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Chromosomes and Mitotic Cell Division Phase In Onion Roots After 24 Hours Acetoorcein Soaking Time

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Abstract

Onions (*Allium cepa*) are usually used *in vitro* to assess effect of chemical subtances by allowing developing roots to come into contact with substances to be tested. Acetic orcein staining of onion chromosomes has remained a standard method of preparation. However, aceto-orcein stain is corrosive and poisonous chemical substances since it containing oxidising agents such as organic peroxides, the toxic substances which are are cyanides, acid corrosives agents, and also radioactive substances. This research study mitotic activity in the roots of onion plants to determine the effects of soaking time of aceto orcein dye on actively dividing root cells. A series of several root tip from each bulb was harvested were soaked in 1, 3 and 24 hours on aceto-orcein stain and processed further for cytological studies by the aceto-orcein squash technique. The research was carried out to study the effect of to mitotic index and chromosomal aberration on onion root. It will determine the percentage of cells that are undergoing mitosis. The squash techniques were used to observe mitosis in the tip of onion root cells during actively mitotic division cells time. Mitotic divisions occur in several phases, consist of prophase, metaphase, anaphase, telophase and interphase. Experiment were repeated six times for every soaking time. The data was analyzed by using T-Test. The result showed that various duration of soaking time significantly influenced the reduction of mitotic index value. The lowest mitotic index on glyphosate concentration 100 ppm i.e. 10. 73% and 7.19% for the duration of soaking time 3 and 6 hours. The highest mitotic index on glyphosate concentration 0 ppm i.e. 37.71% and 32.76% for the duration of soaking time 3 and 6 hours. The result also showed that the chromosomal aberration were increased significantly. The lowest chromosomal aberration obtained i.e. 2.55% and 2.96% for the duration of aceto orcein soaking time 1, 3 and 24 hours. The highest chromosomal aberration obtained i.e. 21.71% and 36.26% for the duration of soaking time 1,3 and 24 hours. The type of chromosomal aberration were abnormal prophase, stickiness, bridge, abnormal anaphase, clumping chromosome, c- metaphase, change of nucleous shape and size.

At 72h, their cytotoxic effects on the root tips showed strong growth retardation in high concentrations of all the wastewaters. Compared to the control, treatment with the wastewaters resulted in root growth inhibition with EC50 values of 35, 50 and 62% for bottling, rubber and brewery effluents respectively, and decrease in mitotic index with increasing concentration for all samples and these were statistically significant ($p<0.05$). Chromosomal aberrations induced in the onion root tip cells were mostly sticky chromosomes and bridges. Chromosomes with disturbed spindles and fragments were also present in appreciable amounts. Based on the EC50 values, the bottling wastewater was most toxic, followed by rubber effluent while effluents from the brewery were least toxic. The findings in this study indicate that there are toxic chemicals present in the wastewaters which are responsible for the observed genotoxic effects on the onion root tip cells. The study also reveals that the *Allium* test is a useful and reliable tool for the genotoxicity screening of industrial effluents which could be employed by environmental managers before these effluents are finally discharged into the environment.

Key words: chromosomes, onion roots, acetoorcein

INTRODUCTION

Onions (*Allium cepa*) are usually used *in vitro* to assess the potential cytotoxicity and genotoxicity effect of chemical subtances by allowing developing roots to come into contact with substances to be tested. Acetic orcein staining of onion chromosomes has remained a standard method of preparation. However, aceto-orcein

stain itself is corrosive and poisonous chemical substances since it containing oxidising agents such as organic peroxides, the toxic substances which are cyanides, acid corrosives agents, and also radioactive substances. Orcein dye can be purchased in both its natural form as extracted from two species of lichens, *Rocella tinctoria* and *Lecanora parella*, and a synthetic form. This research study mitotic activity in the roots of onion plants to determine the effects of soaking time of aceto orcein dye on actively dividing root cells. It will determine the percentage of cells that are undergoing mitosis. The squash techniques were used to observe mitosis in the tip of onion root cells during actively mitotic division cells time.

MATERIAL AND METHODS

Actively dividing cells from an onion root are removed and treated with hydrochloric acid to fix the cells or to stop them from dividing. The cells are then stained, made into a wet mount, and squashed. Squashing spreads the cells into a single layer. Forceps are use to carefully remove several onion root tips and place them in a petri dish. Flooding the root tips with 1 M HCl was done using an eye dropper then allow the root tips to stand in the HCl for 10 minutes. The HCl was removed from the petri dish using the eyedropper and dispose of it. The petri dish were refilled with distilled water. Place a microscope slide on a paper towel. Three drops of aceto-orcein stain were added to the center of the slide. Forceps were used to transfer a prepared root tip from the petri dish to the drop of stain on the microscope slide. Pulverize the tissue by gently but firmly tapping the root tip with the end of a wooden macerating stick and allow the root tip to stain for 10–15 minutes. We can not let the stain dry by adding more stain if necessary. Place the slide on a smooth, flat surface. A coverslip were added to the slide to make a wet mount. Place the wet mount between two pieces of paper towel. The eraser end of a pencil were used to press down on the coverslip. Apply only enough pressure to squash the root tip into a single cell layer. Be very careful not to move the coverslip while you are pressing down with the pencil. Do not press too hard because it might break the glass slide or tear apart the cells. View one slide at a time with a compound light microscope under both low and high power. Select the slide that shows the most cells undergoing mitosis. Observe the slide under high power. Estimated the number of cells in the viewing area were done without moving the slide. The viewing area were divided into three viewing sections and then count the cells in one section**.** In each viewing section, count the number of cells in prophase, metaphase, anaphase, and telophase and record the numbers. Analyzing data was done by calculate the percentage of cells on slide were in the process of mitosis. The following formula was used to calculate percentage of mitosis cells and percentage phase of the cells.

$$\% \text{ cells in mitosis} = \frac{\text{total number of cells in all phases of mitosis}}{\text{total number of cells}} \times 100$$

$$\% \text{ cells in phase} = \frac{\text{total number of cells in phases}}{\text{total number of cells}} \times 100$$

Chromosomal aberrations were determined by scoring cells with fragments,non separated chromosomes, mis-polarized chromosomes in randomly picked 3 zones per slide. For each group of concentrations and control, the mean values were calculated. In order to determine the significance among the maeans, Independent Samples t-test was applied ($p<0.05$).

RESULT AND DISCUSSION

Mitotic divisions occur in several phases, consist of prophase, metaphase, anaphase, telophase and interphase. Prophase indicated by the appearance of the chromatin threads shorten and thicken, called a chromosome. Longitudinal splitting of each chromosome are called chromatids. In early prophase they appear as thin threads. During prophase, chromosomes, consisting of two chromatids each, condense and undergo progressive coiling. During the middle of late prophase the chromosomes can be seen as discrete structures, each with two chromatids and a constricted area called the centromere where the two chromatids are joined. In late prophase the core wall disappears. The transitional period from late prophase to metaphase is called prometaphase, during which the nuclear envelope breaks down and the chromosomes move toward the equatorial

plane (metaphase plate) of the cell. The centromeres will split in anaphase and headed to the cell poles opposite. Chromatids of a chromosome division results are identical. The two poles of the cell has formed a set of chromosomes in telophase as the parent cell chromosomes, then formed the core wall. The occurrence of cytokinesis (division of plasma) is characterized melecul into by cell membrane, and on plant cells coupled with the formation of cell walls. Anaphase was the phase when the centromeres split and headed to the cell poles opposite. Chromatids of a chromosome division results are identical. In telophase, the two poles of the cell has formed a set of chromosomes as the parent cell chromosomes, then formed the core wall. Interphase is a phase of cell division. In this phase of the cell nucleus appears cloudy with chromatin threads are fine. The transition of interphase to cell division (mitosis) and back to interphase is called the cell cycle.

Chromosomes are not visible under the light microscope in non-dividing (interphase) cells. As the cell begins to divide, the threads of chromatin (DNA-protein complex) in the nucleus begin to condense into multiple levels of coiled structures recognizable as chromosomes. There are two modes of cell division: mitosis and meiosis. Mitosis is responsible for the proliferation of body (somatic) cells, whereas meiosis is responsible for the production of gametes. Because mitotic cells are easy to obtain, morphological studies are generally based on mitotic metaphase chromosomes. In the metaphase, the chromosomes are in the equatorial plane of the cell. Chromosomes at metaphase was evident, so it can be calculated. The number of onions chromosomes are 16. Root tips of onion are used because the cells there are actively growing and dividing, giving a good chance to see chromosomes during different stages of cell division. The mechanism of staining may interact at an acid pH with negatively charged groups or possibly interact hydrophobically with chromatin. It binds to histon proteins at chromatin and shows its structure. Nucleoli do not stain with acetic orcein so chromatins can be seen at clear area.

After sufficient exposure of aceto-orcein stain, the cell chromosomes in the onion roots under a light microscope are showing the difference between short and 24 hours of soaking time. It revealing difference of mitotic index and mitosis phase as illustrated in Table 1. Its also detect chromosome abnormalities or cell division abnormalities which can be seen on Fig.1.

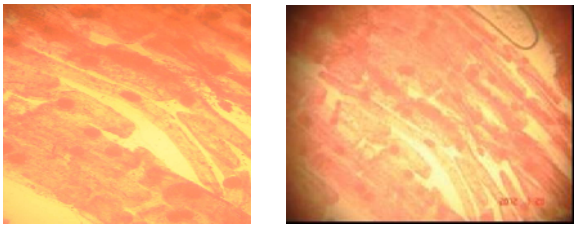

Figure 1. Aceto orcein at 24 hours

Our result are concordance with the result of other researcher. Shamasekar and Gowda (1984) said that toxic substances likes fungicide can cause deviation in mitotic index and mitosis. They stated that the most important effects were mito_depresive behavior in the lowest concentration and total disappearance of mitosis by prophase inhibitin in higher concentration(mitostatic effect). They caused chromosomes breakes, gaps, chromosomes and chromatids changes, lagging chromosomes, chromosomes bridges and as a result of dissapearance of spindle fibers disorders and some irregularities during cell division. Other researchers reported similar results according application time. Fungicides are acting like a mutagen in changing chromosomes structure and behavior.

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