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Colchicine Effect of Mitosis Root Tip by Banana Kepok Yellow (*Musa paradisiaca* L.) Polyploid Explants

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Abstract

Colchicine can inhibit spindle formation so that doubled chromosomes fail to separate during anaphase cytologically to induce mutations that produce polyploid plants. Yellow kepok banana is a triploid banana. The supply of yellow kepok bananas is hampered by conventional cultivation methods and the limited availability of quality seeds. This study aims to determine the effect of adding colchicine compound to tissue culture media on the mitotic index, chromosome number, and chromosomal abnormalities in yellow kepok bananas and the formation of polyploid plantlets. This study was included in the experimental study by conducting mitotic preparation and observing morphology after the test sample was induced by colchicine. The results of the research that has been done show that the addition of the compound colchicine to the tissue culture medium of yellow kepok banana causes a decrease in the mitotic index, an increase in the number of chromosomes, the presence of chromosomal abnormalities, the formation of polyploid plantlets, and an increase in the number of roots, but there is a reduction in root length.

Keywords: colchicine, yellow kepok banana, mitosis, polyploid.

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INTRODUCTION

Colchicine is one of the most commonly used polyploidy plant-forming agents [1]. Colchicine can inhibit the formation of spindle threads during the cell division process so that the number of chromosomes in each new cell doubles or polyploidy occurs. The number of chromosomes that multiply increases the number of genes, thus increasing the metabolic rate. According to Sabana et al. [2], colchicine compounds are antimitotic (inhibit mitosis) because they can cause chromosomes that have replicated to fail to separate in the anaphase process. Cytologically, colchicine can inhibit the formation of spindle threads so that chromosomes that have doubled fail to separate during anaphase.

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The antimitotic properties (inhibiting mitosis) of colchicine are often used in plant breeding and biological studies to induce mutations that produce polyploid plants [3]. Polyploidized plants have the advantage of being able to produce plants that are superior to the original plants [4]. The advantages of polyploid plants are larger cell size, taller plants, wider leaves, larger fruit, higher plant production, large stomata, and more resistance to extreme environments such as drought, pathogen, or disease attacks compared to similar plants that are diploid (2n). Thus, polyploid plants have superior beneficial properties [5].

According to Sartika et al. [1], the high demand for yellow kepok banana is not matched by adequate production so consumer needs are unmet. An effort to overcome the above obstacles is to utilize colchicine compounds in a tissue culture medium for banana plantlet propagation. The tissue culture technique is possible to provide uniform plantlets in large quantities and short time [6]. This study aimed to determine the effect of the addition of colchicine compounds in tissue culture media on mitotic abnormality, chromosome number, and mitotic index, and to determine the number and length of roots in yellow kepok banana plantlets.

METHODS

This research was conducted at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung from May to August 2020. The roots of yellow kepok banana plantlets were obtained from previous research at the MTC Laboratory, PT Great Giant Pineapple. This study used the observation method of mitotic abnormality, chromosome number, mitotic index, number, and length of roots in yellow kepok banana plantlets taken from control (A1), and 0.1% colchicine addiction treatment (A2).

Mitotic Preparation

Mitotic preparations were prepared using the method of Gunarso [7] and Darnaedi [8], i.e. meristem tissue from the root tip of yellow kepok banana plantlets were cut approximately 1 cm from the tip. The root tip is part of the meristem that functions as a nutrient-seeking tool and is actively dividing [9]. Cutting the root tip of yellow kepok banana plantlets was done in a Laminar Air Flow at 08.30-10.00 WIB. Then, 0.3 grams of 8-hydroxyquinoline was added to a 1 L Erlenmeyer with 1 L of distilled water and heated on a hot plate at 600 C. After heating, the solution was homogenized for 15 minutes and the root cells were immersed in 0.03% 8-hydroxyquinoline solution in a flask for 3-5 hours, then stored in a refrigerator at 18-200C. After immersion was complete, the preparations were rinsed using distilled water 3 times.

Fixation is done to kill tissue cells without causing changes in cell components [7]. Fixation was done by immersing the root tip into 45% acetic acid solution for 10 minutes, then rinsed with distilled water 3 times to remove the fixative solution.

Root tip hydrolysis was carried out to lyse the middle lamella so that the cells are separated and dispersed so as to facilitate the calculation of the number of chromosomes. Hydrolysis was carried out by placing the root pieces into a small beaker glass containing a mixture of HCl1N solution and 45% acetic acid in a ratio of 3:1 and then heated on a hot plate for 3-5 minutes at 600 C until the root cells softened. Root tips were rinsed 3 times with distilled water to remove the hydrolysis solution.

Chromosome staining was performed with 2% aceto-carmine. Preparation of 2% acetocarmin solution is by evaporating 45 ml of glacial acetic acid in a glass cup on a hot plate but kept from boiling. Then add 2 grams of orcein to the glass cup while shaking every 15 minutes for 1 hour. After that, add 55 mL of distilled water to the 2% acetocarmin solution and let it stand at room temperature. The root tip pieces that have been cleaned from the hydrolysis solution, were then given 2ml of staining and then allowed to stand for 5-15 minutes. Staining is very important to

give color to chromosomes so that they can be easily observed under a microscope [10].

The tip of the root that has been stained is cut approximately 0.5-1mm and placed on an object glass then dripped with 2% acetocarmin and covered with cover glass. After that, it was tapped using an eraser on a pencil until the cells of the root tip spread and there were no air bubbles. The remaining acetocarmin was absorbed with a tissue on the edge of the glass cover, then squashed (pressed) with the thumb. Observation of root tips using a microscope with a magnification of 100-1000x by adding immersion oil. The mitotic index was determined based on the results of microscopic observations on the cells of the preparations that were being mitotic to observe the differences in each phase of mitotic preparations.

In colchicine treatment, mitotic abnormality (abnormality) was determined by comparing the mitosis with the mitosis of untreated cells (control). The mitotic index (IM) was determined using the formula from Pandey et al., [11]:

$$IM = \frac{\text{Number of cells in mitotic phase}}{\text{Total number of cells observed}} \times 100\%$$

Observation of Plant Morphology

Root morphology taken is the number of roots and root length by counting the number of roots in each replicate explant and measuring the root length with a ruler and the average value is taken.

Data analysis

Data on the mitotic index, chromosome abnormality, number of chromosomes, and the number and length of roots will be analyzed by comparing the average measurement results of the data obtained and displayed with bar charts. Qualitative data is shown in the form of photographs.

RESULTS

The results of cytological observations on root tip cells in yellow kepok banana plantlets (Table 1) showed that treatment using 0.1% colchicine (A2) resulted in an increase in the percentage of mitotic abnormalities compared to plantlets in the control treatment (A1). Treatment with 0.1% colchicine (A2) also resulted in an increase in the number of chromosomes more than the control treatment. The mitotic index in the observation of root tip cells of yellow kepok banana plantlets decreased where the results of the control (A1) were higher than the results of the treatment with 0.1% colchicine (A2). The number of roots in the control treatment plantlets (A1) was less than that of the 0.1% colchicine treatment (A2), but the root length in yellow kepok banana plantlets showed longer results in the control (A1) than the results of 0.1% colchicine treatment (A2).

Tabel 1. Results of Cytological Analysis of Kepok Kuning Banana Planlets

PARAMETER	AVERAGE	
	A1	A2
Mitotic Abnormalities (%)	14. 79%	20. 82%
Number of Chromosomes	33	37
Mitosis Index (%)	54.84%	49.72%
Number of Roots	1	2
Root Length (cm)	3.8	3

Description: A1 = Plantlet from the control treatment
A2 = Plantlets from the treatment of 0.1% colchicine

Mitosis has four stages, namely prophase, metaphase, anaphase, and telophase. In Table 1, mitotic abnormality showed a lower percentage in the control (A1) compared to 0.1% colchicine (A2). In this study, the mitotic stages in yellow kepok banana root tip cells in both the control (A1) and 0.1% colchicine (A2) experienced abnormalities at the mitotic stage, especially at the metaphase and anaphase stages. In the control sample (A1) there was a small amount of mitotic abnormality but the exact cause was unknown. The possible cause of mitotic

abnormalities in the control (A1) is a change in gene structure (mutation) due to the influence of the media used, namely MS (Murashige and Skoog) which contains ZPT [12]. Mitotic abnormalities of root tip cells of yellow kepok banana (Musa paradisiaca L.) plantlets treated with 0.1% colchicine (Figure 1).

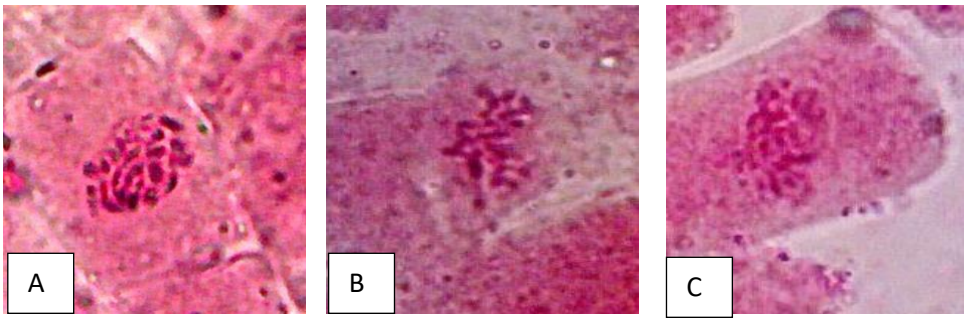

Figure 1. Mitotic abnormality in root tip cells of yellow kepok banana planlets.

Description: (A) Prophase, (B) Metaphase, (C) Anaphase. 100x magnification

The control (A1) produces triploid chromosomes (3x) with a chromosome number of 33, this is also conveyed by Anjasmara [13] that the yellow kepok banana is a group of Musa paradisiaca which has triploid chromosomes with a chromosome number of 33. Whereas the colchicine treatment of 0.1% (A2) increased the number of chromosomes to 37 with a ploidy level of 3x + 4, so it is suspected that yellow kepok banana plantlets with 0.1% colchicine treatment (A2) experienced polyploidization (Figure 2). Polyploidization is the doubling of the entire set of chromosomes during mitosis. The most commonly used artificial polyploidization is using colchicine [14]. **Figure 2**. Results of Observation of Number of Chromosome Cells at the Root Tip of the Yellow Kepok Banana Planlet. Information**:** (A) Colchicine 0,1% (A2) 37, (B) Control (A1) 33. 1000x magnification.

The results of the average value of mitotic index (IM) showed that the control (A1) was higher than the colchicine 0.1% (A2) (Figure 3). This shows that colchicine compounds affect the value of mitotic index allegedly because colchicine compounds inhibit the preparation of spindle threads, causing inhibition of cell division [15].

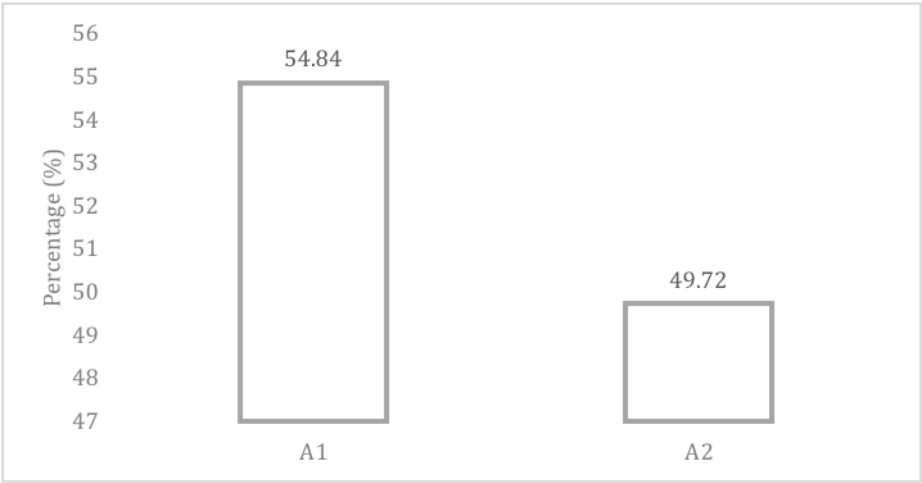

Figure 2. Diagram of the mitotic index of root tip cells of yellow kepok banana plantlets control (A1) and 0.1% colchicine (A2)

Colchicine compounds affect the number and length of roots. The average number of roots in the research that has been done has increased, but the length of the roots produced in the 0.1% colchicine treatment has decreased compared to the control. Giving colchicine can also cause growth hormones in plants that are assisted by colchicine with the right concentration can increase the growth of the number of plant roots [16]. However, although the number of roots in yellow kepok treated with 0.1% colchicine (A2) is more than the control (A1), it is different from the length of the roots, namely the control (A1) has longer roots than 0.1% colchicine (A2). Mitotic abnormality causes unbalanced chromosome division causing cell size to become larger and root length growth to be inhibited. This is supported by the literature of Fajrina et al. [16] state that colchicine treatment causes the time span of root initiation to be longer. The results obtained on andalas plants show that the higher the concentration of colchicine causes the slower the initial initiation of the roots with the same soaking time. Hanayanti and Pramudya [17] also stated that the administration of colchicine will cause inhibition of root growth. The increase in colchicine concentration caused a lower percentage of root growth.

The inhibition of root elongation is thought to be due to a disturbance in the auxin hormone. Crang et al. [18] reported that the hormone auxin functions to spur the process of root formation and root growth. The disruption of auxin hormone is thought to occur due to the presence of colchicine in the roots. This is in accordance with the opinion of Kantama et al. [12], which states that in polyploid plants, the presence of auxin hormone is disrupted (Figure 4).

Figure 3. Diagram of the number and length of yellow kepok roots in the control (A1) and 0.1% colchicine treatment (A2). Notes: (A1) Control (A2) Colchicine 0,1%

CONCLUSION

The conclusion of the research that has been done is the addition of colchicine compounds with a concentration of 0.1% in tissue culture media of yellow kepok banana causes chromosome abnormality with an average value of 20.82%, an increase in the number of chromosomes with an average value of 37, a decrease in mitotic index with an average value of 49.72%, an increase in the number of roots with an average value of 2, and an inhibited root length with an average value of 3 cm

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