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METHODOLOGICAL ADAPTATION FOR GENOTOXIC AND MUTAGENIC EVALUATION USING THE *Allium cepa* TEST

ADAPTAÇÃO METODOLÓGICA PARA AVALIAÇÃO GENOTÓXICA E MUTAGÊNICA ATRAVÉS DO TESTE *Allium cepa*

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Abstract

This study aimed to adapt the genotoxic and mutagenic evaluation protocol of Guerra and Souza (2002). The tests were performed with seeds of *Allium cepa* of the cultivar Baia periforme without agrochemicals, germinated in a photoperiod of 12h, for 72h. After growth, the meristems were collected, stored and kept under refrigeration (2-8°C) in tubes containing 2% acetic orcein, for 1h, 24h, 48h and 72h. Squashes were made to make the slides and observed under optical microscope. We observed that the meristems maintained in up to 72 hours showed satisfactory results regarding the staining and detailing of chromosomes, nuclei and micronuclei. This method differs from the others in that it saves time and resources in the use of equipment and traditional fixing and hydrolyzing agents, also allowing the material to be kept in 2% acetic orcein for up to 72h.

Keywords: Ecotoxicology. Genotoxicity. Mutagenicity.

Resumo

Este trabalho teve como objetivo adaptar o protocolo de avaliação genotóxica e mutagênica de Guerra e Souza (2002). Os ensaios foram realizados com sementes de *Allium cepa* da variedade Baia periforme sem agroquímicos, postos a germinar em fotoperíodo de 12h, durante 72h. Após o desenvolvimento os meristemas, foram coletados, armazenados e conservados sob refrigeração (2-8°C) em tubos contendo orceína acética 2%, por 1h, 24h, 48h e 72h. Foram realizados esmagamentos para confecção das lâminas e observadas ao microscópio óptico em objetiva de 40X. Observou-se que os meristemas conservados em até 72h apresentaram resultados satisfatórios quanto a coloração e detalhamento de cromossomos, núcleos e micronúcleos. Esta técnica difere-se das demais pela economia de tempo e recurso na utilização de equipamentos e agentes fixadores e hidrolisantes tradicionais, possibilitando ainda a conservação do material em orceína acética 2% por até 72h.

Palavras-chave: Ecotoxicologia. Genotoxicidade. Mutagenicidade.

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The first report of the use of the species *Allium cepa* L., popularly known as "common onion", as an organism for biological tests, was from 1920 [1] and, since then, several studies related to toxicity and genotoxicity have used this species in biomonitoring experimentations, because it has few chromosomes, it is easy to obtain cells in division, it responds quickly to environmental variations through changes in cell division patterns, thus indirectly indicates the presence of cytotoxic and genotoxic substances, being the *A. cepa* test a high cost-benefit method compared to other tests that carry out the same assessments, such as the Cometa test and the SOS Chromotest [2,3,4,5,6].

There are several methods that use *A. cepa*, which can vary in the use of seeds [7] or bulbs [8], but the biggest variation found in the literature is the choice of the fixation and staining protocol [9]. Among the methods using *A. cepa* test, Feulgen Reaction [10,11], acetic orcein [2], acetic carmine [12] and Rapid Panotic Kit [13] are the most used reagents.

In order to provide an alternative method for *A. cepa* test, we aimed to develop a simplification of the protocol proposed by Guerra and Souza [14] for genotoxic and mutagenic evaluation, with regard to storage, time and resources reduction needed for its performance.

The tests were carried out with *A. cepa* seeds, of the cultivar Baia periforme, same lineage and free of agrochemicals, acquired from Isla Sementes® (Porto Alegre, RS, Brazil). The experiment was carried out with water samples from the urban region of the São Francisco stream in the city of Rio Branco, Acre, Brazil.

For each water sample, two petri dishes with germination paper were used, containing 100 seeds each. The seeds were kept in a controlled environment at 20 to 24°C, with a 12h photoperiod, being irrigated once a day with water from the collected sample.

After 72 hours, the meristems were collected with approximately 10mm to 20mm in length and placed in centrifugation microtubes containing 2% acetic orcein, this conservation in acetic orcein is not described in the protocol proposed by [14].

To assess the feasibility of the technique, prior to the preparation of the slides, the meristems were exposed to the dye for 1h, 24h, 48h and 72h and kept in a refrigerator (2-8°C).

In slides preparation, the meristems were placed on it, the apical portion was sectioned, added two drops of 2% acetic orcein, and then covered with a coverslip. Contrasting Guerra and Souza [14] protocol, fixing agent (Carnoy - Alcohol and Acetic Acid 3:1) and hydrolyser solution (HCl 1N) were not used. The slides were heated in flame, three times for 3 seconds each, after that the meristems were squashed and then visualized and photographed at 0.63x magnification using a Zeiss® Axiocam A1 optical microscope.

Figure 1 shows the result of the technique, performed with different time intervals (1h, 24h, 48h and 72h), noticing that the quality of the staining did not change, in addition, the slides that remained between 24 to 72 hours, presented the most evident chromosomes and nuclei. In a very clear way, it was able to observe chromosomal alterations and micronuclei with satisfactory quality.

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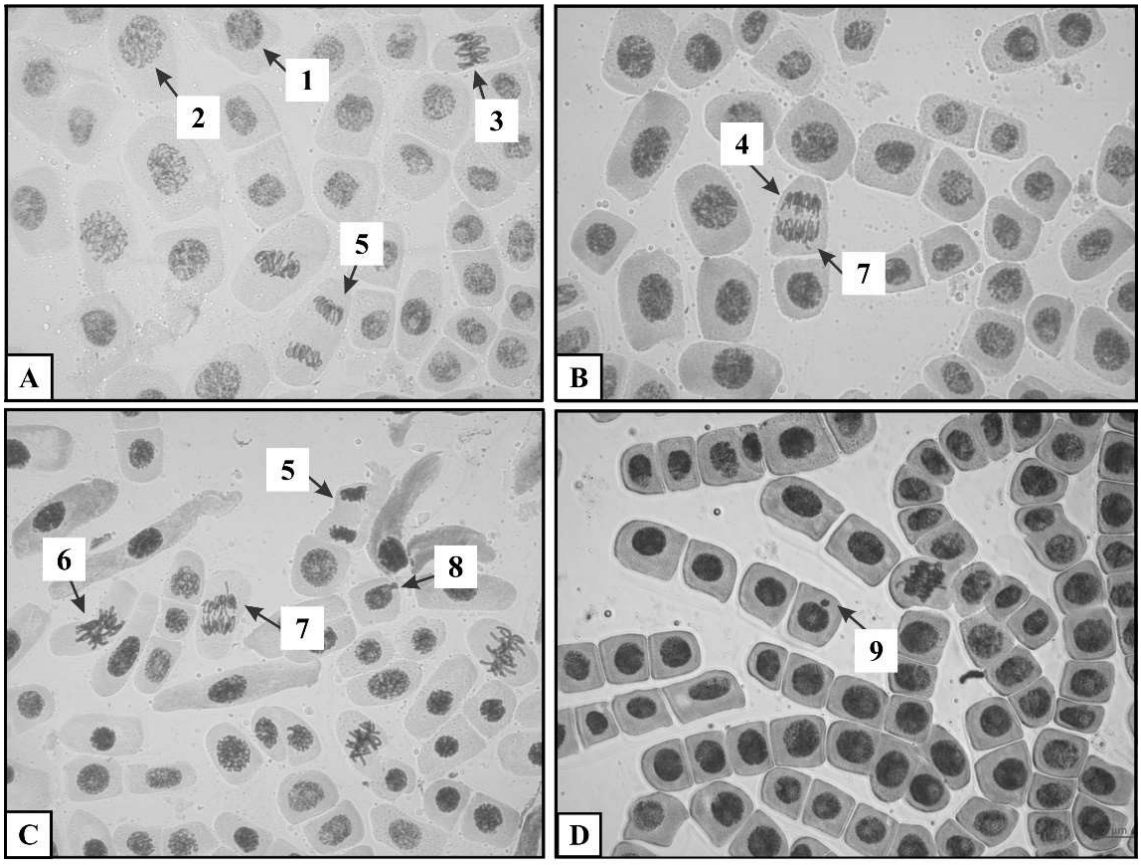

Figure 1: Images of *A. cepa* meristem slides at different maintenance periods, in 2% acetic orcein solution: 2g of orcein + 55 ml of water + heating + 45 ml of absolute acetic acid. A) 1h; B) 24h; C 48h; D) 72h. 1 - Prophase, 2 - Prometaphase, 3 - Metaphase, 4 - Anaphase, 5 - Telophase, 6 - C-Mitosis, 7 – Metaphase with chromosomal breakage, 8 - Nuclear budding, 9 - Micronuclei.

This protocol, adapted from [14], differs from it and others, due to the use of acetic acid present in the acetic orcein instead of the Carnoy solution and does not use a hydrolyzing agent (HCl 1N), thus decreasing the preparation time, in addition it dispenses the use of water-bath equipment. Acetic acid, present in acetic orcein, works as a fixing agent, since it acts in the denaturation and / or coagulation of proteins [15].

This adaptation offers the advantage of stocking and preserving meristems for up to 72 hours, without losing quality. In comparison with other works that performed *A. cepa* test, that presented photos of the cells stained with usual methods, as Feulgen reaction, acetic carmine, acetic orcein with fixator or Rapid panoptic kit [9,16,17,18], we noted that this simplification of the protocol guarantees reliability to the chromosomal morphological aspects, in order to allow the interpretation of potential environmental changes through the technique, reducing the costs for the protocol and greater flexibility in relation to the analyzes after the collection of the meristems.

The elaboration of new techniques and adaptations is important to provide new protocols, leaner and more efficient to carry out genotoxic tests with *A. cepa*. This study proposed an adaptation in contrasts to the other used methodologies, in order to facilitate and offer effectiveness for this test.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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