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Response of Pineapple Callus (*Ananas comosus* Merr.) through *In-Vitro* Colchicines Treatment

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abstract

Pineapple Plant (*Ananas comosus* (L.) Merr) is a fruit plant which has a high economic value. Increased variety of pineapple preparations lead to increased demand for pineapple fruit. Polyploidation is one way to increase the varieties using colchicine. This study aims to know the pineapple explants of callus response after being treated by colchicine using the *in vitro* method. This research used Group Randomized Design (GRD) which variations concentration of Colchicine 0%, 0.01%, 0.05%, and 0.1%. Parameters were observed to response and thickness of callus of pineapple explants. Data were analyzed using the F test and DMRT test in which the significance level of 5% with SPSS 17 program. The results showed that pineapple plant explant able to respond colchicine concentration marked with thicker callus. The best colchicine treatment to induce pineapple explants callus was concentration 0.1% of colchicine.

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1. Introduction

Pineapple Plant (*Ananas comosus* (L.) Merr) is a fruit plant that has a high economic value. The increased variety of pineapple preparations results in an increased demand for pineapple fruit. In order to increase the production of pineapple, it is necessary to assemble superior varieties through plant breeding. During this time, seed multiplication is an important step in the development of new varieties. New varieties obtained from plant breeding can be reproduced by *in vitro* cultures. From the results of *in vitro* culture, it can be seen the reaction of plant resistance that can be cultivated through *in vitro* or directly in the cultivation area. New varieties that have been developed by research experts can be perceived benefits when they are reproduced and distributed to the community. The new varieties should be reproduced in such a way as to fit three requirements: prime quality, competitive prices, and consistent availability, in the sense that the product can be available on time and proportional. These three conditions are possible to achieve when production is made on a commercial

scale (Wurschum et al., 2012; Santoso and Sobir, 2013; Al-Khagh et al., 2015; Hossain, 2016).

The improvement of pineapple plants quality can be done by assembling pineapple plants through genetic mutations (doubling the number of chromosomes). The number of chromosomes in a normal or diploid pineapple has a number of chromosomes $2n = 50$ (Sousa, Carlier, Santo, and Leitão, 2013). Plants of polyploid may use colchicine as a mutagenic material (Friska and Daryono, 2017, Sari et al., 2017). According to Takahira et al. (2011), colchicine may be used to multiply the number of chromosomes to induce polyploidy. Ascough et al. (2008) suggested that colchicines can inhibit the formation and activity of spindle threads at the time of cell division of mitosis and prevent the nucleus and cells dividing so that the number of cell chromosomes multiplies. The mechanism of this compound is by binding β -tubulin dimer and inhibit the assembly of microtubules, but colchicines do not inhibit the work of bounded microtubules. The effects are the multiplication of chromosomes in the cells due to microtubule failure in pulling chromosomes toward the poles (Eigsti and Dustin, 1957). According to Dhooghe et al. (2011), multiplication of chromosomes using colchicines really depends on the concentration of the given colchicines.

Polyploidy is an organism which has more than two sets of chromosomes or genomes in its somatic cells. Some features of polyploidy plants include nuclei and larger cell contents; leaves and flowers which grow larger; and it may undergo changes in chemical compounds including an increase or change in the size or proportion of carbohydrates, proteins, vitamins, or alkaloids (As'adah et al., 2016 ; Sivakumar et al., 2017). Based on several studies, it appears that colchicine can cause ploitation of the *Ponkan Mandarindan* citrus (Dutt et al., 2009), tomato plants (Adelanwa et al., 2011), garlic root (Pharmawati and Waitiani, 2015), maize (Winaryo et al , 2016), and red ginger (Friska and Daryono, 2017) marked by chromosomal changes as well as increasing chromosome number, species change in rose plants (Sarwar and Butt, 2015), regeneration and variation of leaf number on *Bacopa monnieri* (Kharde et al , 2017) and induce embryonic development (Azmi et al., 2015). Beside colchicines concentrations, the timing of colchicines treatment may also affect polyploidy of plants. Silva et al. (2000) reported that the immersion of protokorm *Cattleya intermedia* in colchicine 500 and 1,000 mg / L for 4 days can produce tetraploid plants. It is also reported by

Kerdsuwan and Techato (2012) that the *Rhyncostylis gigantea* plant var. the tetraploid rubrum was produced from the immersion of protocorm in colchicine 2.000 mg / L for 3 days.

One way for colchicines applications is by applying tissue culture techniques. Tissue culture is a method to produce identical large-scale disease-free plantlets (Khan et al., 2004). Therefore, it is necessary to innovate the treatment of colchicines through tissue culture techniques so that it can produce polyploidy plants quickly (Sarwar and Butt, 2015; Shivakumar et al., 2017).

On the basis of the mindset about the potential of pineapple in Indonesia and colchicines as polyploidy mutagen, so it is expected to improve the quality of pineapple fruit after immersion with colchicines through tissue culture techniques. The purpose of this research is about to find out explants phenotypic response of the pineapple plant (*Ananas comosus* Merr.) through *in-vitro* colchicines treatment.

2. Method

This research is an experimental research with Randomized Block Design with 0%, 0.01%, 0.05% and 0% concentration of colchicines, and immersion period of 72 hours. Each treatment was repeated 3 times with three explants per treatment. The sample used in this research is the explosion of pineapple varieties of Smooth Cayenne obtained from pineapple plantation of *Kelud* mountain slope. Colchicine was diluted with MS + ZPT media (2,4 D 0.5 ppm / l and BAP 1 ppm / l) based on the concentration.

2.1 Media MS + ZPT Manufacturing

Manufacturing of MS + ZPT media by dissolving MS medium with distilled water and added 2,4 D 0,5 ppm / l and BAP 1 ppm / l and sugar 30g / l. The solvent was adjusted to a pH of 5.9 by adding KOH or HCl 1 N. The solvent was added 8 g / l gelatine, then cooked until the solvent is boiled. After boiling the solvent, put it in a bottle and autoclaved with a temperature of 121⁰C.

2.2 Colchicines Solvent Manufacturing

Manufacturing of colchicines solvent is done by weighing colchicines based on treatment concentration using an analytical scale, then dissolved into liquid MS medium. The solvent

pH was measured to 5.6 using pH meter. Sterilization of colchicines solvent using Millipore 0.20 μm in laminar air flow cabinet (LAFC).

2.3 Colchicine Induction

Induction of pineapple explants colchicine was performed in LAFC by cutting the previously pinned pineapple crown and then immersed in a treated colchicine solvent of 0%; 0.01%; 0.05%; and 0.1% were soaked for 72 hours while shaken by using a shaker to give the colchicine solvent about the entire explant part. The treated explants colchicine was then rinsed with sterile aquades and dried with filter paper then planted on MS medium without ZPT and stored in the culture room by irradiation using 20 W lamps for 16 hours / day with an average light intensity of 1,500-2,000 lux, with a temperature of 16-20⁰C for two weeks then subcultured to growth media ie MS + ZPT media (2,4 D 0,5 ppm / l and BAP 1 ppm / l) and stored back into the culture room. The explants are planted on a bottle for about 3 explants per bottle.

2.4 Observation

The parameters observed in this study include the number of explants that form callus and callus thickness for 1 month after planting.

2.5 Statistical Analysis

The data in this research is in the form Quantitative data of callus thickness then analyzed statistically using analysis of variance (F test) with a 5% significance level. When F arithmetic is greater than F table. It is continued to Duncan's Multiple Range Test (DMRT) with a 5% significance level. This test is used to determine optimal treatment.

3. Result and Discussion

Colchicine is a synthetic mutagenic agent commonly used in agriculture. Its wide application is usually used to make larger shape shifts of fruits, vegetables and other cultivated plants (Wu et al., 2012; Hsiao-Hang et al., 2017). In this study, the medium used in callus growth was a medium enriched with 2.4 D and BAP hormones with concentrations of 0.5 ppm and 1 ppm. The role of the hormone is to modulate CDKs at the root tip in order to provide control of cleavage and endoreduplication in plants (Tank and Thaker, 2014;

Simmons and Bergmann, 2016). The hormones present in the callus growth medium have a synergistic effect with colchicine, so the callus in pineapple plants has mass and size increases (Ikeuchi et al., 2013; Kai et al., 2017). The result of the observation on the explants response of queen pineapple varieties shows the influence of concentration level on callus response and callus thickness. The treated callus response can be seen in table 1 below.

Table 1 The Response Percentage of Callus Pineapple Formation of Smooth Cayenne Varieties After Colchicine Treatment

Concentration	Percentage Average of Callus Formation Response
0 %	11.1 % a
0,01 %	33.3 % ab
0,05 %	88.9 % cd
0,1 %	100 % d

Notes: Numbers with the same letters show that there is no significant difference based on Duncan Test 0,5%, n=3

Based on table 1, it can be seen that the concentration of colchicines gives a good response to the development of explants callus of pineapple. The best concentration to give the response of explants callus of pineapple is 0,1% concentration of colchicines solvent that is all explants 100% are smooth after soaked with colchicines. It is noted by the increasing of thickness as the concentration of colchicine is given. Sari et al., 2017 stated that the treatment of colchicines at various concentrations of morphological characters and the cytology of the Bombay silk plants was found to have an effect of wet weight gain on plants. The effects of colchicines treatment also affect the germination response of Marigold Pot (*Calendula officinalis* L.) (El-Nashar and Asrar, 2016). The thickness of callus after given various colchicine can be seen in table 2 below.

Table 2 Callus thickness and Number of Chromosm Pineapple Variety Smooth Cayenne After Colchicine Treatment

Concentration	Average of Callus Thickness (cm)	Number of Chromosome
0%	0,107 a	51 a
0,01%	0,113 ab	67 bc
0,05%	0,132 cd	60,3 ab
0,1%	0,153 d	75,3 c

Notes: Numbers with the same letters show that there is no significant difference based on Duncan Test 0,5%, n=3.

Based on table 2, it is known that 0.1% colchicine concentration gives the best treatment for explants callus of pineapple initiation. Because at the concentration, it is optimal to induce polyploidy explants callus of pineapple. This can be known from the results of chromosome calculations after being treated with colchicines, it is increased almost 1.5 times of pine chromosome that is not treated with colchicines that are approximately 51 pieces. Plants with triploid chromosomes have morphological characteristics of large organs, large biomass and resistance to environmental stress conditions, as well as an increase in photosynthetic energy (Wang et al., 2016; Shanko, 2017).

The number of chromosomes in the colchicine treatment of 0.1% concentration has the largest number of chromosomes when compared to other treatments. The number of chromosomes in a normal or diploid pineapple has a number of chromosomes $2n = 50$ (Sousa, Carlier, Santo and Leitão, 2013). The combination of given colchicine can increase the production of multiple haploids on tetraploid chromosomes and increase fertility in plants (Palva et al., 2013; Aurelia, et al., 2017). Based on Table 2, it is known that pineapple callus has 75.3 pieces (1.5 X from normal) after being treated with colchicine. Hence, it can be said that the pineapple callus has increased in the number of chromosomes after being given colchicines for 72 hours. According to Avery et al. (1947), he stated that the changes that occur in plants vary widely due to the treatment of colchicines. The presence of different effects on plant cells due to colchicine is only effective in the cells that are being actively dividing (Nilanthi et al., 2009; Wurschum et al., 2012). This is in line with research conducted by Pandey et al., 2014 which shows that the growth of the four different species of fungi (*Glomus mossae*, *Glomus fasciculatum*, *Gigaspora margarila* and *Gigaspora gilmores*) has increased in the colchicine-added medium of *Gloriosa superba*.



Figure 1 Callus Response after Treatment (1) Callus Response without Treatment and (2) Callus Response after Treatment of Colchicine

Colchicine in plants causes binding of β -tubulin dimer and inhibits the assembly of bound microtubules. So the effect is the multiplication of chromosomes in cells due to microtubule failure in pulling chromosomes toward the poles (Eigsti and Dustin, 1957; Nara et al., 2013). It can cause the proliferation of chromosomes in the cell. The multiplication causes the cell to increase in size. This is in accordance with the results of the study that the concentration of colchicine affects the thickness of the pineapple explants callus which is shown in Figure 1. It is reinforced by Wardhani and Wiendi (2012) research that the long treatment of immersion with colchicine has a very significant effect on callus induction *Glycine max* L. Merr varieties Wilis which has a long period of 24 hours immersion at a concentration of 0.01% with the percentage of largest callus of 95% at week 8 of MST. Another study conducted by Haryanti et al. (2009) concluded that the cell size of the plant by treatment of colchicine with a concentration of 0.10%; 0.15% and 0.20% is greater than controlled cell plant, or in the 0.05% concentration. Tiwari and Mishra (2012) explained that colchicines can give a different morphological character on *Phlox drummondii* of Polemoniaceae family in the form of thickening on the leaves, the darker color on the flowers and the growth of the more compact organ. Colchicine in *Actinidia chinensis* plants can increase the size and shape of the fruit (Wu et al., 2012). It is evident that colchicine can cause ploitation in meristematic cells.

The increase of these chromosomes leads to an increase in the size of the cell. It can be known from the findings that the higher concentration of colchicines, the thicker will be the callus on pineapple explants. The increase of these chromosomes causes the cell size to grow larger and result in an increase in the size of the vegetative organs such as roots, stems, leaves, flowers, and fruit (Burns, 1972, Schwarzacher, 2016).

4. Conclusion

Based on the findings and discussion above, it can be concluded that the concentration of Colchicines can affect the growth of pineapple callus. It can be identified from the increased callus thickness compared to the controlled treatment. The best treatment for callus induction is on colchicines of 0.1%.

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