION

According to the newspaper O Globo [1], Brazil occupies the seventh position in the world ranking of uranium reserves, however, due to many complaints involving the nuclear fuel and its environmental impacts and health risks [2], the public policies are not constant in encouraging the exploration of the Brazilian reserves.

Certain issues such as the origin and the effects of natural radioactivity are not common in usual conversations in our country. The absence of reliable information about this topic is the responsible for the inattention given to its impact on moments that deserve concern, directing the blame of any radiation abnormalities found to the Brazilian nuclear industry.
Studies indicate that regions with large uranium ore concentrations present their natural environmental radiation exposure levels higher than in other locations [3].

It is known that ionizing radiation can be harmful to health, taking into account the exposure time and intensity. Prolonged exposure to nuclear radiation can cause various injuries and seriously compromise the health of people, animals and the ecosystem, in addition, it can trigger genetic mutations, especially gamma radiation.

In this context, a good portion of natural radioactivity in the soil can be incorporated into foods, as with dairy and potatoes, which contains ^{40}K [4, 5]. Uranium frequent intake through water or contaminated food brings many health risks, which may lead to its accumulation in bones, kidneys and also the total volume of bone marrow cells hematopoietic stem [6].

Damages in the environment, and also, to the health of neighboring population, by the radioactive sites, without any means of control or prevention, can be occurring. There are several strategies to do environmental impact evaluation [7] and, one of them is the monitoring environmental conditions through biological indicators. The use of plants as bio-indicators has provided interesting results in researches related to air pollutants and the presence of natural or artificial radionuclides. This study aims to identify potential impacts of the uranium in natural environmental conditions, to observe if there is any effect in onion roots (*Allium cepa*) when they are exposed to uranium natural radiation. The *Allium cepa* is a great bio-indicator of water quality and air, being used in the micronucleus test to indicate possible change in the DNA, qualitative or quantitative manner. In this study we used the micronucleus assay to analyze potential damage that could cause uranium dioxide in onion plant cell, by bioassays micronucleus test. [8]

2. MATERIALS AND METHODS

2.1. Bioassays with *Allium cepa* germinated in the presence of uranium dioxide (UO_2) present in nature

The research was carried out in the cytogenetic laboratory of the State University of the Southwest of Bahia, in Vitoria da Conquista, using the micronucleus test and Allium Cepa as bioindicator.

For the micronucleus test, bioassays were performed to detect possible alterations of the genetic material of A. cepa in the presence of uranium dioxide (UO_2). And, the uranium used in the study was collected in Lagoa Real, in the municipality of Caetite, Bahia.

2.1.1. Bioassays

The bioassays were assembled in four different treatments and three replicates in each. The petri dishes were first prepared by lining them with germinating paper and sequentially led to the fume hood where they were sterilized by ultraviolet radiation for 20 minutes.

Sequentially, each treatment was performed according these strategies: treatment (T1) is the negative control treatment of the experiment, where 10 seeds of A. cepa were used and irrigated with 5 ml of mineral water; Treatment 2 (T2) is the positive control treatment, in which 10 onion seeds were subjected to 30 minutes of UV radiation, with a wavelength of 2500 Å, which is capable of promote DNA damage, and then added 5 ml of water; The treatment 3 (T3) was carried out also using 10 onion seeds, which were placed to germinate in the presence of 0.5 g of uranium dioxide and 5 ml of water added. Each treatment was maintained at room temperature until the roots reached approximately 1 cm in length and were collected at approximately 10 am and fixed in Carnoy (3 ethanol: 1 acetic acid) for 24 hours at room temperature and then stored in 70% alcohol at 4 ° C for further analysis.

2.1.2. Cooloring Methods

For cytogenetic analysis, the staining with 1% acetic orcein for the coloring of the meristems was carried out first, proceeding to squash with 45% acetic acid. Then, the Feulgen staining method, in which the tips of the Carnoy roots were removed, were washed twice in distilled water for five minutes each wash; The material was then placed in HCl (1N) and then in a water bath (60 ° C) for 11 minutes; once the time period has finished the material was washed in distilled water for five minutes, dried the roots and placed in weak with Schiff's Reagent for two hour. Latter one the root tips were washed by dripping with water for five minutes and the root tip was placed in a small petri dish containing 45% acetic acid for two to five minutes; crushed the root in the blade and covered with coverslip. Subsequent to staining, the permanent slides were prepared with Xylol, Canada Balm and Etellan (Merck) and analyzed in the optical microscope at a magnitude of 400x, using a scanning technique, evaluating the occurrence of chromosomal aberrations for detection of qualitative DNA damage, such as delayed chromosomes, chromosomal bridges and chromosomal fragments, among others, in 1000 cells in anaphase-telophase by slides, in addition to the quantification of detected micronuclei, with a total of approximately 3000 cells evaluated in each treatment .

3. RESULTS AND DISCUSSION

For the analysis of the data, approximately 3000 cells were counted in each treatment during the interphase and the presence of micronuclei and the structural alterations of the plant cells were analyzed.

The results in the quantitative analysis showed that UV was an excellent positive control [9,10,11], because it correlated with the treatments in the presence of uranium, it was inferred that uranium causes change in cells similar to the UV control. That is, as the cell is exposed to radiation, it induces higher rates of changes in cell structure.

It was not possible to identify if the plant cell incorporated the uranium, since in this study this parameter was not evaluated.

Structural alterations of the A. cepa cells were observed, chromosomal changes were observed in treatments 2, 3 and 4, and in treatment 1, a negative treatment, as expected, there was no change in cell structure. In treatment 2, which is the positive treatment, it was observed changes predicted for this type of control according to literature. Chromosome

breaks in the mitotic phase, chromosomal bridges in the anaphase, telophase with DNA bridges found [10] indicate that it is a clastogenic agent, that is, the action of agents that lead to chromosome breakage, which in the case can be inferred. The agent is the ultra violet radiation, used in the positive control. According to Evseeva et. Al. [14] the anaphase bridges formations are due to the adhesion between the chromosomes during the division [12] when they break down can lead to loss of genetic material and, according to Fiskesjö [13,16] can lead to aneuploidy and polyploidy . And for whole chromosomes released from spindle fibers at metaphase, smaller nucleus because the DNA is tightly packed, cells with nuclei in the elongate form, and large piece of DNA outside the nucleus still further bound by the DNA molecule to nuclear DNA, was not Found nothing explanatory on the subject in the researched literature.

The presence of micronuclei and interphase cells with elongated nuclei, abnormal anaphase, telophase with micronucleus, and pieces of DNA loose from the nucleus were observed in the treatment with 0.5 grams of uranium, treatment 3 (Fig. 1). Being that, in a slide did not find any abnormality in its structure, event occurred probably due to the absence of cells suffering from cell division mitosis. The observed micronuclei appear as a function of the mutagenic action, and the pieces of DNA loose from the nucleus [12,15] are probably due to the action of agents that are clastogenic. Cells with elongate nuclei, abnormal anaphases and micronucleus telophase, no explanations were found for their cause.

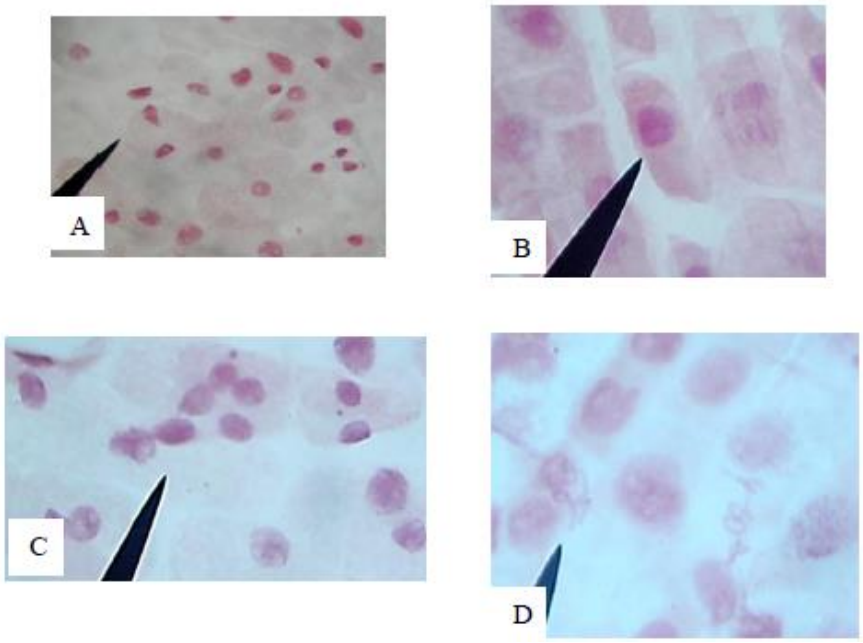

Figure 1: Structural alterations in the cells of A. strain of the treatment with 0.5 grams of uranium: (A) Micronuclei and interphase cells with elongated nuclei; (B) Abnormal anaphase; (C) Telophase with micronucleus and (D) Pieces of loose nucleus DNA;

The cells in telophase, suggesting synchronization, were found in great quantity. E, the C-metaphases according to Vidakovié-Cifrek et al [17] and Fernandes [16] arise from problems in the cytoplasmic microtubules. The C-metaphases also originate from irregular metaphases that occur by the rupture of the mitotic spindle. The presence of this alteration is important because, according to Fiskejö [13] and Fiskejö [16], its presence is evidence of complete

inactivation of the cellular mitotic spindle, because the equatorial plates are not organized, promoting an impediment or a delay in the division of the centromere.

The formation of micronuclei, structural changes of cells, such as C-metaphases, chromosome fragments and bridges, leads to indications that mutations occurred in the genetic content of the organism studied, which in the case was to the onion, due to an exposed genotoxic agent, uranium in this research.

Cellular DNA or its chromosomes can be altered by mutagenic agents, these agents lead to the promotion of toxicity to cells, inducing actions such as interference in mitotic or meiotic cell division and incorrect cell division. These mutagenic agents may increase or accelerate the onset of mutations and, with subsequent cell divisions, may lead to accumulation of mutations and lead to cellular decontrol, which can subsequently lead to the onset of cancer [13].

4. CONCLUSIONS

Adequate results were obtained regarding the use of cytogenetics, through bioassays. In that, it can be observed that the ultraviolet was a good positive control for the experiment, mainly when related to the treatment with uranium dioxide the concentration of 0.5 grams.

It can be observed when the concentration of uranium in contact with the cells was increased, in the analysis of the micronucleus test , the amount of micronuclei present in the plant cells was increased, indicating that an increase of the radiation dose in the cells of the onions .

Regarding chromosomal structural alterations, these have very much resembled the results of UV treatments and those in contact with uranium.

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