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Research Article

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Chromosomes and the Mitotic Cell Cycle Phase in Onion Roots

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Abstract

Onions (Allium cepa) are commonly employed in vitro to evaluate the effects of chemical agents. Onion chromosomes have long been prepared using the acetic orcein staining technique. However, aceto-orcein stain is corrosive and deadly since it contains oxidizing agents like organic peroxides, toxic compounds like cyanides, acid corrosives, and radioactive materials. This study examined the mitotic activity in the roots of onion plants to ascertain the impact of the aceto orcein dye's soaking period on actively dividing root cells. Using the aceto-orcein squash procedure, a number of root tips from each bulb were collected, soaked for 1, 3, and 24 hours in aceto-orcein stain, and then processed further for cytological analyses. The purpose of the research was to examine the impact of chromosomal aberration and mitotic index on onion roots. It will reveal how many cells are actually going through mitosis. The tip of onion root cells was observed going through mitosis while actively undergoing mitotic division using the usual methods. There are various phases involved in mitotic divisions, including prophase, metaphase, anaphase, telophase, and interphase. At each soaking time, the experiment was run six times. The outcome shown that different soaking time durations had a substantial impact on the decline in mitotic index value.

Keywords: Cell Biology, Chromosomes, Cell Division Metaphase & Allium Root.

1. Introduction

Usually, developing roots of onions (*Allium cepa*) are exposed to chemical compounds in vitro to evaluate the effects of those substances [1]. Prevost and Dumas (1824) made the discovery of the cell cycle while researching the frog zygote's cleavage [2]. To divide and create new cells, a cell must go through a number of steps. The fundamental structural and functional unit of all living things is the cell [3]. It is the fundamental component that, when united with other cells of a similar type, creates tissues and organs [4]. The cytoplasm, cytoskeleton, endoplasmic reticulum (ER), Golgi apparatus, lysosomes and peroxisomes, mitochondria, nucleus, plasma membrane, and ribosomes are among the organelles that make up a cell. The cell goes through a sequence of processes that cause the cell to divide and produce two copies of its DNA. The cell cycle is referred to as this. Let's examine the activities that occur during a cell cycle while the cell divides [5]. The term "cell cycle" describes the sequence of activities that occur within a cell that lead to the duplication of DNA and the division of the cytoplasm and organelles to form two daughter cells. Proper cell division at the ends of the stem or roots is one factor that influences plant growth. Furthermore, it is well recognized that precise cytoskeleton alignment during cell divisions is essential for mitosis to continue properly [7]. Root growth also requires cell divisions because only freshly produced cells are responsible for an organ's elongation by enlarging in size mainly in line with long axis of the root [8,9]. Every creatures' chromosomes or individual DNA molecules have their own genetic code. Human cells have 46 chromosomes, compared to the eight found in an onion cell [10]. In the cell cycle, the newly generated DNA is divided during the process of mitosis, which also results in the formation of two new cells with the same number and type of chromosomes as the parent nucleus. Asexual reproduction is a process called mitosis that is seen in unicellular organisms [11]. The fundamental mechanism of reproduction at the cellular level is cell division. With the exception of germ cells, which have a ploidy or chromosome count that is halved during division, most eukaryotic cells divide in a way that keeps the number of chromosomes constant [12]. When a cell goes through mitosis, its nucleus splits into two daughter nuclei, each of which contains an equal amount of genetic material. Following the separation of the nucleus, it follows the G2 phase and is followed by cytoplasmic division. Mitosis is necessary for cell proliferation and for replacing damaged or worn-out cells. The DNA may be altered by abnormalities during mitosis, leading to a genetic disease. Cell division is necessary for organisms to develop, expand, maintain tissues, heal, and create new cells. Meiosis and mitosis are the two kinds of cell division. The Eukaryotic cells' nuclei split in half during mitosis, dividing the parent cells into two daughter cells as a result. Hence, there are two main steps to every cell division: - Cytoplasm division during cytokinesis, and nucleus division during karyokinesis

2. Interphase

A cell spends a portion of its growth cycle in interphase before initiating mitosis. When in the interphase, it goes through the following phases:

- **G1 Phase:** This is the time frame prior to DNA synthesis. Between mitosis and the start of the cell's genetic material replicating, a cell is in the G1 phase. The cell is metabolically active and continues to expand at this stage without copying its DNA (Maclean & Hall 1987).
- **S Phase:** It is during this stage when DNA synthesis occurs. If the cell's starting DNA content is represented as 2N, replication results in 4N. Nonetheless, the number of chromosomes does not change; for example, if 2n chromosomes were present during the G1 phase, they would still be present by the conclusion of the S phase. In cells that contain centrioles, the centriole also splits into two centriole pairs.
- **G2 Phase:** Between the conclusion of DNA synthesis and the start of the prophase is the G2 Phase. The cell prepares to enter the mitotic phase during this phase by producing the RNA, proteins, and other macromolecules needed for the expansion of cell organelles, spindle formation, and cell development.

3. M phase

The cell undergoes a full reconfiguration during the mitotic phase, also known as the equational division phase, to produce a progeny with the same number of chromosomes as the parent cell. The procedure of cytokinesis, which is preceded by mitotic nuclear division, divides the other organelles in an equal number as well. The four stages that overlap during the mitotic phase are as follows:

3.1 Stages of Mitosis: The majority of the cell's life is spent in the interphase, which occurs just before prophase and is when mitosis begins (the DNA is copied). The prophase is technically the initial stage of this process because the nucleus is divided during the actual process;

i. Prophase

Prophase, which immediately follows the S and G2 phases of the cycle, is characterized by the condensing of genetic material into compact mitotic chromosomes made up of two chromatids connected at the centromere. When the prophase is complete, the mitotic spindle, microtubules, and proteinaceous cytoplasm components that aid in the process are beginning to assemble. Disintegration of the nuclear envelope begins. At this point, the chromosomes begin to coil and thicken; also, the nucleolus and nuclear membrane begin to contract and eventually vanish, signaling the beginning of the mitotic phase [13].

ii. Prometaphase

The nuclear envelop ruptures in the prometaphase. The centromere to the chromosome microtubules is now free to expand. The kinetochores are attached to by the microtubules, allowing the cell to transport the chromosome. When a group of fibers group together to form the spindle fibers, the stage has reached its final condition.

3. 2 Metaphase- At this point, the chromosomes begin to be pulled by the microtubules equally, and they end up at the center of the cell. The metaphase plate refers to this area. As a result, every cell receives a fully functional genome. The chromosomes thicken during this stage. Each chromosome has two different chromatids, each is attached to the spindle fibers on its side, and the chromosomes line up along the center of the cell.

i. Anaphase

Anaphase begins with the separation of the sister chromatids. These sister chromatids develop into the daughter nuclei's chromosomes. The fibers connected to each chromosome's kinetochores then tug the chromosomes in that direction. Each chromosome's centromere leads at the edge while the arms follow. Every chromatid pair separates from the centromere and moves towards the opposite end of the cell through the spindle fiber. The cell membrane at the center is currently being compressed [14].

ii. Telophase

As the nuclear membrane begins to form surrounding it, the chromosomes that are clustered at the two poles start to coalesce into an undifferentiated mass. After disappearing after prophase, the nucleolus, Golgi bodies, and ER complex begin to return. Cytokinesis, or the division of the cytoplasm into two daughter cells, occurs after telophase. It so signifies the end of cell division. The other end of the cell has been colonized by chromatids. the spindles' absence, the uncoiling of daughter chromosomes results in the formation of chromatin fibers. 1. The reformation of the nucleolus and nuclear membrane, which results in the emergence of two daughter nuclei at the opposing ends, Cytokinesis or cell splitting could potentially take place at this stage.

The following phase of the cell cycle after mitosis is known as interphase, and it occurs between two successive cell divisions. Most of a cell's existence is spent in the interphase. The G1, S, and G2 stages are included.

3.3 Features of Mitosis

Because the number of chromosomes in the parent and daughter cells are the same, this process is known as equational cell division. Two daughter cells are produced from the parent cell during each cycle of cell division. Mitosis causes the vegetative portions of plants, such as the root tip and stem tip, to grow. This procedure does not include segregation or combining. Certain cells, such as the heart cells in adult animals, do not divide, while others only divide to replace cells that have been lost or injured due to cell death. After leaving the G1 phase, those cells that do not divide further enter the inactive G0 phase, also known as the quiescent phase. While still metabolically active, these cells do not divide unless necessary.

3.4 Functions of Mitosis

The following are the two crucial roles that mitosis plays: An organism's development is aided by mitosis. Asexual reproduction occurs during mitosis in single-celled organisms, and it aids in the repair of damaged tissues. When the surrounding cells cannot perceive the injured cells, mitosis starts in those cells. The cells that are dividing eventually overlap and cover the harmed cells.

3.5 Significance of Mitosis

The zygote's transformation into an adult is the result of mitosis. Each daughter cell receives an equal number of chromosomes during this process. It is in charge of a person's personal development and growth. All of an organism's bodily cells continue to contain the same number of chromosomes thanks to this. Asexual reproduction, vegetative plant reproduction, and the repair and regeneration of damaged tissues are all dependent on mitosis. As no recombination or crossing over occurs, mitosis aids in maintaining the integrity of the genome. It is also important for the repair and regeneration of old and damaged cells in animals, such as the gut epithelium and blood cells.

3.6 Theory of the Experiment

Why are onion root tips being used in this experiment to show mitosis? The most desirable and appropriate raw material to investigate the different stages of mitosis is provided by the meristematic cells that are located in the tip of the roots. A monocot plant is the onion. Large, readily discernible chromosomes are found in monocotyledonous plants. Hence, their root tips are employed. Depending on the species and the type of cell, mitosis can take a variety of times. Is there anything that can affect mitosis? Indeed, mitosis and the cell cycle are influenced by a variety of factors, including time and temperature.

4. Material & Methods

4.1 Required

Compound microscope, Acetocarmine stain, Water, Burner, Hydrochloric acid, N/10, Filter paper, Coverslip, Aceto alcohol (Glacial acetic acid and ethanol in the ratio of 1:3), Glass Slide, Onion root peel, Forceps, Blade, Watch glass, Dropper, Needle &Vial.

4.2 Procedure of the Experiment

Put a tile with an onion on it. With a sharp blade, carefully cut the onion's dry roots, Put the bulbs in a water-filled beaker to encourage the growth of the root tips. The new roots may take four to six days to develop and show. Cut the newly formed roots to around 3 cm, then place them in a watch glass. Move it to a vial containing freshly made aceto-alcohol, which is a mixture of glacial acetic acid and ethanol in a ratio of 1:3. Let the root tips a full day to rest within the vial. Pick one root with forceps and place it on a fresh glass slide. Let one drop of N/10 HCl to contact the tip of the root with the aid of a dropper. Add around 2 to 3 drops of the acetocarmine stain as well. Lightly warm it on the hob so as to prevent the stain from drying out, using filter paper, a substantial Pick one root with forceps and place it on a fresh glass slide. Let one drop of N/10 HCl to contact the tip of the root with the aid of a dropper. Add around 2 to 3 drops of the acetocarmine stain as well. Just warm it on the burner in a manner that prevents the stain from drying, using filter paper, a substantial stain might be carefully treated. With the use of a blade, you can trim the root tip's more stained portion. the more stained portion should be kept and the less stained portion discarded. Include a water drop therein, It can have a coverslip fixed on it with the aid of a needle, In order to correctly crush and straighten up the meristematic tissue of the root tip that is present under the coverslip as a fine cell layer, lightly touch the coverslip with the un-sharpened end of a needle, The slide for the onion root tip cells has been created and is ready to be inspected for various mitotic stages. Place the slide beneath the compound microscope to see and learn about mitosis Focus as desired to obtain a distinct and clear image.

5. Results

After 24h the 3 cm fixed onion roots were squashed and stain with hematoxylins and eosin stains. Images are taken by Metzger binocular microscope (Fig. 01, Fig. 02 & Table 01).

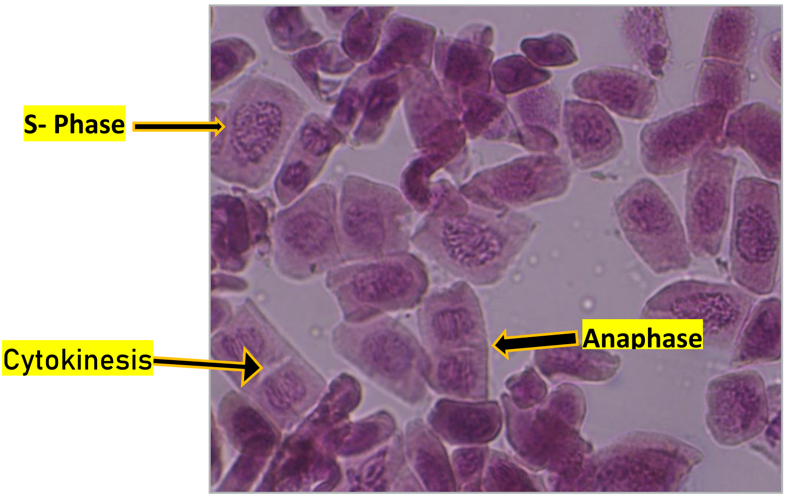

Figure 1: Microphotograph Showing the S-Phase, Anaphase & Cytokinesis 40X

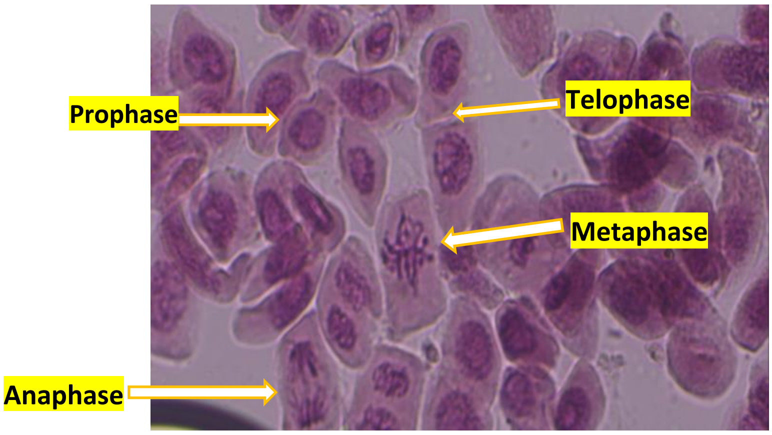

Figure 2: Microphotograph Showing the Pro-Phase, Anaphase, Metaphase & Telophase 40X

Name of Cell Divisional Stage	No. of Stages	Total Cell Count
Interphase	66	100
S phase	75	100
Prophase	48	100
Metaphase	69	100
Anaphase	58	100
Telophase	96	100
Cytokinesis	67	100

Table 1: Showing the Total Cell Count in Different Cell Divisional Stages

6. Conclusion and Discussion

On the stage of the compound microscope, the slide containing the dyed root tip cells is inserted, and any changes are observed and drawn. You may watch the many stages of mitosis, including prophase, metaphase, anaphase, and telophase. There are all the cell cycle stages are showing in the slides or microphotographs.

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Conflict of interest

There is no conflict of interest

Data availability statement

Data will be available upon request

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Authors Contribution

Ms. Gupta is the main author of this manuscript; she has done all experimental setup and completed the all objectives of this paper and compiled the data also.

Remaining author is only help to formatting this manuscript.

Key Message

Cell biology helps us to every cellular organism, is it how many chromosomes and genes are present on that species of that genera. It tells us how the genetic materials are exposed with any kind of toxicants or mutagens. This work is focused only on the different cell divisional stages of cell cycle in onion root tip. It is only the for depicting all the stages of cell cycle as well as mitosis division using onion root squash preparation, is very important and mostly used in labs for cell biology experiments.

Hence it should be very helpful for new coming students to understand the methodology and experimentation behind it.

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