Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 2, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CYTOGENETIC STUDIES IN *Amaranthus retroflexus* - CHROMOSOME NUMBERS AND PHYLOGENETIC ASPECTS

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Abstract

The study of the cell and cell division is of great importance, because it makes it possible to identify the genetic material, the mechanism by which genes are transmitted from the mother cell to the daughter cells, from ascendants to descendants, how genetic recombination is carried out and how they are produced mutations at gene level, as well as restructuring at chromosome level. The study of the preparations is carried out in bright light under a microscope. For the study of the chromosomes of the Amaranthus retroflexus species (2n = 34), the fast Feulgen-Rossenbeck staining method was used, which uses a bleached basic fuchsin solution (Schiff's reagent) as a dye.

Key words: *cell, genetic, chromosomes, Amaranthus retroflexus.*

INTRODUCTION

The cell (lat. *celulla* = room) constitutes the fundamental morpho-physiological unit of living matter and has a very complex structure.
The study of the cell and cell division is of great importance, because it makes it possible to identify the genetic material, the mechanism by which genes are transmitted from the mother cell to the daughter cells, from ascendants to descendants, how genetic recombination is carried out and how they are produced mutations at gene level, as well as restructuring at chromosome level (Chadoeuf-Hannel, 1982; Schonbeck, 1980).
The variety of cells existing in the living world is extremely large, however, it was possible to establish a general scheme of the cell structure, valid for all organisms, consisting of membrane, cytoplasm and nucleus (Ahrens & Stoller, 1983; Allemann et al., 1996).
The cell membrane - represents the external covering of the cell, through which the separation and communication with the external environment is achieved (Drzewiecki, 2001, Horak et al., 2000). It has, in most plants, a skeletal and selective role, regulating the circulation of substances towards and outside the cell (Brenner et al., 2000; Horváth, 1991; Gutterman et al., 1992).
The cell membrane also has a physiological and biochemical role, through its participation in the regulation of cellular metabolism, ensuring substance exchanges from one cell to another through a system of canaliculi (plasmodesmata) (Buhler et al., 1996, Bürki et al., 2001, Ferreira et al., 1991).
The permeability of the membrane, with the help of which the exchange of substances is carried out, represents one of the most complex functions of the membrane (Eberlein et al., 1992; McLachlan et al., 1995; Pandey, 1999).
Cytoplasm represents the cellular headquarters contained between the cell membrane and the nuclear one. It consists of a relatively homogeneous semi-viscous liquid, which forms a colloidal system (Buhler et al., 1996; Bürki et al., 2001; Ferreira et al., 1991). From a chemical point of view, proteins, lipids and carbohydrates are found in the cytoplasm, and besides these, mineral salts and water are also present (Aguyo, 2000; Greizerstein, 1992; Senesac, 1985).
The nucleus - is usually in the middle of the cell and is surrounded by the nuclear envelope. It is a spherical or ovoid corpuscle present in almost all plant and animal cells and measures 1/4-1/3

of the volume of a cell (Aellen, 1959; El Aydam, 1997; McWilliams et al., 1968). Inside is the karyolymph, the nuclear chromatin made up of nucleoproteins (DNA and histone and non-histone proteins), one or more nucleoli, ribosomes (Chadoeuf-Hannel, 1981).
The electronic microscope is an optical magnifying device that uses the photon as a source of radiation, an element of the spectrum of electromagnetic waves (Chadoeuf-Hannel, 1983; Schonbeck, 1981).
Cytological examination in photon microscopy is limited by the power of separation or resolution of the apparatus, the most valuable quality of a microscope. It is believed that the maximum limit for the resolving power of the photon microscope is 0.2 microns, a value that cannot be exceeded due to the long wavelength of the photon.
By fitting special devices to the ordinary electron microscope, it is possible to achieve:
- phase contrast microscopy;
- microscopy in fluorescent light;
- microscopy on a dark background;
- microscopy in polarized light.

The usual electron microscope consists of three parts: mechanics, optics and the light source.

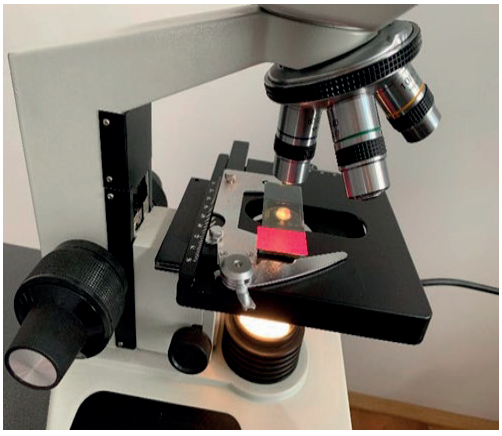


Figure 1. The Kruss type electron microscope, used in the laboratory for viewing microscopic preparations of the *Amaranthus retroflexus* species (2n = 34)

The main reason for the study is to understand the structure and functions of cells which leads to the progress in technology, such as biotechnology and genetic engineering, which have many practical applications in medicine, industry and agriculture**.**

MATERIALS AND METHODS

The study of the preparations is carried out in bright light under a microscope (Figure 1). Place the preparation on the microscope table, adjust the sharpness for the 10x objective and look for the desired structure. A dividing cell is brought to the center of the microscopic field. Raise the condenser a little and switch to higher objectives (20x, 40x, 60x, etc.). Usually, the study of mitotic and meiotic cell division is performed with the 40x and 60x objective.
In order to study the entire preparation, one starts from one part of it and walks in front of the objective from one end to the other of the slide and from bottom to top until all the cellular structures are covered.
For the study of the chromosomes of the *Amaranthus retroflexus* species (2n=34), the fast Feulgen-Rossenbeck staining method was used, which uses a bleached basic fuchsin solution (Schiff's reagent) as a dye.
In this sense, the following stages were completed:
Obtaining the biological material.
Chromosomes are visible only during the phases of cell divisions. Thus, they can be highlighted in the young meristematic tissues at the tips of roots, stems or young leaves. They can also be highlighted during meiosis, during the formation of gametes.
In order to obtain meristematic tissues from the species *Amaranthus retroflexus*, seeds of the respective species were germinated in disposable plastic pots, on moist filter paper, as well as on a support of 100% cotton disks, moist (Figures 2 and 3) (of mentioned that, in the case of the latter variant, the germination percentage was much better).

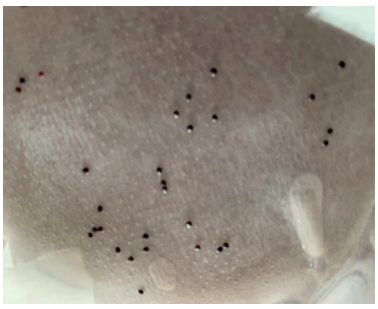


Figure 2. *Amaranthus retroflexus* seeds germinated on filter paper

Figure 3. *Amaranthus retroflexus* seeds germinated on 100% moist cotton pads

In order to keep a humid atmosphere inside the pot and to prevent the penetration of sunlight, filter paper and wet cotton pads were also placed on the cover of the germination pots, in order to promote germination. When the roots reached a length of about 8-10 mm, they were harvested with tweezers, moving to the next stage.

Fixation. It has the role of killing the cells and ensuring the coagulation of the cellular constituents, avoiding as much as possible the modification of the internal and external structure of the cells. Acetic acid 45% was used as a fixative (glacial acetic acid 45% and distilled water 55% at a temperature of 20C. The ampoules with the biological material were then kept in the refrigerator for 24 hours (Figure 4).

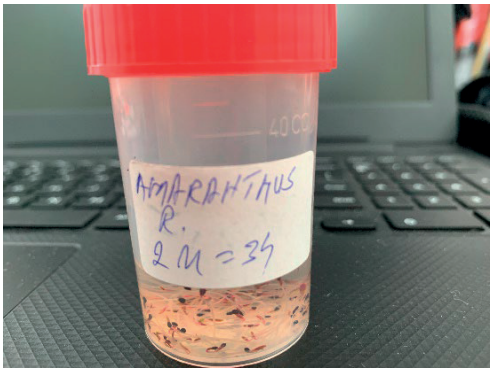


Figure 4. The biological material consisting of meristematic roots of *Amaranthus retroflexus*, in the fixation stage

Hydrolysis. It has the role of macerating the tissues by partially dissolving the intercellular pectic substances, thus facilitating the coloring process. It was performed in a solution of normal HCl at a temperature of 60°C, for 15 minutes.

Coloring. It was performed with a bleached basic fuchsin solution (Schiff's reagent), with selective staining at the chromosomal chromatin level.

The temporary preparations were analyzed under the optical microscope during the next 2-3 hours, until complete staining of the cytoplasm (Figure5).

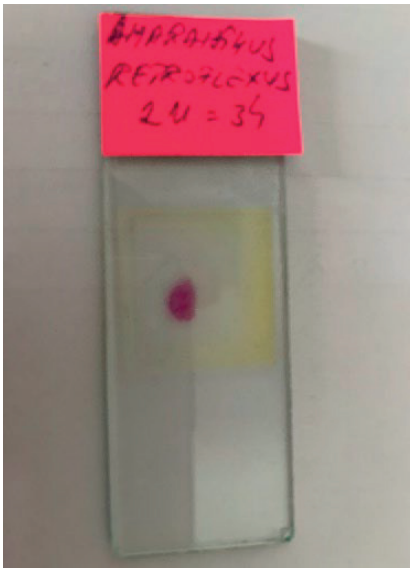
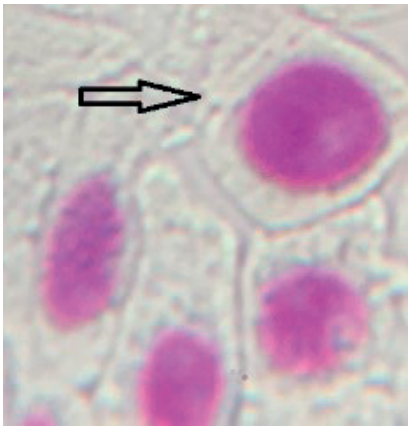
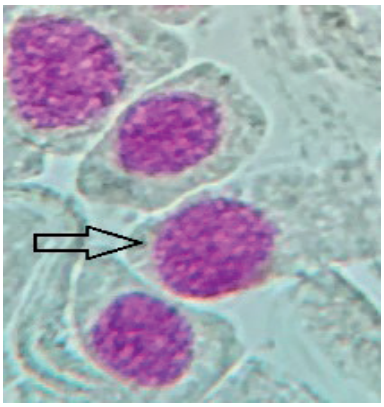


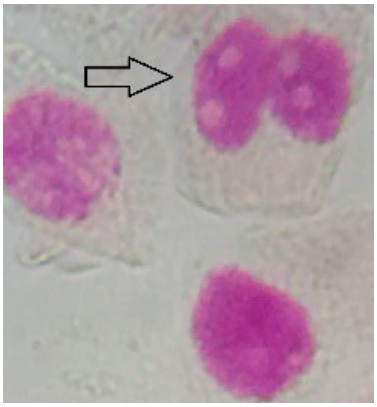
Figure 5. Temporary microscopic preparation of *Amaranthus retroflexus* prepared for viewing



a



b



c

Figure 6. The normal appearance of mitosis in the species *Amaranthus retroflexus* (60x), highlighted in some stages: a- interphase; b-prophase; c-telophase

RESULTS AND DISCUSSIONS

The preparation of meristematic roots from *Amaranthus retroflexus* using the described method yielded suitable material for the study of mitotic cell division. The Feulgen-Rossenbeck staining method successfully highlighted the chromosomes in the cells, enabling their observation under the microscope. The use of a 40x or 60x objective was found to be suitable for studying mitotic and meiotic cell division in this species (Figure 6).

The obtained results demonstrate the effectiveness of the described method for preparing and staining meristematic tissues from *Amaranthus retroflexus*. The use of acetic acid as a fixative and HCl for hydrolysis proved to be appropriate for preserving the cellular structure and facilitating the staining process. The selective staining of chromosomal chromatin with Schiff's reagent allowed for clear observation of the chromosomes under the microscope.

The successful observation of mitotic and meiotic cell division in *Amaranthus retroflexus* provides valuable information for understanding the cellular mechanisms involved in the growth and reproduction of this plant species. Further studies using this method could explore variations in the chromosome number or structural abnormalities in different individuals or populations of *Amaranthus retroflexus*, contributing to a better understanding of the genetics of this species.

CONCLUSIONS

The study of cells and cell division is indeed crucial for understanding many biological processes. Through this study, we can identify genetic material and mechanisms of inheritance, such as mitosis and meiosis, and how they contribute to genetic recombination and mutations.

The *Amaranthus retroflexus* species, with a chromosome number of 2n = 34, can be studied using various methods, including staining techniques. The Feulgen-Rossenbeck staining method is a widely used technique that involves staining DNA with Schiff's reagent, a bleached basic fuchsin solution. This staining technique allows for the visualization of the chromosomal material within the cell, providing insights into the structure and behavior of chromosomes during cell division.

Overall, the study of cells and cell division, as well as the use of staining techniques like the Feulgen-Rossenbeck method, can help researchers better understand the genetic processes that underlie many biological phenomena.

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