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Cytological Analysis of Polyploid Kepok Abu Banana Planlets (Musa paradisiaca L.) Due to the Administration of Colchicine in Tissue Culture Media

Istiqomah[1], Sri Wahyuningsih[2], Eti Ernawiati[3], Rochmah Agustrina[4]

[1, 2, 3]Biology Study Program, Department of Biology, FMIPA, University of Lampung, Indonesia
Jl. Prof. Dr. Sumantri Brojonegoro No. 1 Bandar Lampung 35145

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***Correspondence email:**

istiqomahqomah4@gmail.com

ABSTRACT

This research aimed to determine the mitotic abnormalities, changes in the number of chromosomes, mitotic index, and the number and length of kepok abu banana plantlet roots due to administration of 0.1% colchicine in tissue culture media. This research was performed at the Botanical Laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences, UNILA. This research compared the cytology by employing the Squash method between root tip cells of kepok abu banana plantlets after administering 0.1% colchicine and the control group. The researchers analyzed the data descriptively and presented them in tables and bar charts. The results showed that the administration of 0.1% colchicine resulted in mitotic abnormalities. It also increased the number of chromosomes in the controls 2n=3x and 2n=3x+4. The mitotic index decreased, and the average number of roots was lower. However, the size was longer compared to the control group.

Analisis Sitologi Planlet Pisang Kepok Abu Poliploid (Musa paradisiaca L.) Akibat Pemberian Kolkisin Pada Media Kultur Jaringan

Abstrak: Tujuan penelitian ini untuk mengetahui kemunculan abnormalitas mitosis, perubahan jumlah kromosom, indeks mitosis, serta jumlah dan panjang akar planlet pisang kepok abu akibat pemberian kolkisin 0,1% pada media kultur jaringan. Pelaksanaan penelitian di Laboratorium Botani Jurusan Biologi FMIPA Unila. Penelitian ini membandingkan sitologi dengan metode *Squash* antara sel ujung akar planlet pisang kepok abu setelah penambahan kolkisin 0,1% dengan kontrol. Data dianalisis secara diskriptif dan ditampilkan dalam bentuk tabel serta diagram batang. Hasil penelitian menunjukkan bahwa pemberian kolkisin 0,1% memunculkan kelainan mitosis (abnormalitas mitosis) serta meningkatkan jumlah kromosom pada kontrol 2n=3x, dan perlakuan kolkisin 0,1% yaitu 2n=3x+4. Indeks mitosisnya menurun dan rata-rata jumlah akar lebih sedikit, namun ukurannya lebih panjang dibandingkan dengan kontrol. Dari hasil tersebut dapat disimpulkan bahwa pemberian kolkisin 0,1% mampu menginduksi sel-sel poliploid pada planlet pisang kepok abu.

INTRODUCTION

Banana is a horticultural commodity the community favors (Hapsari dan Lestari, 2016). The banana known today is a type of banana that comes from two ancestral species, namely Musa acuminata with an A genome and Musa balbisiana with a B genome (Bello *et al*, 2005; Komaryati dan Adi, 2012). One of the bananas with the highest accession level is the Kepok banana, which is widely distributed in Lampung is kepok abu banana with the AAB genome. Kepok abu banana is more dominant to genome A so that the properties possessed by genome A (hard-textured fruit with a slightly sour-sweet taste) can be consumed as fresh or processed fruit (Hapsari *et al*., 2015). However, banana cultivation is currently processed conventionally through the natural propagation of tillers and only produces one or two shots (saplings) in one cycle. Therefore, the availability of the seed is insufficient to meet the cultivation target. Besides, the nature of parthenocarpy, sterility, ploidy in bananas, and their long vegetative period are other obstacles in banana cultivations (Manzo-Sanchez *et al*., 2015). It is necessary to make breakthroughs in biotechnology through mutated breeding to overcome the limitations of superior kepok banana seeds, especially kepok abu banana.

Suyanti dan Supriyadi (2007) claims that vegetative banana shoots can be handled by culturing suckers that grow from banana weeds through tissue culture techniques influenced by the composition, formulation, and the type of media (Yusnita, 2003). One method that can be used is administering colchicine compounds into tissue culture media. Colchicine is an alkaloid compound (Głowacka *et al*., 2009), which inhibits the process of cell division (antimitosis) by interfering with the formation of spindles or spindle threads during chromosome separation at metaphase. It also inhibits the cytokinesis process. This condition causes inhibition of cell division and the formation of cells with three or more sets of chromosomes to become polyploid cells (Crowder, 1997).

Colchicine compounds are often used as plant breeding agents by inducing mutations and producing polyploid plants (Alemu and Daba, 2016; Nautiyal, 2011). Polyploidy plants have more than one set of chromosomes in their somatic cells. Cytologically, polyploid plants vary in shape, several chromosomes, and cell sizes. Furthermore, colchicine compounds can reduce the mitotic index (Davidson and Macleod, 1966) and cause cell abnormalities (Kundu and Ray, 2017). One of the efforts to produce polyploid plants is administering colchicine compounds to tissue culture media.

Griesbach (1981) placed the orchid protocorm on MS media (Murashige and Skoog) administered by colchicine for ten days, resulting in better flower colors. In line with the research results by Fahriz *et al*. (2018), immersing the embryonic callus of Pontianak Siamese colchiploid oranges in colchicine solution for ten and seven days showed changes in the number of stomata and the number of chromosomes. This research aimed to determine the mitotic abnormalities, changes in the number of chromosomes, the mitotic index, and the number and length of roots in polyploid kepok abu banana plantlets due to the administration of colchicine in tissue culture media. This research also aimed at producing polyploid Kepok abu banana plantlets with superior properties.

METHOD

This research was conducted at the Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Lampung from February to April 2020. The roots of the kepok abu banana plantlet were obtained from previous research at the MTC Laboratory, PT. Great Giant Pineapple PG 4. The researchers employed the observation method on the mitotic abnormalities, chromosome number, mitotic index, number

of roots, and root length of kepok abu banana plantlets taken from negative control (A1), and the treatment of 0.1% colchicine administration (A2)

Mitosis Preparation

The researchers performed the cytological observations using the Squash method (Gunarso, 1988) and (Darnaedi, 1991). The researchers cut the root tips of the kepok abu banana plantlets 1 cm long in the morning at 08.30 to 10.00 WIB; then, they put them in 8-hydroxyquinoline 0.03% solution for three to five hours. They were stored in a dark bottle at 18-20ºC in the refrigerator. The root tips were fixed using 45% glacial acetic acid for 10 minutes at room temperature and then washed using distilled water three times to remove the fixative solution. The root tips were soaked in a hydrolysis solution consisting of 1N HCl and 45% glacial acetic acid (3:1 ratio) and then heated on a hot plate for three to five minutes at 60°C. Hydrolysis served to dissolve the middle lamella so that the cells are only one layer thick (Setyawan dan Sutikno, 2000). The root tips were stained with 2% acetocarmin for fifteen minutes at room temperature in the next step. The colored root tips were placed on a glass object and then covered with a cover glass. The squash was done with the thumb and tapped with a rubber pencil. The remaining acetocarmin was absorbed by the outside of the cover glass with a tissue.

The objects were observed under a microscope with a 100x to 1000x magnification. The microscopic observations of 1000x magnification were done using immersion oil. Observation of mitotic abnormality was done by comparing the mitosis of untreated cells (A1) with mitoses of cells treated with 0.1% colchicine (A2).

Chromosomal counting was carried out on ten roots in the 0.1% colchicine treatment and ten roots in control. Meanwhile, the mitotic index parameter was calculated in 10 fields of view, and the average value was taken using the mitotic index (IM) formula by Pandey *et al*. (1994).

$$IM = \frac{\text{The number of cells in the mitotic phase}}{\text{The total number of cells observed}} \text{x } 100\%$$

Plant Morphological Observations

The root morphology taken was the number of roots and root length by counting the number of roots in each iteration explant, measuring root length with a ruler, and calculating the average value.

Data Analysis

Mitotic index data were analyzed by comparing the average treatment shown with a bar chart. Root morphological data, such as the number of roots, were counted, and the length was measured using a ruler. The results were then analyzed by comparing the average treatment shown with a bar chart.

RESULTS AND DISCUSSION

The observation on the root tip cells of banana kepok gray plantlets obtained that 0.1% colchicine treatment (A2) caused mitotic abnormalities with a higher percentage than the control (A1). The 0.1% colchicine treatment could increase the number of chromosomes more in the root tip cells of the kepok abu banana plantlet. However, the mitotic index of the 0.1% colchicine treatment (A2) tended to decrease compared to the control (A1). At the same time, based on the number of roots, the control (A1) produced more plantlet roots than the 0.1% colchicine treatment (A2). However, 0.1% colchicine treatment (A2) increased more root length compared to control (A1) (Table 1).

Table 1. The Results of Cytological Analysis of Kepok Abu Banana Planlets

Information	Average value	
	A1	A2
Mitotic Abnormalities (%)	13.67	23.89
Chromosome Number	33	37
Mitosis Index (%)	35.14	21.86
Number of roots	3	2

Root length	2.4	2.97

Note: A1 = Planlet control, A2 =Planlet with 0.1% colchicine

Mitotic abnormalities are the mitotic processes that are different from normal mitotic stages. In observing the mitotic phases of 0.1% colchicine treatment, the average mitotic abnormality was higher. It was assumed that the chromosomes were scattered between the two poles and did not move towards the opposite poles due to the absence of the spindle fibers. Spindle fibers play an important role in cell division as a place for chromosomes to move towards opposite poles so that a new cell is formed. According to Suryo (1995), cell mitotic abnormalities are caused by colchicine compounds that prevent spindle fibers' formation so that chromosome sets cannot be pulled towards opposite poles and result in scattered chromosomes in the equatorial plane during metaphase. Mitotic abnormalities caused by colchicine are called C-mitosis (Colchicine-mitosis), more specifically known as c-prophase, c-metaphase, c-anaphase, and c-telophase (Ernawiati, 2008) (Figure 1).

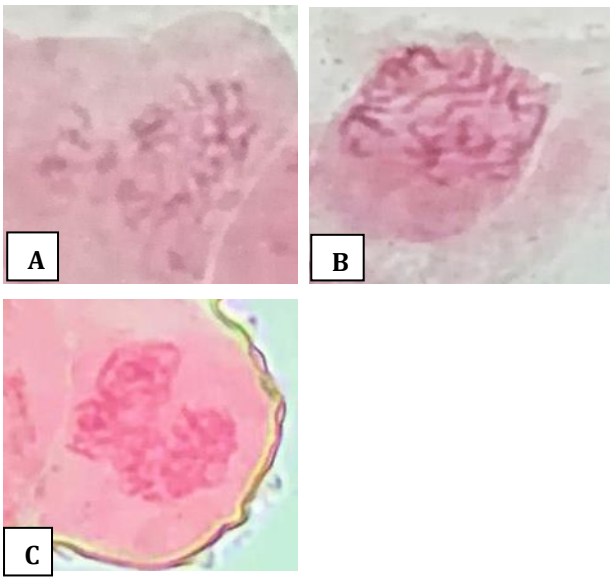

Figure 1. Abnormalities Due to the Administration of 0.1% Colchicine (A2)
Description: (A) C-Prophase, (B) C-Metaphase, C) C-Anaphase

The observed data showed that 0.1% colchicine treatment caused chromosome doubling (polyploidization) in the root tip cells of the kepok abu banana plantlet. Therefore, the plant had more chromosomes but fewer cells with larger cell sizes. The number of chromosomes due to 0.1% colchicine treatment can be seen, which has increased to 37 with a ploidy level of 3x+4. Ernawiati, *et al.* (2018) believe that kepok abu banana is a group of Musa paradisiaca L. with a chromosome triploid with a chromosome number of 33 (Figure 2). Rugini and Fedeli (1990) support this result that polyploid plants have more than a set of chromosomes in the somatic cell. The characteristics of polyploid plants are that they have a larger nucleus, cell contents, and a greater number of chromosomes.

Based on the research conducted by (Purnama, D. I. A. R., dan Pharmawati, 2018), the administration of 0.1% colchicine could increase the number of marigold plants' chromosomes twice as much as the control. This research indicates that the administration of 0.1% colchicine can increase the number of banana plantlet chromosomes (Figure 2).

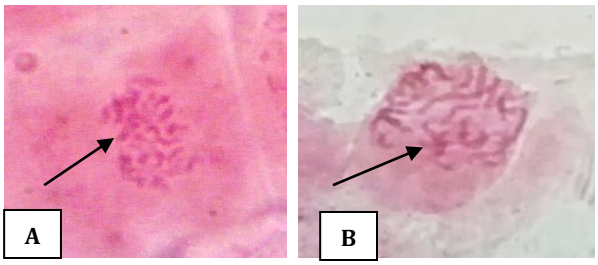

Figure 2. The Number of Chromosomes in Root Tip Cells of Kepok Abu Banana Planlets under 1000x magnification.
Description: (A) Control 3x = 33, (B) 0.1% colchicine treatment (A2) 3x+4, and (B)Colchicine 0.1% 2n=3x+4= 37

After increasing the number of chromosomes in kepok abu banana plantlets, administering 0.1% colchicine can also reduce the mitotic index. This theory was indicated by the average mitotic index in control, higher than the average value of 0.1% colchicine administration (Table 1, Figure 3). The decrease in the mitotic index in the root tip cells of the kepok abu banana plantlet was thought to be due to the presence of colchicine compound with a concentration of 0.1%, which caused the root tip cells to experience depression due to lack of water. The decrease in the mitotic index in kepok abu banana plantlets was solely due to

soaking time in colchicine compounds. Therefore, the longer the immersion, the lower the water content in the cell. Thus, it will reduce the water pressure in the cell and inhibit cell division.

Research on colchicine has also been done by Davidson and Macleod (1966). They found that the administration of colchicine with a concentration of 0.025% within three hours of immersion on the roots of Vicia faba reduces the mitotic index. According to Crowder (1997), the decrease in the mitotic index is thought to be due to the influence of colchicine compounds which inhibit the process of cell division (antimitosis) by interfering with the formation of spindles or spindle fibers during chromosome separation in metaphase and inhibiting the cytokinesis process (Table 1, Figure 3). The average value of the mitotic index (IM) can be seen in Figure 3.

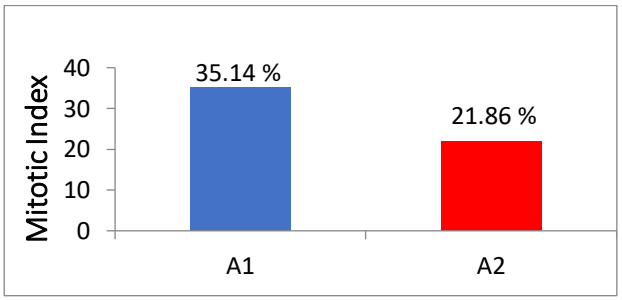

Figure 3. The Mitotic Index Diagram of Kepok Abu banana plantlet root tips
Description: (A1) Control, (A2) Colchicine 0.1%

Furthermore, the administration of 0.1% colchicine also inhibited the growth of the kapok abu banana roots plantlets. The data can be seen from the observation that the average value of the plantlet roots growth was higher in control (A1) compared to the 0.1% colchicine administration (Table 1, Figure 4). It was suspected that the administration of colchicine with a concentration of 0.1% (A2) did not present optimal effects on root growth (Figure 4).

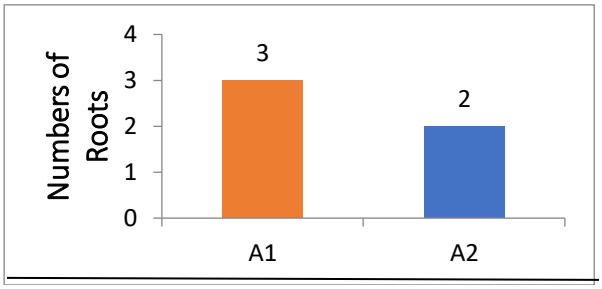

Figure 4. The Numbers of Plantlet Root of Kepok Abu Banana
Description: (A1) Control, (A2) Colchicine 0.1%

However, the root length due to administration of 0.1% colchicine (A2) had a higher mean value than the control (A1) (Table 1, Figure 5). It was suspected that the administration of 0.1% colchicine compound affected the polyploid cells, resulting in longer root length growth than the control (A1). The administration of 0.1% colchicine (A2) also resulted in a root growth delay, resulting in fewer roots. However, root development was much better (Figure 5).

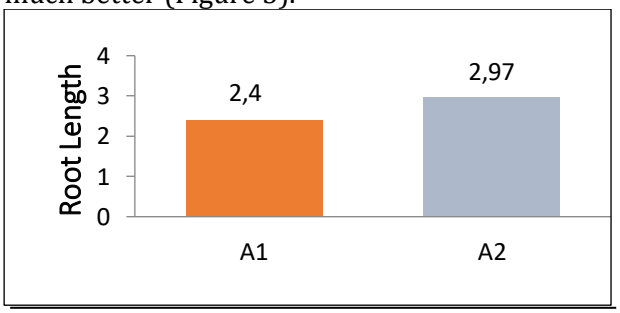

Figure 5. Plantlet Root Length of Kepok Abu Banana

Description: (A1) Control, (A2) Colchicine 0.1%

Research conducted by Murni (2010) shows the effect of doubling the number of chromosomes, affecting the morphology of curly chili plants. The size of the tetraploid curly chili plant is much larger than the diploid curly chili plant. Furthermore, Kuckuck *et al.* (1991) claim that the administration of colchicine can change the physiological properties of plants along with the increase in the size of plant cells. These changes can be seen in the growth and development of plants.

CONCLUSIONS AND SUGGESTIONS

Based on the analysis, the administration of 0.1% colchicine can induce polyploid cells in the root tip cells of the kepok abu banana plantlets by causing mitotic abnormalities, increasing the number of chromosomes, lowering the mitotic index, decreasing the number of roots, and extending the root size.

Based on the information obtained, it is hoped that further research will be conducted in a wider range of concentrations so that the right concentration can be obtained to induce polyploid cells in banana plantlets.

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