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The Mitotic Index of *Cajanus cajan* from Kisar Island, in the Southwest of Maluku

Kristin Sangur, Alwi Smith*, Meike Tomasoa

Biology Education Study Program, Faculty of Teacher Training and Education, Universitas Pattimura, Indonesia
*Corresponding Author: smith.alwi2020@gmail.com

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Abstract. The mitotic index of the roots of pigeon pea can be the basis for determining the growth of pigeon pea. The purpose of this research was to determine the time of root cell division, to observe the mitotic phases, and to determine the mitotic index of pigeon pea root cells. The preparation of the pigeon pea was carried out for 4 days to grow the roots. The roots were cut off at 08.00, 08.15, and 08.30 WIT (Eastern Indonesian Time). The roots were cut 0.5-1cm. Carnoy's solution was used as the fixative solution using the Squash technique. The prepared roots were then observed using an Olympus cx-22 microscope and an OptiLab camera with a magnification of 100x40. The data were descriptively analyzed to describe the images of mitotic phases and the mitotic index presentation in the root cells of pigeon pea. The results of this research showed that the cell division of the pigeon pea roots began at 08.00 WIT, which was marked by the presence of a lot of prophase. The next phases that appeared were prometaphase, metaphase, and anaphase which occurred from 08.15 to 08.30 with different numbers. The highest mitotic index occurred at 08.15, when most of the root cells underwent metaphase. This study succeeded in revealing that the optimum time for pigeon pea root cell division is 08.15 WIT. In the future, this research can help pigeon pea farmers in Southwest of Maluku to carry out vegetative reproduction which is closely related to this mitotic study.

Key words: *Cajanus cajan*; mitotic index; pigeon pea; Southwest Maluku

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INTRODUCTION

Pigeon pea (*Cajanus cajan*) is one of the biodiversity of legumes from Kisar Island, Southwest Maluku, in addition to peanuts, red kidney beans, green beans, and cowpeas. The seeds of the pigeon pea are round or lens-shaped, the color of the seed coat varies from dirty white to silver white, light brown to brown, speckled with dark brown and dark pink, and yellow cotyledons (Saxena et al., 2010). The term pigeon pea appeared in the 15th century, originating from America because the seeds were favored by pigeons (Upadhyaya et al., 2015). Nowadays this plant is only consumed as a vegetable and used as a side crop, when compared to other types of plants grown by the people on Kisar Island. In fact, pigeon pea has several superior characteristics to be studied and further developed, so that it can be more optimally used. Pigeon pea contains several nutrients such as 20-22% protein, 1.2-4.43% fat, 65% carbohydrates, 7.16% fiber, and 3.76-3.80% ash (Sharma et al., 2011; Akande et al., 2010). In addition, it also contains several kinds of vitamins such as thiamine, riboflavin, and niacin; minerals such as potassium, phosphorus, iron and zinc, calcium, manganese, and copper (Adepoju et al., 2019). The pigeon pea is more tolerant of drought and high temperatures than other types of legume. It has woody and sturdy trees and taproots that grow fast and deep (Flower & Ludlow, 1987; Mathew et al., 2015). Mligo and Craufurd (2005) also added that the flowering characteristic of polycarpic pigeon pea is a way to respond to high environmental stress. The research by Deeplanaiki et al. (2013) reported that the cultivar pigeon pea Asha (ICPL 87119) has a DLP gene that determines its function in environmental stress tolerance.

To optimize the use of pigeon pea, genetic improvement can be done as an effort to improve plant quality, namely through chromosome manipulation (Khoiroh et al., 2015). According to Corneillie et al. (2019), the indirect use of genetic information in plant breeding is in the form of increasing knowledge of the genetic makeup of a particular type of plant, and the direct use of genetic information in plant breeding is in the form of applying cytogenetic techniques to improve plant traits. Chromosomes are nucleoprotein structures, carrying genetic material in the form of DNA as a unit of heredity and carrying information for cell regulatory activity (Crow & Crow, 2002; Francis, 2007). The main function of chromosomes is to be responsible for separating the same amount of DNA and ensuring that the offspring carries the traits of both parents at each cell division (Bass & Birchler, 2011).

The first step in studying chromosomes is to observe the phases of mitosis. Mitosis is the process of genome division that has been duplicated by a cell into two identical cells produced by cell division. Mitosis consists of five phases, namely prophase, prometaphase, metaphase, anaphase, and telophase (Muhlisyah et al., 2014; Sharma & Vig, 2012). It is essential that mitosis research be conducted in order to obtain the best cell phase, which is when the chromosomes are clearly visible and scattered, so that they can be easily counted. Chromosomes can be well observed when the cells are in the late prophase stage (El-Ghamery et al., 2000). The mitotic phase is closely related to the time of cell division in these plants. The research by Raja et al. (2015) reported that the time for cell division of *Capsicum annuum* was between 06.00 and 08.15 o'clock. The research by Abidin (2014) showed that the most active mitotic activity in the genus Allium varies such as the mitotic activity in *A. sativum* and *A. fistulosum* occurs in the morning at 09.00 and 06.00 WIB (Western Indonesian Time), while the mitotic activity in *A. cepa* occurs at noon at 12.00 WIB. The research by Willie and Aikpokpodion (2015) showed that the most active mitotic division in *Vigna unguiculata* occurs at 07.00-14.00, with the peak occurring at 11.00-13.00. This shows that the time for cell division of each plant is different and not constant. Another contributing factor is the time of cutting the roots.

Meanwhile, some information related to cell division, including the time of cell division, characterization of the mitotic phase, the number of cells undergoing mitosis, and the mitotic index of pigeon pea root cells from the Kisar island of Southwest Maluku has not been carried out. In fact, the calculation of cells undergoing mitosis can be the basis for determining the magnitude of the mitotic index of these pigeon pea root cells. Tabur and Oney (2009) reported that the mitotic index value increases when there is an increase in cell division. The mitotic index is the ratio of the number of cells that undergo mitosis in each phase (Rossato et al., 2010). Abidin (2014) also added that the mitotic index is an indicator of growth, where the greater the mitotic index, the better the plants grow. Therefore, determining the time of cell division and observing the mitotic phase of pigeon pea root cells are very essential, in order that the calculation of the mitotic index of pigeon pea root cells can also be done. The purpose of this research was to determine the time of root cell division, to observe the mitotic phases, and to determine the mitotic index of pigeon pea root cells (*C. cajan*). This research reveals that scientific information about the optimum time for pigeon pea cell division so as to help farmers in Southwest of Maluku in vegetative reproduction.

METHODS

Sample preparation

The seeds of Pigeon pea (*C. cajan*) used were black seeds from North Romleher Hamlet, Wonreli village, Kisar island, Southwest Maluku regency (Figure 1). This research began with soaking the pigeon pea seeds for 12 hours, and then the pigeon pea seeds were sprouted for 4 days. The root cutting was carried out at 08.00, 08.15, and 08.30 WIT (Eastern Indonesian Time). The roots were cut 0.5–1 cm. The cut roots were then put in a flacon bottle and then pre-treated by soaking the roots in distilled water for 24 hours. The roots were then fixed with 2 ml of Carnoy's solution at room temperature for 24 hours, after which the roots were washed using distilled water. The roots were then hydrolyzed with 2 ml of HCl 1 N solution in an oven at 60°C for 30 minutes. After that, the roots were washed using distilled water again. The roots were stained using 2 ml of *aceto orcein* 2% and soaked for 30 minutes at room temperature (Setiawan et al., 2018).

Figure 1. Pigeon pea (*C. cajan*) plant. (a) Flowering plants; (b) Pigeon pea pods; (c) Appearance of pigeon pea seeds in pods; (d) Black pigeon pea seeds; (e) Sprouted pigeon pea seeds.

Root cell observation

The preparation was carried out using the Squash method. The prepared roots were then observed using an Olympus CX-22 microscope and an OptiLab camera with a magnification of 100x40. The data of the cleavage of each mitotic phase were then observed, calculated, and recorded.

Calculation of the mitotic index

The mitotic index was calculated using the formula following Darbelley et al. (1989), namely: $IM = \frac{Nm}{N} x\ 100\%$

Description:
IM = Mitotic Index
Nm = Number of cells undergoing mitosis from prophase to telophase in one mitotic preparation
N = Number of all cells observed

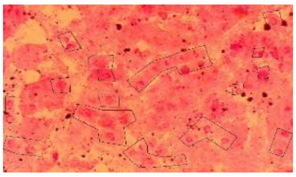
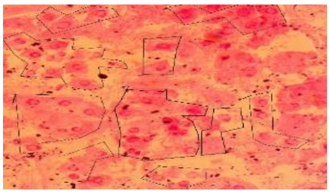
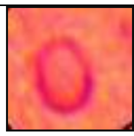
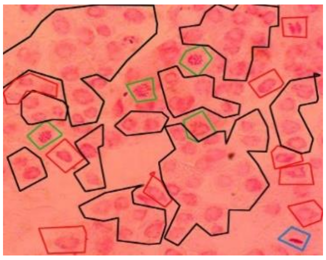
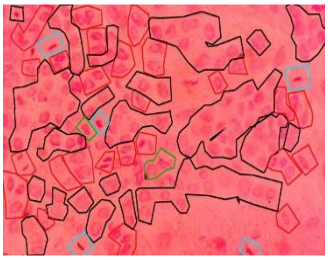
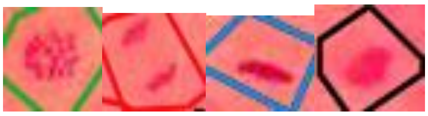
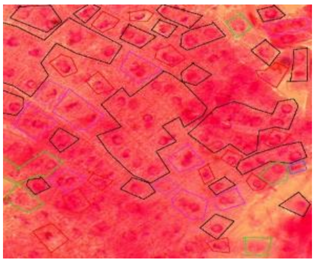
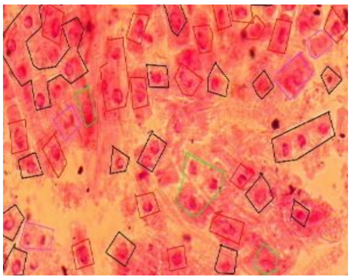
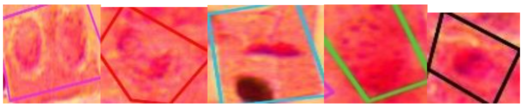
Data analysis

The data were descriptively analyzed to describe the images of mitotic phases and the presentation of the mitotic index of the pigeon pea root cells.

RESULTS AND DISCUSSION

Every plant has its own biological clock for mitotic division. The cutting time is a crucial stage because it can affect cell division (Etikawati & Setyawan, 2000). In addition, the cutting time also affects the duration of mitosis and the mitotic index (Yadav, 2007). In the previous research, the roots of *Allium sativum* were cut at 08.00 WIB (Western Indonesian Time) (Setyawan & Sutikno, 2000), while the roots of *A. ascalonicum* L. were cut at 09.00 WIB (Western Indonesian Time) (Abdullah et al., 2017). In addition, Iriani et al. (2020) in their research reported that the leaflets of *Hibiscus rosa-sinensis* (L.) were cut at 08.00 WIB (Western Indonesian Time). Based on the results of this research, the mitosis of the root cells of pigeon pea (*C. Cajan*) began at the time of the root cutting at 08.00 WIT (Eastern Indonesian Time).

Table 1. Images of the prepared pigeon pea roots (*C. cajan*) based on differences in cutting time

Cutting Time (WIT)	View 1	View 2	Phases Found
08.0			
08.15			
08.30			

Color description: (▬:Prophase; ▬:Prometaphase; ▬:Metaphase; ▬:Anaphase; ▬:Telophase)

The prophase in the mitotic division of pigeon pea root cells started at 08.00, and even the prophase continued until 08.30 (Table 1). According to Zoller et al. (2004), the prophase stage in mitotic division is characterized by the chromosomes condensing linearly, shortening, and increasing in diameter. The results of the observation showed that both prometaphase and metaphase appeared at the cutting time from 08.15-08.30. Prometaphase is the final stage of prophase and the beginning of metaphase. The metaphase stage is characterized by the chromosomes being scattered into strands and having a longer chromosome size and clearly observed chromosome structure. The research by Yuniastuti et al. (2021) reported that the optimal time for pigeon pea root cell division is at 08.15 o'clock, when the metaphase is occurring. The same result was also found in this study that the root tip of pigeon pea from Kisar Island had a metaphase time of 08.15.

This is characterized with the chromosomes being evenly spread, and the length can be easily observed. Furthermore, Wanjari et al. (2016) explained that at the time of metaphase, the chromosomes were in a condensed condition, so that they provided detailed information regarding the optimal chromomeric pattern and size. The metaphase stage is also characterized by the formation of spindle thread. The spindle thread plays a role in the separation of chromosomes, where metaphase is characterized by the alignment of chromosomes in the cell equator plane (Hartl & Jones, 2005). Whereas at the anaphase stage, the chromosomes begin to separate and the chromatids will go to the poles of the cell. The anaphase and telophase were also found during the division of pigeon pea root cells at 08.15-08.30. The migration of the two sets of daughter chromosomes that move to opposite poles indicates that the anaphase process has been completed and the two daughter cells have the same number of chromosomes (Enger et al., 2012). The final stage of the mitotic phase is telophase. According to Elrod and Stansfield (2002), in the telophase phase, each separate chromatid set converges at the two poles of the cell and these chromatids now turn into chromosomes.

Further analysis was carried out to determine the number of cells undergoing mitotic division from each phase. The number of cells undergoing different phases of mitosis at each root cutting time can be seen in Table 2.

Table 2. The number of mitotic phases of pigeon pea (*C. cajan*) based on the difference of cutting time

Time	View	Mitotic Phases				
		Prophase	Prometaphase	Metaphase	Anaphase	Telophase
08.00	I	33				
	II	48				
Average±Standard deviation		40.5±10.61				
08.15	I	73	4	1	11	
	II	113	2	5	48	
Average±Standard deviation		93±28.28	3±1.41	3±2.83	29.5±26.16	
08.30	I	51	7	1	14	10
	II	24	5	0	19	4
Average±Standard deviation		37.5±19.09	6±1.41	0.5±0.71	16.5±3.54	7±4.24

Prophase was found at any cutting time in both types of fixative solutions and had a very large and dominant amount when compared to the other mitotic phases. This shows that the root division time of pigeon pea originating from Southwest Maluku has a long cell division time starting from 08.00-08.30. This is in accordance with the research results by Kusumaningrum et al. (2012) that prophase is a long phase among other phases, this is indicated by the chromatin threads that experience spinning and thickening to form chromosomes, then in the middle of prophase the chromosomes are seen as two chromatids that are bound by the centromere until the final prophase where the cell nucleus is degraded. Moreover, Cooper (2000) stated that prophase takes the longest time compared to the other stages in the mitotic phase, which is the half time of one whole mitotic phase.

Prometaphase is a phase of mitosis following prophase, so that this phase was not found at the 08.00 cutting time, but it was found at the 08.15 cutting time. This shows that the time for cell division of pigeon pea roots began at 08.00, indicated by the appearance of prophase. Previous research on prometaphase in several other plants has reported that the highest prometaphase in the roots of *Passiflora edulis* was found at 10.20 after prophase (Muhlisyah et al., 2014). Rindyastuti and Daryono (2009) found that the highest prometaphase in *Coccinia grandis* species was at 09.50-10.15 after prophase, while the prometaphase in lime and kaffir lime was found mostly at 08.00 WIB (Western Indonesian Time) after prophase (Arisuryanti et al., 2007).

Metaphase and anaphase were only found at the cutting time of 08.15-08.30 WIT (Eastern Indonesian Time), while telophase was only found at the cutting time of 08.30 WIT (Eastern Indonesian Time). When compared with the number of prophase, the number of the other three phases (metaphase, anaphase, and telophase) was fewer (Table 2). This may happen because the three phases tended to have short duration. Anaphase is a short-time stage which includes the separation of sister chromatids, and morphological changes in the inner cytoskeleton of cells which include astral microtubules as the main driver of spindle elongation, while spindle middle zone microtubules hold or regulate the rate of polar separation. The results of the same study were also presented by Rosculete et al. (2019) that the percentage of prophase remains higher than the percentage of metaphase, anaphase, and telophase despite exposure to herbicides. In addition, Ito et al. (2001) reported the percentage of all phases of mitosis in tobacco root tip cells, which was prophase 11%, metaphase 2%, anaphase and telophase 1%. Some of this research could corroborate the finding that pigeon pea (*C. cajan*) has a percentage of prophase higher than the other phases of mitosis.

Table 3. Mitotic index of pigeon pea root (*C. cajan*) based on the difference in the cutting time

Cutting Time (WIT)	Number of Cells Observed	Number of Cells Undergoing Mitosis at Each View		Mitotic Index of Pigeon pea Roots (*C.cajan*) (%)
	41	View I	33	80.49
08.00	53	View II	48	90.57
	Average±Standard	deviation		85.53±7.13
	100	View I	89	89
08.15	180	View II	168	93.33
	Average±Standard	deviation		91.17±3.06
	92	View I	83	90.22
08.30	62	View II	52	83.87
	Average±Standard	deviation		87.05±4.49

The mitotic index is a comparison of the number of cells undergoing mitosis with the total number of cells in an observation. Based on the percentage of the mitotic phases of pigeon pea, it shows that the highest mitotic index value is when the percentage of metaphase increases at 08.15 WIT (Table 3). This is in accordance with the research results conducted by Osuji and Owei (2010), who examined the mitotic index of *Treculia africana,* and the research by Adesoye & Nnadi (2011), who examined the mitotic index of *Sphenostylis stenocarpha* (Hochst. Ex. A. Rich.). Both of the research concluded that the highest mitotic index occurred during the high percentage of cell metaphase. Moreover, Ekanem and Osuji (2006) explained that the highest mitotic index is in the metaphase phase, such as what was found in the Caladium varieties. Shervani and Mishra (2020) stated that different conditions may affect the mitotic index in the root tips of *Tinospora cordifolia*. Similarly, it is also indicated that different cutting time causes differences in the mitotic index. This research revealed that the cutting time of pigeon pea root cells (*C. cajan*) from Southwest of Maluku was at 08.15 WIT with the highest mitotic index. This research is a basic research to be a reference for the analysis of the mitotic index of other pigeon pea varieties in Southwest of Maluku. In addition, the research results can be used as scientific information for vegetative reproduction of pigeon pea.

CONCLUSION

The results of observations and analysis of the mitotic index of Pigeon pea root cells (*C. cajan*) show that the cell division began at 08.00 WIT (Eastern Indonesian Time), marked with the appearance of prophase. The cell division continued until 08.30 WIT, which was marked with the appearance of telophase. The highest mitotic index occurred at 08.15 WIT, which corresponded to the high number of root cells undergoing metaphase.

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