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Studies on Mitotic Division of *Allium ascalonicum* L. Based on Observation Time

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Abstract. Cell is the little functional structure of living thing. It has important benefit of life. All of body activity is controlled by the genetic material in cell, as an example is cell reproduction. Cell Reproduction is an enhancing of cell generation by cell division (mitotic). Cell division of iving thing has different time each other. It depend to composition of material genetic comlpex and the environment factor such as nutrition, temperature and light intencity. So that it make difficult to learn about the process of mitotic division. Therefore this research has purpose to find the effective time of mitotic divison and learn about the mitotic division process. This research was conducted by using chromosomal preparation method and mitotic index calculation. The sample of this research is *Allium ascaloniicum* L. (shallot). Based on the research that has been done, *Allium ascalonicum* L. has highest mitotic index in afternoon at 12.00 WIB with percentage 31,6%. In evening at 15.00 WIB it has 28,3% and in morning at 09.00 WIB it has 18,4 %. The mitotic division consist of 5 phase, prophase, prometaphase, metaphase, anaphase, and telophase.

Keyword : genetic material, reproduction, mitotic division, index mitotic, chromosome.

Running Title : Studies on Mitotic Division of *Allium ascalonicum* L.

INTRODUCTION

The smallest structure of the living body is the cell. Cells are often referred to as functional and structural units in living things. This is because in the cell there are several biological processes that involve the cell organelles. One of the processes that occurs is the reproduction of cells that play a role in the growth of living things (Campbell, 1999).

Mitotic division in a cell has different time each other. It depend to composition of material genetic comlpex and the environment factor such as nutrition, temperature and light intencity. So that it make difficult to learn about the process of mitotic division. Such studies must pay attention to appropriate observation times to be more effective at observing the index of mitotic division. According to Mardliyyah (2016) the mitotic index is the percentage of the total number of cells undergoing division in a cell cycle. However, the reference to the mitotic index is still limited, so research on the mitotic index is needed to add references (Abidin, 2014). Therefore this research has purpose to find the effective time of mitotic divison and learn about the mitotic division process.

This research was conducted at three observation times, namely at 09.00 WIB, 12.00 WIB and 15.00 WIB. This is because the cleavage time in each species is different and not constant. Each species has the optimum biological clock for mitosis (Diana, 2018). Thus, the observations made at three times can be explored more about the optimal time to serve cells undergoing mitosis

The sample used in the study of cell mitosis is the part of the plant that is actively experiencing growth (meristematis). This section is most easily found at the root tip. Therefore, the material used in this study is the root tip of shallot (*Allium ascalonicum* L.). Root cells are easy to grow and are uniform, root cells also do not have chlorophyll so they are easily dyed (Abidin, 2014). Whereas parts other than roots usually have pigmentation, for example in the tubers which have red and purple pigments so that it will be difficult to be dyed (Sinta, 2016). Apart from being easy to obtain and inexpensive, this shallot species is a good material for processing into mitosis preparations. This is because the chromosomes of these species are large and have a small number of autosomes, namely 16 chromosomes, so that the chromosomes are easy to observe (Fukui, 1996). Therefore this research has purpose to find the effective time of mitotic divison and learn about the mitotic division process.

MATERIALS AND METHODS

The materials used in this research are shallots, Glacial acetic acid 45%, HCL 1 N, Absolute alcohol, Aceto orcein 1%, Glycerin, aquades. The tools are used in this research are binocular microscope, lab opti, razor blade, Drop pipette, Petri dishes, Cotton, Flacon bottle, Glass objects, Cover glass, Toothpick, tissue.

The research used the mitotic index study method and the development of Cell Reproduction worksheet teaching materials. The mitotic index study was carried out at three observation times, namely morning (09.00 WIB), afternoon (12.00 WIB), and evening (15.00 WIB). The study used chromosome preparation techniques with a sample of the root of a shallot (*Allium ascalonicum* L.). This technique consists of making chromosome preparations which include fixation, maceration, staining, squashing, and calculating the mitotic index.

The chromosome preparation starts from the growing shallot roots which are cut using a razor blade at the tip (3-4 mm) and put in a flacon bottle. The time of the deduction is made at three times, namely at 09.00 WIB, 12.00 WIB and 15.00 WIB. After that, washed the root tips with distilled water until clean and fixated using 45% acetic acid at 40^0C for 15 minutes. After being fixated, the root tips were washed using distilled water and macerated with 1 N HCl at a temperature of 55^0C for 2-5 minutes. The root tips were washed again using absolute alcohol, then stained

with 1% aceto-orcein for 2 hours. The root tip is placed on a glass object and the edges are absorbed with a tissue. After that, drop the glycerin and squash it, then cover the preparations with a closing glass. The preparations were observed under a binocular microscope and then images were taken using an Optilab. Preparations were made in 3 repetitions each time.

The preparations that have been made were observed using a microscope and documented using micro optics. The total number of preparations was 9 preparations, namely mitosis preparations at 09.00 WIB, mitotic preparations at 12.00 WIB and mitosis preparations at 15.00 WIB, each of which amounted to 3 pieces (carried out 3X repetitions on different plant roots). Each preparation was observed in 5 fields of view with a magnification of 100 X and a scale of 1 micron. Mitosis observation data were analyzed qualitatively using the mitotic index formula (percentage of cells currently in mitosis) below (Pancasakti et al, 2012).

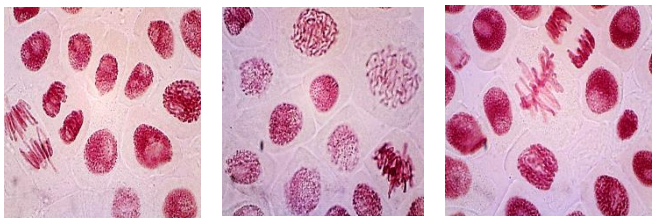

Figure 1. Cross section of the shallot root chromosome

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

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

Based on the preparations made, the percentage of cells that are in the mitosis stage is calculated. Each preparation was calculated as a percentage of the five mitotic phases

RESULTS AND DISCUSSION

The mitotic index study was carried out at three observation times, namely morning (09.00 WIB), afternoon (12.00 WIB), and evening (15.00 WIB). The following is the percentage value of the mitosis index at three observation times

No	Observation / Repetition Time		Cells in Mitosis Phase				
			% P	% PM	% M	% A	% T
1.	09.00 WIB	I	8,6	2,7	4,3	1,2	5,0
		II	6,9	4,0	3,3	0,9	2,4
		III	3,8	2,8	2,0	6,3	1,1
	Average		6,4	3,2	3,2	2,8	2,8
	Mitotic Index (%)		18,4				
2.	12.00 WIB	I	8,3	10,8	1,3	3,9	7,0
		II	18,0	2,8	5,1	7,1	3,1
		III	8,8	1,3	3,1	7,7	6,6
	Average		11,7	5,0	3,2	6,2	5,6
	Mitotic Index (%)		31,6				
3.	15.00 WIB	I	9,4	1,8	3,9	5,8	2,9
		II	6,2	-	4,6	10,8	8,3
		III	6,0	4,4	4,5	4,7	11,6
	Average		7,2	2,1	4,3	7,1	7,6
	Mitotic Index (%)		28,3				

Table 1. Percentage of Mitosis Index at Three Observation Times
Note:
% P = Percentage of cells at the prophase stage
% PM = Percentage of cells that are at the Prometaphase stage
% M = Percentage of cells that are at the Metaphase stage
% A = Percentage of cells that are at the Anaphase stage
% T = Percentage of cells that are at the Telophase stage

Based on the Table above, it can be seen that the mitotic index value at 09.00 WIB, namely 18.4% with a prophase percentage of 6.4%; prometaphase and metaphase 3.2% respectively; and anaphase and telophase 2.8% each. At 12.00 WIB the mitotic index value was 31.6% with a prophase percentage of 11.7%; prometaphase 5.0%; metaphase 3,2%; anaphase 6,2; and telophase 5.6%. Meanwhile, at 15.00 WIB the mitotic index value was 28.3% with a prophase percentage of 7.2%; prometafase 2.1%; metaphase 4,3%; anaphase 7,1%; and telophase 7.6%.

The highest mitotic index value of shallots (*Allium ascalonicum* L.) is at noon (12.00) while the lowest mitotic index value is in the morning (09.00). This is in accordance with Abidin (2014) that the highest mitotic index value of shallots is 12.00. During the day, the temperature gets higher. This supports cell division which is controlled by enzymes, which work optimally at the optimum temperature (Fukui, 1996). According to Taylor and Clawes (1978). The highest mitotic index of the genus Allium is between 24 and 30 ° C.

CONCLUSIONS

From this discussion, the effective time for mitosis in shallot (*Allium ascalonicum* L.) can be concluded. The effective time for cell mitosis is 12.00 WIB 12.00 which is 31.6%; followed at 3:00 p.m. with a mitotic index percentage of 28.3%; and at 09.00 with a mitotic index percentage of 18.4%.

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