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Adaptation of the Micronucleus Technique in *Allium Cepa*, For Mutagenicity Analysis of the Jamari River Valley, Western Amazon, Brazil

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Abstract

This study aimed to make technique adjustment of micronuclei in *Allium cepa*, recommended by Fiskejö [1], by replacing methanol and Giemsa by triarilmetano the 0,1%, xantenos the 0,1% and tiazinas the 0,1%. We obtained good results with the replacement, as its efficiency in stain nucleic acids, obtaining a good visualization of cells, mitotic index, micronuclei, anaphase and telophase bridges, and this methodology is indicated in future examinations of mutagenicity in the river Vale do Jamari.

Keywords: Mutagenicity; Micronucleus; *Allium cepa*

Introduction

Toxicity is related to the detection, chemical composition and biological action of toxic substances, the toxicity of a substance can be considered as the ability to be harmful, causing serious damage to the body [2]. The route of administration, duration and frequency of exposure, are the most important factors that influence the toxicity to the body, even being found inside the cells, the genetic material is not free to suffer constant changes and mutations [3].

The change is defined as any change in deoxyribonucleic acid (DNA), can be sudden and heritable, once occurred, is maintained and transmitted to daughter molecules in the structure of genetic material, and in most cases, can develop a number of problems detrimental, but to survival of the species, the mutation is also a source of genetic variability of living beings, due to structural changes in the genetic material [4].

Mutations can be observed through the formation of micronuclei that are small bodies containing (DNA), located in the cytoplasm, manifested in cell division, with results of chromosome breaks, forming acentric fragments, or sequences of whole chromosomes that are not linked to the spindle mitotic and thus do not reach the cell poles during mitosis or meiosis [5]. A whole chromosome or acentric chromosomal fragment does not if integrate to new core; this can also constitute a small individual core, called the micronucleus [6-7], (Figure 1).

The micronucleus test detects mutagenesis in eukaryotic organisms of type clastogenicity, aneugênese and damage to the mitotic spindle. Micronuclei are identified in any cell type; the micronuclei can be evaluated for diagnosis of hematologic malignancies in epithelial cells of the mouth, urinary tract and also monitor environments by testing on animals and plants [8].

For that the micronucleus be viewed is necessary Cell division after the occurrence mutagenic, being necessary to make cell culture or using cells that are multiplying constantly, as bone marrow and roots [9].

The Allium cepa species has been used as an efficient standard organism to run genetic tests for cytoxicity, especially cytogenetic and chromosome aberration tests [1,10-20].

This study aimed to make technique adjustment of micronuclei in Allium cepa, recommended by Fiskejö [1,10], by replacing methanol and Giemsa by triarilmetano the 0,1%, xantenos the 0,1% and tiazinas the 0,1%.

Material and Methods

Study area

The Vale do Jamari (Figure 2), is located central northern in the state of Rondonia, being composed by cities: Campo Novo of Rondônia, Buritis, Monte Negro, Alto Paraíso, Cacaulândia, Cujubim, Rio Crespo, Machadinho of west and Ariquemes [21].

The hydrography of the region is formed by several rivers and streams, where they are dumped every day human feces, waste of dairy and meat, justifying an analysis of mutagenicity, since they are sources of water supply of the above municipalities.

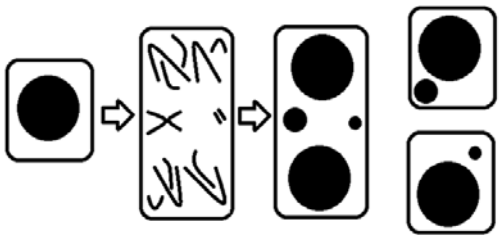

Figure 1: Formation of micronucleus in eukaryotic cells (Figure of: Dionatas Ulises de Oliveira Meneguetti).

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Experimento

The experiment had 20 units of *Allium cepa* small, uniform, of the same origin, ungerminated and healthy, being that 10 were germinated on 50 ml vials, with the bottom immersed in water collected from the water and sewerage company of the state of Rondonia (CAERD) and 10 in mineral water (Figure 3).

In about 72 hours after the start of testing, the meristems were collected at approximately 0.5 to 3.0 cm long, being washed in distilled water, followed by hydrolysis with 1N HCL for 10 minutes in water bath at 60°C, with tubes cooled in running water. After further washing of the meristems hydrolyzed were in distilled water made smears on two slides by *A. cepa* and waited for 30 minutes to dry, then they were stained with Panotico Rapido LB kit that consists of three containers: first 0.1% triarylmethane, second 0.1% xanthones and third 0.1% thiazines, slides were immersed 10 times in each container with submersion of one second in duration in the sequence described above.

Subsequently the slides were washed in deionized water with pH 7.0 and dried at room temperature. In each slide were counted micronuclei found in 1000 cells.

Results and Discussion

In cells of the meristems germinated in water CAERD was an index of 2,8 micronuclei per 1000 cells while the mineral water index was 2,3 / 1,000. After ANOVA analysis, Tukey test performed by GraphPad Prism 5.0, it was observed that there was no statistical significance between samples ($p > 0.05$).

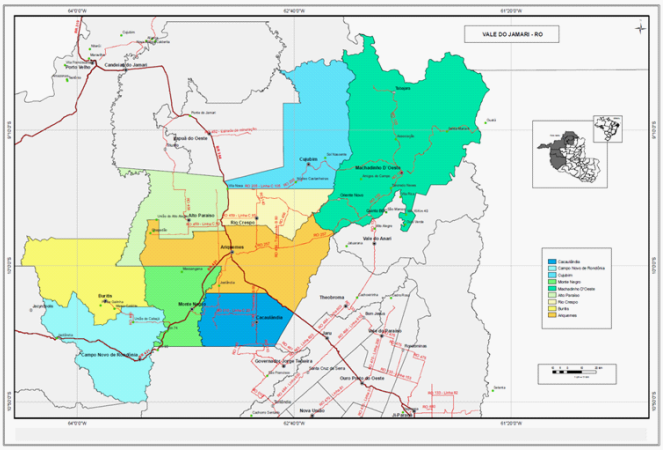

Figure 2: Map the region of Vale do Jamari, Rondônia [21].

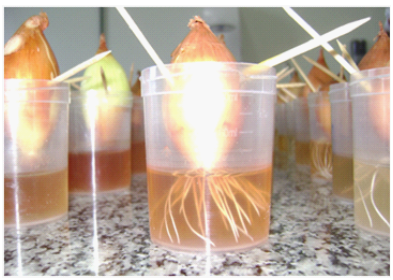

Figure 3: Germination of *A. cepa* (Figure of: Dionatas Ulises de Oliveira Meneguetti).

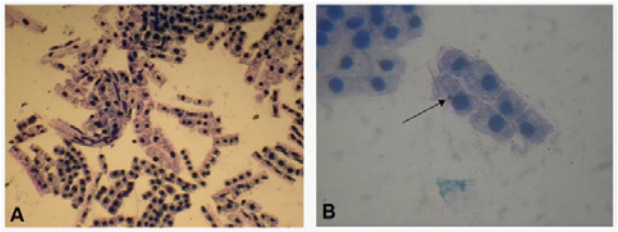

Figure 4: A – cells of *A. cepa* (ocular:10x, objective:10x), B – Micronucleus in cell of *A. cepa* (ocular:10x, objective: 40x).

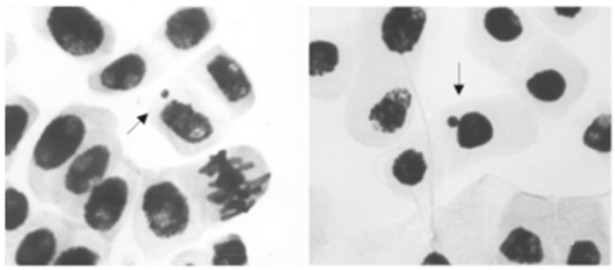

Figure 5: Micronucleus in cell of *A. cepa* [22].

The blades were clearly visible, with a nice color of the nuclei, and good visual the limits of cells, can be observed all stages of cell divisions occurred in the process of mitosis, in particular the formation of micronuclei, as can be seen in (Figure 4).

The quality of the slides prepared with this method is equivalent the methodology described by Fiskejö [1], as seen in (Figure 5), in study conducted by Matsumoto et al. [14] emphasizing that (Figure 4) was not retouched in software, can even have higher quality as indicated therein.

Conclusion

We obtained good results with the replacement of methanol and Giemsa by triarilmetano the 0,1%, xantenos the 0,1% and tiazinas the 0,1%, as its efficiency in stain nucleic acids, obtaining a good visualization of cells, mitotic index, micronuclei, bridges anaphase and telophase , and this methodology is indicated in future studies of mutagenicity in the river Vale do Jamari.

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