AgroScience Today

Popular Article

e-ISSN: 2583-0147

Volume 5 Issue 8
Page: 0931 – 0935

Observation of Pollen Mother Cells (PMC) and Microspores at Meiotic Cell Division in the Inflorescence of Rice (*Oryza sativa* L.)

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Published on: August 31, 2024

ABSTRACT

As a part of the course, PBG 112 – Principles of Genetics and Cytogenetics (2+1), by the students of B.Sc., (Hons.) Horticulture (2023 Batch) at Adhiparasakthi Horticultural College, the study was undertaken on the behaviour of chromosomes at meiosis. In this

context, we have observed the Pollen Mother Cell (PMC) and Microspores. For which, the inflorescence of rice was used as an experimental material for studying the behavior of chromosomes at meiotic cell division. Both the cells were observed at 40 X magnification.

INTRODUCTION

A fundamental aspect of the life cycle of every sexually reproducing eukaryote is meiosis. The process of cell division in germ cells produces male and female gametes, or pollen grains and eggs, respectively. Meiosis is typically investigated in relation to plant species' squash preparation of anthers. The process of dividing a cell nucleus into four daughter nuclei, each with half as many chromosomes as the parent nucleus, is known as meiosis. Since it lowers the number of chromosomes in the cell from the diploid number (2n) to the haploid number (n), it is also known as reduction division. Similar to mitosis, it also involves DNA replication in the parent cell during interphase. However, Meiosis I (reduction division) and Meiosis II (equational division) are the two cycles of nuclear and cytoplasmic division that come after. Thus, four haploid cells are produced from a single diploid cell.

MEIOTIC PHASES

MEIOTIC I

Prophase I: In meiotic division, it is the longest phase. It is divided into five substages: Leptotene, Zygotene, Pachytene, Diplotene, and Diakinesis.

Leptotene: Chromosomal fibers condense and coil gradually. Chromosomes are haphazardly dispersed throughout the nucleus.

Zygotene: The chromosomes get thicker and shorter. When a synaptonemal complex forms, homologous chromosomes are positioned next to one another and start pairing with one another. Synapsis is the term for the pairing of homologous chromosomes. This stage of DNA synthesis accounts for 0.3% of the total.

Pachytene: Crossing over is the exchange of chromosomal segments between non-sister chromatids of the homologous chromosome pair. Chiasmata (plural: chiasmata) are the sites where non-sister chromatids exchange chromosomal segments during crossing over. Chiasmata connect the homologous chromosomes to one another. The two chromatids on each homologous chromosome in the pair are referred to as bivalents or tetrads.

Diplotene: From the centromere to the end of the chromosome, homologous chromosomes repel one another and separate. We refer to this procedure as chiasma terminalization. Only at the chiasmata of homologous chromosomes are they held together. Further condensing causes chromosomes to grow thicker and shorter. The nucleolus gets smaller.

Diakinesis: Following the full terminalization of chiasmata, this stage starts. Condensed and dispersed chromosomes can be found in every cell. The nuclear membrane and nucleolus vanish. At the conclusion of this phase, the spindle fibers start to form.

Metaphase I: Spindle fibers bind the homologous chromosomes, which are located on either side of the equatorial plate. Each chromosome's centromeres point in the direction of the poles opposite the equator as a result of spindle fiber contraction.
Anaphase I: The centromeres maintain the sister chromatids' unity during the initial stages of anaphase rather than splitting. Chromosome segregation is the process by which the individual chromosomes of a homologous chromosome pair split off and migrate to opposing poles. As a result, the number of chromosomes decreases from the diploid (2n) to the haploid (n) stage.

Telophase I: Chromosomes relax and uncoil, causing them to regroup. Nuclear membrane and nucleolus reappear. There are two haploid daughter nuclei produced. In Telophase I, a diploid mother cell undergoes cytokinesis to produce two haploid (n) daughter cells.

Meiosis II

The second meiotic division is similar to mitotic division. However meiosis II differs from Mitosis in the following ways.
(i) The interphase, or interkinesis, is brief before Meiosis II. Given that each chromosome already has two chromatids, it lacks the "S" period.
(ii) Each chromosome contains two chromatids that are not sisters throughout. Put differently, recombination has resulted in alternating segments of non-sister chromatids in certain chromatids.
(iii) Normal mitosis deals with the number of diploid chromosomes, while meiosis II deals with the number of haploid chromosomes.

Meiosis II has four stages. They are Prophase II, Metaphase II, Anaphase II and Telophase II
The spindle apparatus reappear in prophase II. The centromeres are aligned on the equatorial plane by metaphase II. Each chromosome's centromere divides during anaphase II, enabling sister chromatids to split apart. Two cells undergo cytokinesis and then divide into four meiotic products during telophase II.

PREPARATION OF FIXATIVES

The word "killing" in cytology refers to the abrupt end of a tissue's individual cells' life cycle. The main agent that kills is alcohol. Cells or tissues are abruptly killed during the killing and fixing process, leaving the chemical makeup and morphological structure of the cells largely unchanged. Despite being separate processes, both are typically achieved using a single fluid, which is typically a blend of compatible chemical reagents.

Carnoy's Fluid I (Glacial Acetic Acid – 1 Part; Absolute Ethanol – 3 Part). It works well for mending all materials made of plants, animals, and people. Fixation times range from 15 minutes to 24 hours. The specimen should be cleaned in 95% ethanol with two or three times after fixation in order to get rid of any acetic acid that might have interfered with the staining process. The components of Carnoy's fluid I and Farmer's solution are identical.

PREPARATION OF STAINS

Staining is the process of coloring the cells using specific inorganic or organic dyes. The choice of dye or stain for a given material is determined by the substance's chemical makeup, the

fixative's pH level, and the stain's chemical reactivity with the material. The majority of cytological stains are dye solutions made of aromatic organic compounds with chromophoric and auxochromic groups as their active chemical groups. The auxochromic group allows the dye to stick to the tissue or material, while the chromophoric group gives the dye its color.

Acetocarmine: Carmine stain is dissolved in acetic acid to create acetocarmine stain. Gently boil 45 milliliters of glacial acetic acid and 55 milliliters of distilled water to obtain 45 percent acetic acid. After bringing 45% acetic acid to a boil, 1g of carmine powder is added and the mixture is left to boil for a few minutes. The solution is taken off the burner and allowed to cool to room temperature after it has boiled. After that, the mixture is filtered through Whatman No. 1 filter paper and placed in a clear bottle. The filtrate has a color of light red. For deep staining and preservation, ferric acetate and chloride may be added as needed.

Figure 1: Microspores

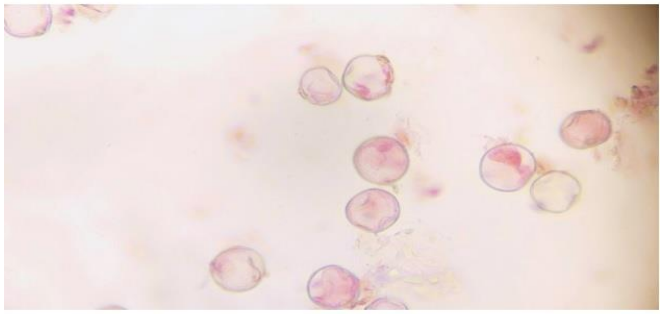

Figure 2: Pollen Mother Cells (PMC)

PROTOCOL

1. Young flower buds of rice are fixed in Carnoys Fluid I solution between 8 and 11 a.m. After 5 hours the flower buds are transferred to 75% ethanol for preservation.
2. The preserved buds are washed with distilled water.
3. Anthers are removed from the flower buds by viewing under a dissection microscope.
4. The anthers are cut transversely and the microsporocytes are squeezed out by pressing the anther with a scalpel.
5. Anther walls and debris are removed using a clean needle.
6. A drop of aceto-carmine is added to the preparation.
7. A cover slip is placed on the squash without any air bubble.
8. Excess stain is removed using blotting paper.
9. The slide is heated over a spirit lamp flame for a few seconds to have good spread of cells.
10. The slides are observed under a microscope (10x) for meiotic stages.

EXPERIMENT FINDINGS AND CONCLUSION

As per the above protocol we have used the Rice inflorescence as an experimental material for the study of meiotic phases. In that, we have observed the Pollen Mother Cell (PMC), and Microspores at 40 X magnification. The image have been captured and given in Figures 1 and 2.