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The Morphology Character of Japanese Taro (*Colocasia esculenta* var. *Antiquorum*) In Induction of Polyploidization Mutations In Vitro: A Case Study of Increased Concentration and Duration of Immersion of Colchicine Mutagens

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Abstract

Japanese taro is an alternative food commodity and has prospects as an export commodity, especially to Japan. Colchicine is a mutagen that can be used in polyploid mutations but can be toxic in plants. The purpose of this study was to determine the effect of various concentrations of colchicine and immersion time on the growth and morphological characters of Japanese taro plantlets in vitro. In this study, there were 2 focus factors of treatment. The first factor is colchicine concentration consisting of 0.0% (K0/control), 0.1% (K1), 0.2% (K2) and 0.3% (K3). the second factor is the immersion time consisting of 48 hours (T1), 72 hours (T2) and 96 hours (T3). The treatment of in vitro shoot immersion with 0.1%, 0.2% and 0.3% concentration of colchicine solution for 48 hours, 72 hours and 96 hours was significantly different from the control. Explant growth decreased every week and died at 6 MSI on colchicine treatment. The level of concentration and duration of immersion with different colchicine caused different color changes of the explants, the higher the concentration of colchicine and the duration of immersion, the color of the explants would quickly turn brown.

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Introduction

Japanese taro (*Colocasia esculenta* var. *Antiquorum*) or Satoimo taro (Japan) is an alternative food commodity that is currently gaining popularity in Indonesia. The popularity of Japanese taro cultivation is due to its good economic value and prospects, especially as a food ingredient and export commodity to Japan. Most of the Japanese population consume Japanese taro as a staple food. Based on data from SEAMEO (2013), the demand for Japanese taro in Japan reaches ±360,000 tons per year, while production capacity in Japan continues to decline to 250,000 tons per year. The decrease was due to limited land and climatic factors that made it impossible to plant throughout the year. This opens up opportunities for tropical countries, such as Indonesia, to export Japanese taro plants to Japan.

The Indonesian government continues to encourage local governments to increase the productivity of Japanese taro. Several local governments such as Kepahiang, Cisarua, Bantaeng, Malang and Buleleng (Maretta et al., 2016; Wahyuni, 2019) encourage their

farmers to develop Japanese taro as an export commodity. This results in the need for a large and continuous supply of seeds.

Fulfillment of seeds in large quantities and sustainable is certainly something that must be fulfilled to support productivity. One method that can be used is by in vitro propagation. In vitro propagation can produce seeds in large quantities and in a relatively short time and uniformly so that it can be one solution. On the other hand, the productivity of Japanese taro in Indonesia is still very low so that innovative solutions are needed to increase the productivity of Japanese taro.

Research to increase the productivity and quality of Japanese taro can be done among others through the induction of polyploidization mutations. Induction of polyploidization aims to increase crop productivity because polyploid (tetraploid) plants are known to have a larger figure, fruit size, tuber or flower than diploid plants (Suryo 2007).

Polyploidization can occur in plants either naturally or induced by antimitotic chemicals, such as orizalin, trifularin, amiprofos methyl, and colchicine. Induction of polyploidization can be done by giving chemical mutagens such as colchicine to plant meristem tissue. Colchicine can not only change the number of plant chromosomes but can cause gene mutations on the scale of seeds and vegetatively propagated plants. This induces polyploidy by inhibiting the formation of spindle fibers during cell division, while the number of chromosomes increases but cell division does not occur, resulting in the production of polyploid cells (Manzoor et al., 2018).

The success of polyploidization induction using colchicine application, depends on the part of the plant used, the species of the plant, the concentration of colchicine used and the duration of exposure to the plant. Concentrations that are too high often cause problems in the form of abnormalities in developing seedlings (Manzoor et al., 2018). So this needs to be done very carefully by a plant breeder.

Polyploidy induction of Japanese taro has never been done. Induction of polyploidy is expected to produce plants that have high productivity and are resistant to various diseases after various stages of selection so that they are superior to diploid taro. This study aimed to study the effect of various levels of colchicine concentration and immersion time on the growth and morphological characters of Japanese taro plantlets in vitro.

Materials and Methods

This research was conducted at the Tissue Culture Laboratory, Department of Agrotechnology, Faculty of Agriculture, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10 Tamalanrea Makassar. The study used a 2-factor Completely Randomized Design (RAL2F) with 3 replications and 5 explants for each treatment. The first factor is the treatment with colchicine concentration and the second factor is the soaking time treatment. The concentration of colchicine used as the first factor was 0% (control), 0.1%, 0.2% and 0.3%. The immersion time used as the second factor was 48 hours, 72 hours and 96 hours. Concentration of 0% (control) was still soaked according to the second factor with sterile distilled water.

Table 1. Combination of Treatment between Colchicine Concentration and Soaking Time

Concentration	Immersion Time		
	T1	T2	T3
K0	K0T1	K0T2	K0T3
K1	K1T1	K1T2	K1T3
K2	K2T1	K2T2	K2T3
K3	K3T1	K3T2	K3T3

Description: K0 (0%), K1 (0.1%), K2 (0.2%), K3 (0.3%), T1 (48 hours), T2 (72 hours), T3 (96 hours)

Plantlets used as material for polyploidy induction were in vitro shoots of Japanese taro from 8 weeks old subculture which were then used as explants. The in vitro shoots were removed from the midrib to 1 cm in size. The shoots were immersed in a culture bottle containing 25 ml of colchicine solution and shaken at 100 rpm on a shaker. After immersion, the explants were washed with sterile distilled water 3 times, removed the outer midrib, and then planted into shoot and root propagation media. The shoot and root propagation medium used was Murashige and Skoog (MS) media with the addition of 2 mg.L-1 BAP and 1 mg.L-1 IBA. The medium contains sugar (30 g.L-1), the pH of the media is adjusted to 5.8 and compacted with agar (7 g.L-1). The shoots were kept in an incubation room at a temperature of 25-26°C with 16 hours of light per day.

In vitro shoot growth observations of Japanese taro after colchicine immersion were carried out during incubation (1-6 MSI). Observation parameters include the number of compound shoots (saplings), the number of leaves, the number of roots and the number of live/dead shoots. Observations were also made on plantlet color after treatment. Observational data were analyzed using Analysis of Variance (ANOVA) followed by a real difference test using 5% BNJ. The 5% BNJ test was carried out using the Statistical Tool for Agricultural Research (STAR) application.

RESULTS AND DISSCUSSION

Percentage of Induction Live Shoots

Colchicine is a mutagen that can affect the ability of a plant such as slowing down the ability to sprout, root or leaf formation. In this study, treatment with different concentrations of colchicine with different soaking times affected the percentage of live Japanese taro shoots (Table 2).

The percentage of live shoots in the control treatment and colchicine 0.1% and 0.2% for 2 days of immersion did not show any shoot death up to 2 MSI, but at 5-6 MSI at a concentration of 0% shoot death occurred. At concentrations of 0.3% (48 hours immersion), 0.2% and 0.3% (72 hours immersion) and 0.1%, 0.2%, 0.3% (96 hours immersion) started to cause death from 1 MSI. Observations at 1 MSI showed that 100% of shoots were alive in the control and treatment with colchicine immersion for 2 days at 0.1% and 0.2% and 3 days immersion in 0.1% colchicine. However, there was shoot death of all explants at a concentration of 0.3% (48 hours immersion) at 3 MSI and 0.3% colchicine concentration (72 hours immersion) at 2 MSI. At 6 MSI all colchicine treatments experienced shoot death except in controls.

Table 2. Average Life Percentage of Japanese Taro Shoots Resulting from Colchicine Treatment and Soaking Time at Age 1 to 6 MSI

Immertion Time (hours)	Colchicine Consentration (%)	% Shoots Live At Age (Week)					
		1	2	3	4	5	6
Kontrol	0	100,0	100,0	100,0	100,0	86,6	86,6
	0,1	100,0	100,0	53,3	33,3	33,3	00,0
48	0,2	100,0	100,0	33,3	20,0	13,3	00,0
	0,3	86,6	33,3	00,0	00,0	00,0	00,0
	0,1	100,0	66,6	53,3	33,3	20,0	00,0
72	0,2	93,3	86,6	46,6	20,0	13,3	00,0
	0,3	53,3	00,0	00,0	00,0	00,0	00,0
	0,1	60,0	60,0	46,6	46,6	20,0	00,0
96	0,2	86,6	66,6	40,0	13,3	13,3	00,0
	0,3	93,3	86,6	33,3	20,0	13,3	00,0

The percentage of live shoots decreased with increasing colchicine concentration and soaking time. The results obtained from this study on Japanese taro were an increase in the concentration and duration of immersion of colchicine caused a decrease in the survival percentage of taro shoots to 5 MSI and resulted in the death of all plantlets at 6 MSI. In some studies using colchicine can produce shoots to form plantlets. However, in this study, they did not grow to become plantlets. This is presumably due to the different conditions of the cells in each plant so that the sensitivity of the cells also differs in responding to the treatment of high concentrations of colchicine and longer immersion time. In addition, the difference in the age of the plantlets used as explants was suspected to be the cause of the difference in tolerance between explants and colchicine so that the explants became weak and reduced their survival percentage. The smaller growth of induced shoots was thought to be due to damage to plant tissue after exposure to colchicine so that it took a longer time for recovery (Damayanti and Mariska 2003).

Number of Induced Roots and Shoots

Root growth in immersion for 48 hours showed an increase in concentration of 0% (control) at the age of 2 MSI and constant until the age of 3-4 MSI, but decreased at the age of 5-6 MSI. The decrease occurred due to wilting which then died in Japanese taro plantlets. Root growth in the control was greater than the colchicine treatment. At 48 hours of immersion, all concentrations (0.1%, 0.2% and 0.3%) increased at the age of 5-6 MSI and 4 MSI at concentrations of 0.1% and 0.2% (figure 1a). The highest number of roots in shoots treated with colchicine was found at a concentration of 0.1% with 2 roots and gave the highest average number of roots of 0.8 for colchicine treatment.

At 72 hours of immersion, there was also a decrease in the concentration of 0% (control) due to wilting which then died. at 72 hours immersion all concentrations increased and were constant (figure 1b). At the concentration of 0.1%, 0.2% and 0.3% at the age of 1-4 MSI did not increase the number of roots. At 0.1% and 0.2% immersion there was an increase at the age of 5-6 MSI with 1 root each. At a concentration of 0.3% there was no increase until the age of 5-6 MSI. The highest average number of roots for soaking colchicine for 72 hours was at concentrations of 0.1% and 0.2% with an average of 0.2, respectively. At 96 hours of immersion, the concentration of 0% (control) did not decrease until the age of 5-6 MSI. In colchicine immersion for 96 hours, it was constant and increased with each age (figure 1c). At

concentrations of 0.1%, 0.2% and 0.3% there was no increase in the number of roots from 1 MSI to 4 MSI. However, there was an increase at the age of 5-6 MSI, namely 1 root each.

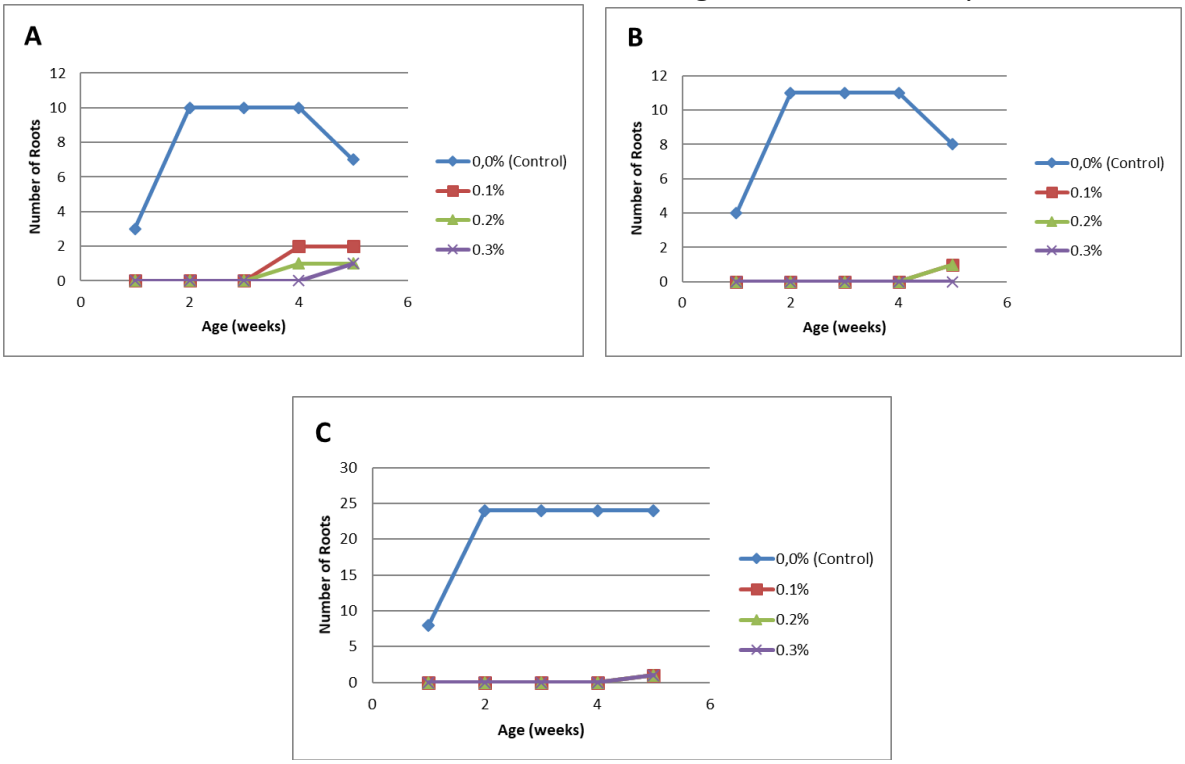

Figure 1. Graph of Root Number of Japanese Taro Explants Resulting from Colchicine Induction for 6 MSI

Description: a) Immersion 48 hours; b) Immersion 72 hours; c) Immersion 96 hours

The results of the ANNOVA test analysis showed that the 0% colchicine concentration treatment (control) was significantly different from the 0.1%, 0.2% and 0.3% colchicine treatments in influencing the number of root explants. Soaking and concentration of colchicine were significantly different in influencing the number of roots. The interaction between soaking time and colchicine concentration was significantly different in influencing the number of roots.

Table 3. Results of the 5% BNJ Test Analysis of Total Roots of Japanese Taro Shoots Induced by Colchicine Treatment and Immertion Time

Treatment	Average number of Japanese taro shoots at the age of 6 MSI			NP BNJ 5% (K)
	T1	T2	T3	
K0	2,33 $^{a}_{q}$	2,67 $^{a}_{q}$	8,00 $^{a}_{p}$	1,56
K1	0,67 $^{b}_{p}$	0,33 $^{b}_{p}$	0,33 $^{b}_{p}$	
K2	0,33 $^{b}_{p}$	0,33 $^{b}_{p}$	0,33 $^{b}_{p}$	
K3	0,33 $^{b}_{p}$	0,00 $^{b}_{p}$	0,33 $^{b}_{p}$	
NP BNJ 5% (T)		1,56		

Notes: Numbers followed by the same letter in the same column are not significantly different in the 5% BNJ test; MSI = Week After Induction

The results of the 5% BNJ follow-up test showed that the addition of the highest number of roots in Japanese taro explants obtained K0T3, namely control (0%) with 96 hours of immersion and significantly different from other treatments at the age of 6 MSI (Table 3). The roots formed in this study were still in the form of root nodules which were identified by paying attention to the shoots. The color of the root nodules is white so that it can be distinguished from compound shoots. Root growth was stunted during the study and some were damaged. At 6 MSI, the explants died so that the roots did not grow again. According to Mugiono (2001), physiological damage caused by colchicine treatment can be caused by chromosomal damage or cell damage outside the chromosome. Colchicine is known to be toxic to plants, thereby reducing the vegetative growth of post-induction polyploid plants, such as mung bean (Haryanti et al., 2009).

Number of Induced Shoots

Leaf growth in colchicine treatment 0.1% (K1T1, K1T2, K1T3), 0.2% (K2T1, K2T2, K2T3) no leaves. While in K0T1 at 1 MSI there were 11 leaves, 2 MSI 24 leaves, 3 MSI 29 leaves, 4 MSI 28 leaves, 5 MSI 17 leaves and 6 MSI 17 leaves. In K0T2 there were 1 MSI 18 leaves, 2 MSI 32 leaves, 3 MSI 33 leaves, 4 MSI 35 leaves, 5 MSI 31 leaves and 6 MSI 31 leaves. In K0T3 there were 1 MSI 7 leaves, 2 MSI 16 leaves, 3 MSI 30 leaves, 4 MSI 32, 5 MSI 65 leaves and 6 MSI 65 leaves. The decrease in the number of leaves at K0T1 and K0T2 at 5 MSI was due to some leaves dying and wilting, but some when entering 6 MSI the leaves began to grow again (Figure 2).

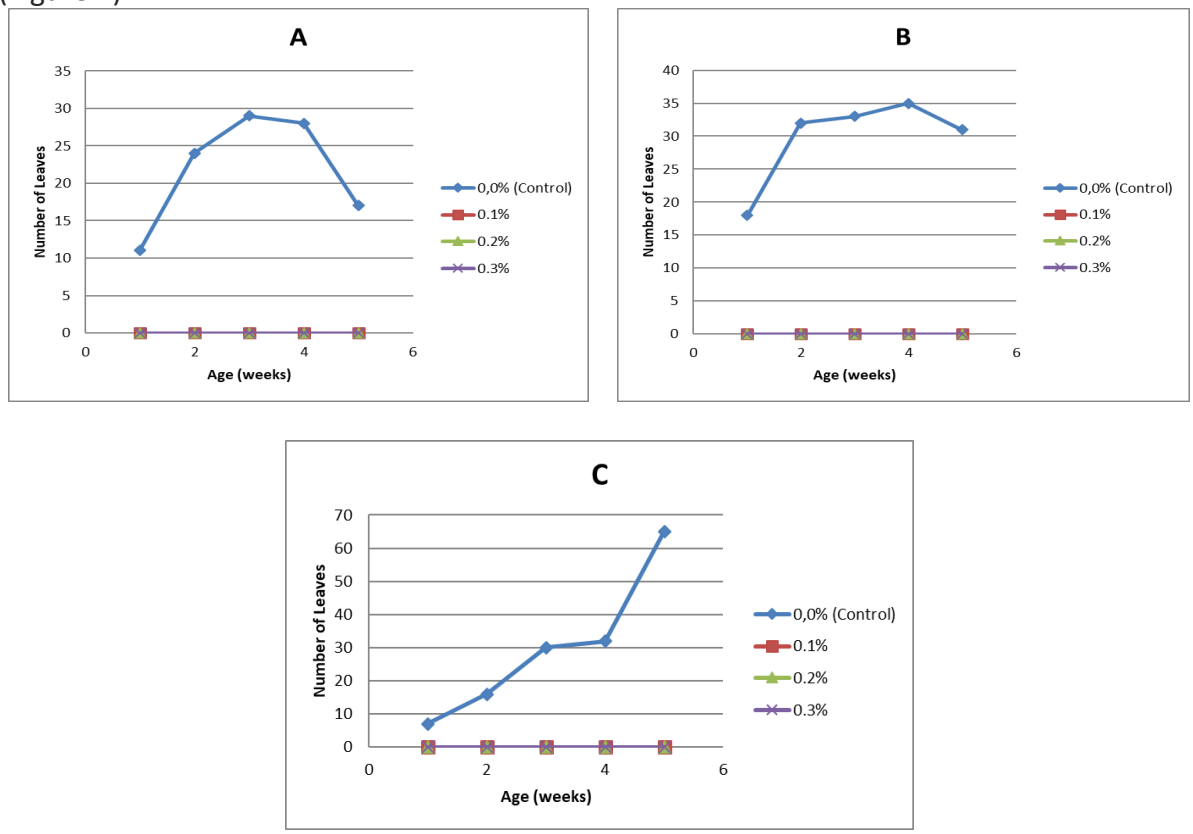

Figure 2. Graph of Leaf Number of Japanese Taro Explants Result of Colchicine Induction for 6 MSI

Description: a) Immersion 48 hours; b) Immersion 72 hours; c) Immersion 96 hours

The results of the ANNOVA test analysis showed that the treatment with 0% colchicine concentration (K0T1, K0T2, K0T3) was significantly different from 0.1% colchicine treatment (K1T1, K1T2, K1T3), 0.2% (K2T1, K2T2, K2T3) and 0.3% (K3T1, K3T2 , K3T3) in influencing the number of leaf explants. Soaking had no significant effect, but the concentration of colchicine was significantly different in influencing the number of leaves. The interaction between soaking time and colchicine concentration was significantly different in influencing the number of leaves.

Table 4. Analysis Results of 5% BNJ Test Number of Japanese Taro Leaf Shoots Induced by Colchicine Treatment and Immertion Time

Treatment	Average number of Japanese taro shoots at the age of 6 MSI			NP BNJ 5% (K)
	T1	T2	T3	
K0	5.67 $^{a}_{q}$	10.33 $^{a}_{q}$	21.67 $^{a}_{p}$	7.16
K1	0.00 $^{a}_{p}$	0.00 $^{b}_{p}$	0.00 $^{b}_{p}$	
K2	0.00 $^{a}_{p}$	0.00 $^{b}_{p}$	0.00 $^{b}_{p}$	
K3	0.00 $^{a}_{p}$	0.00 $^{b}_{p}$	0.00 $^{b}_{p}$	
NP BNJ 5% (T)		7.16		

Notes: Numbers followed by the same letter in the same column are not significantly different in the 5% BNJ test; MSI = Week After Induction

The results of the 5% BNJ follow-up test showed that the addition of the highest number of leaves in Japanese taro explants was obtained by K0T3 and was significantly different from other treatments at the age of 6 MSI (Table 4). In observing the number of induced shoots, it was found that 0.1% (K1T1, K1T2, K1T3), 0.2% (K2T1, K2T2, K2T3) and 0.3% (K3T1, K3T2, K3T3) treatments had no leaves while at 0% (K0T1 , K0T2, K0T3) there are leaves. The highest number of leaves was found in K0T3 with a total of 21,67.

Number of Induced Tillers

The growth of tillers (compound shoots) in the colchicine treatment of 0.1% (K1T1, K1T2, K1T3), 0.2% (K2T1, K2T2, K2T3) and 0.3% (K3T1, K3T2, K3T3) showed no tillers. While in control (K0T1) at 1 MSI there were 6 tillers, 2 MSI 16 tillers, 3 MSI 17 tillers, 4 MSI 15 tillers, 5 MSI 7 tillers and 6 MSI 7 tillers. In K0T2 there were 1 MSI 10 tillers, 2 MSI 32 tillers, 3 MSI 32 tillers, 4 MSI 32 tillers, 5 MSI 25 tillers and 6 MSI 25 tillers. In K0T3 there were 1 MSI 6 tillers, 2 MSI 24 tillers, 3-6 MSI 29 tillers. The decrease in the number of tillers at 0% concentration (K0T1 and K0T2) at 5 MSI was due to some leaves dying and withering, but some when entering 6 MSI the leaves began to grow again.

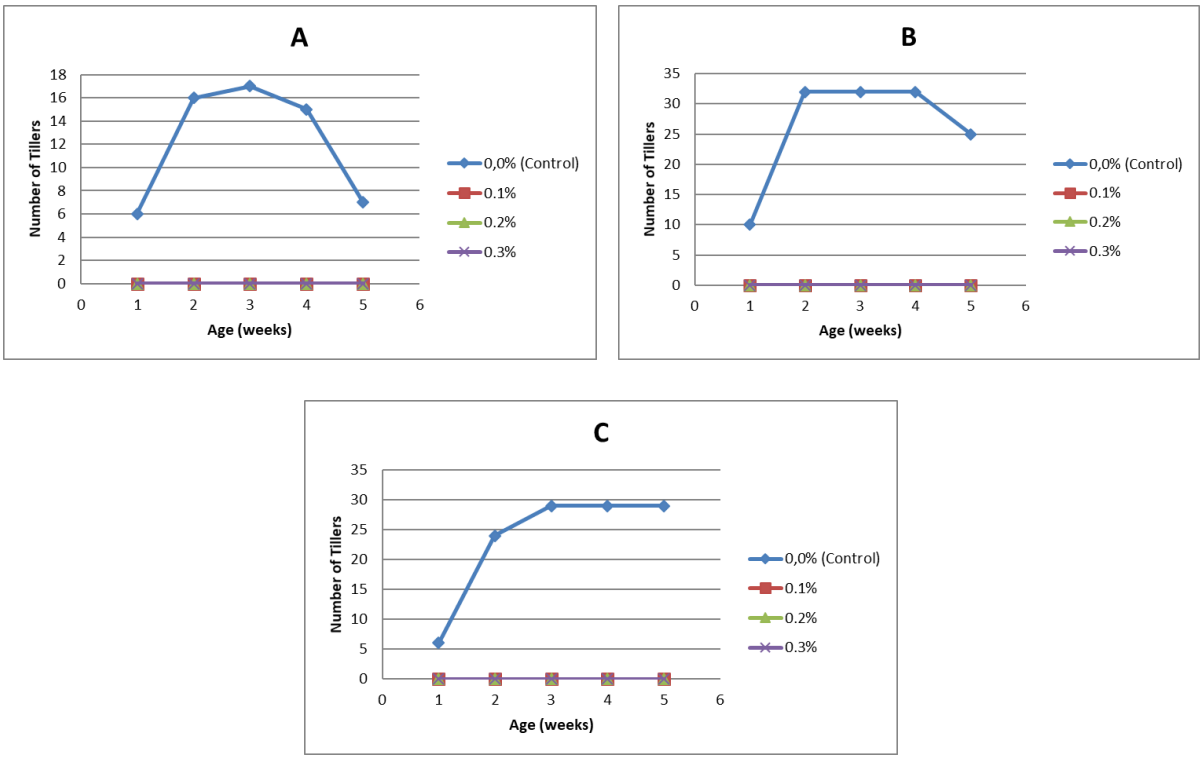

Figure 3. Graph of the number of tillers of Japanese taro explants produced by colchicine induction for 6 MSI

Description: a) Immersion 48 hours; b) Immersion 72 hours; c) Immersion 96 hours

The results of the ANNOVA test analysis showed that the treatment with 0% colchicine concentration (K0T1, K0T2, K0T3) was significantly different from 0.1% colchicine treatment (K1T1, K1T2, K1T3), 0.2% (K2T1, K2T2, K2T3) and 0.3% (K3T1, K3T2 , K3T3) in influencing the number of explant tillers. Soaking had no significant effect, but the concentration of colchicine was significantly different in influencing the number of tillers. The interaction between soaking time and colchicine concentration was significantly different in influencing the number of tillers. The results of the 5% BNJ follow-up test showed that the addition of the highest number of tillers in Japanese taro explants was obtained by K0T3 and was significantly different from other treatments at the age of 6 WAP (Table 5).

Table 5. Results of the 5% BNJ Test Analysis of the Number of Japanese Taro Shoots Induced by Colchicine Treatment and Immertion Time

Treatment	Average number of Japanese taro shoots at the age of 6 MSI			NP BNJ 5% (K)
	T1	T2	T3	
K0	2.67 $^{a}_{q}$	8.33 $^{a}_{q}$	9.67 $^{a}_{q}$	1.78
K1	0.00 $^{a}_{p}$	0.00 $^{b}_{p}$	0.00 $^{b}_{p}$	
K2	0.00 $^{a}_{p}$	0.00 $^{b}_{p}$	0.00 $^{b}_{p}$	
K3	0.00 $^{a}_{p}$	0.00 $^{b}_{p}$	0.00 $^{b}_{p}$	
NP BNJ 5% (T)		1.78		

Notes: Numbers followed by the same letter in the same column are not significantly different in the 5% BNJ test; MSI = Week After Induction

Explant Color During Incubation

Observation of the color of the explants was carried out to see the effect of the concentration of colchicine and the duration of immersion on the color of the explants that occurred. In the study, it was found that the average colchicine treatment had a slightly brownish color in the explants at the end of the immersion and at 2 MSI to 6 MSI they experienced browning (brown) and when they entered 6 MSI, all explants died except for controls (Figure 4).

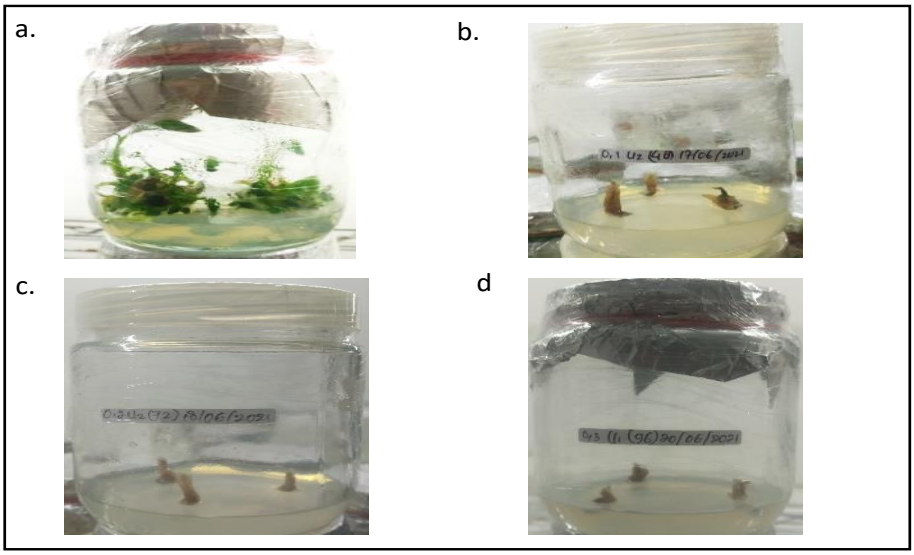

Figure 4. Colors of Japanese Taro Explants Result of Colchicine Induction for 6 MSI Description: a) Colchicine 0% (Control); b) Colchicine 0.1%; c) Colchicine 0.2%; d) Colchicine 0.3%

Based on observations of the color of Japanese taro shoots during polyploid induction (figure 4), it was found that there was a change in shoot color in explants treated with various levels of colchicine concentration. The response to changes in explant color every week was different for each concentration. In the control treatment (0% with immersion 24 hours, 72 hours and 96 hours) the color of the explants tended to be stable from the beginning of incubation to the end of incubation. Meanwhile, in the treatment of various concentrations of colchicine (0.1%, 0.2% and 0.3% with immersion time of 48 hours, 72 hours and 96 hours) changes were seen every week. The color of the explants at the beginning of incubation was greenish but at 1 MSI there was a treatment that showed a color change, namely in some explants at concentrations of 0.2% and 0.3% with a soaking time of 72 hours and 96 hours. At the age of 3 MSI to 6 MSI, color changes continued to occur at all concentrations of colchicine treatment. At 6 MSI, all treatment explants at the colchicine concentration level experienced overall browning and died. Some explants that have shown root nodules have also become browning, so that in this study no cytology test (chromosomal analysis) was carried out.

Colchicine not only helps in the doubling of chromosomes, but also causes mutations in plants. Plants that have mutated through colchicine are known as colchi-mutants (Ari et al., 2015). The concentration of colchicine for seed treatment is usually in the range of 0.1%-0.8%, but high doses cause malformations and reduce the production of tetraploid plants. So, it is recommended to use colchicine with the lowest possible concentration (Pirkoohi et al., 2011). Since coccicin is highly toxic to plants, therefore low doses with a long exposure period are considered reliable to reduce its toxic effects and increase the rate of polyploid production (Sajjad et al., 2013).

CONCLUSIONS

The treatment of in vitro shoot immersion with 0.1%, 0.2% and 0.3% concentration of colchicine solution for 48 hours, 72 hours and 96 hours was significantly different from the control. Explant growth decreased every week and died at 6 MSI on colchicine treatment. Shoot growth after colchicine treatment was slower than control. The level of concentration and duration of immersion with different colchicine causes different color changes of explants, the higher the concentration of colchicine and the duration of immersion, the color of the explants will quickly turn brown. Colchicine has toxic properties that can kill plants when applied in high concentrations. Each concentration of colchicine and soaking time gave different effects on Japanese taro explants. The higher concentration affects the inhibition of the growth of explants and can cause damage to the explants.

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