Agrotech Journal
Url: http://usnsj.id/index.php/ATJ
Email: editor.atj@usnsj.com

The Effect of BAP Concentration on *In-Vitro* Mutant Taro Regeneration

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ARTICLE INFO

e-ISSN: 2548-5148
p-ISSN: 2548-5121
Vol. 7 No. 2, December 2022
URL: http://dx.doi.org/10.31327/atj.v7i2.1873

Abstract

Plant regeneration through invitro culture is strongly influenced by the use of growth regulators. BAP is a class of cytokinins that affect cell division which is very well used to stimulate plant growth. The aim of the study was to determine the effect of BAP concentration on the regeneration of various *in vitro* mutant taro. The study was carried out at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar. The method used was factorial completely randomized design with 2 factors as the environmental design. The first factor was the BAP concentrations of 0.0 mg/L, 1.0 mg/L, 1.5 mg/L, 2.0 mg/L and 2.5 mg/L. The second factor was mutant plants consisting of control plants (diploid), mutant 1 (*Putative-Tetraploid),* mutant 2 (Mixoploid), mutant 3 (Mixoploid), and mutant 4 (*Putative-Tetraploid*). The results showed that the best regeneration was obtained by BAP concentration of 0 mg/L with control plants on the parameters of root length (2.41 cm), number of roots (11.66 pieces) and plantlet formation rate (13 days). The BAP concentration of 1 mg/L was shown by mutant plant 1 on the parameters of the number of shoots (26 pieces) and number of leaves (16 pieces). BAP concentration of 0 mg/L in mutant 4 had a shoot height of 1.83 cm. The results of ploidy flow cytometry analysis of mutant plants *with Putative-Tetraploid* parental traits after regeneration resulted in detectable diploid chromosomes that were different from the parent types in all BAP concentration treatments

Keywords: BAP, taro, mutant, in vitro

A. Introduction

One commodity that currently has high prospects with increasing international market demand, especially in Japan, is taro satoimo (*Colocasia esculenta* var. antiquorum). The Satoimo taro commodity belongs to the genus Araceae which is easy to cultivate and has a fairly high level of productivity. The high demand for satoimo taro commodity from Japan opens wider opportunities in satoimo taro cultivation (Amelia & Yumiati, 2016).

Satoimo (*Colocasia esculenta* (L.) Schott var antiquorum) is a type of taro that is classified as small corm taro or known as Japanese taro which is traded internationally. About 50% of Japan's population of ±120 million people, consume satoimo as a staple food other than rice. Thus, the current demand for Japanese taro reaches ± 360,000 tons per year, while production capacity in Japan continues to decline to 250,000 tons per year due to limited land and climatic factors that do not allow farming throughout the year (SEAMEO, 2013). This condition opens up opportunities for Indonesian exports to Japan and has encouraged local governments in Indonesia, including Kepahiang, Cisarua, Bantaeng, Malang, and Buleleng to encourage farmers to develop satoimo as an export commodity (Maretta, D., Handayani, D. P., Rosdayani, H., dan Tanjung A., 2016). The productivity of Japanese taro in several districts in South Sulawesi province during 2019-2020 can be seen in the table 1.

Table 1. Planted Area, Harvest Area, Productivity and Production of Japanese Taro in South Sulawesi Province

No	District/City	Planted Area (ha) MT 2020/2021	Harvest Area (ha)	Productivity (kw/ha)	Production (ton)
1.	Maros	27.49	27.49	167.33	460
2.	Gowa	25.11	25.11	156.11	392
3.	Takalar	16.75	16.75	277.01	464
4.	Jeneponto	19.75	19.75	312.52	635
5.	Bone	20.90	20.90	342.11	715
6.	Soppeng	15.60	15.60	971.15	1.515
7.	Wajo	3.25	3.25	455.38	148
8.	Luwu	10.25	10.25	190.24	195
9.	Luwu Utara	19.60	19.60	119.90	235
10.	Luwu Timur	19.30	19.30	696.89	1.345
11.	Enrekang	20.00	20.00	341.50	698
12.	Toraja Utara	10.00	10.00	280.30	380
13.	Makassar	1.00	1,00	28.82	32
	Total	209.00	209.00	345	7.214

Source: Food Security Service of South Sulawesi, 2021

The development of Japanese taro cultivation has resulted in the need for continuous availability of sufficient seeds, but is often constrained by season, land availability, and long planting duration. Therefore, plant tissue culture has been developed to produce plants in large quantities and in a short time, free of pests and diseases, does not depend on the season and requires less initial seeds (Maretta *et al*., 2016). One of the important stages in tissue culture is shoot multiplication which is the most basic method that must be mastered to be able to produce plants in large quantities. Growth regulators and other compounds such as appropriate vitamins in *in-vitro* culture are needed so that they can produce plantlets in large quantities efficiently.

One of the growth regulators that plays a role in shoot multiplication is Benzylaminopurin (BAP). BAP is one of the cytokinin group hormones with the ability to induce tiller shoots which is higher than other types of cytokinins such as kinetin and 2-ip, because BAP is not easily damaged and is more stable in the media so that the amount available in the media is more than other type of cytokinin. The BAP-driven growth includes faster cell division and enlargement (Ilham, M., Sugiyono dan Prayoga, L., 2019).

The use of BAP and several other compounds in culture media may stimulate shoot proliferation because of the synergism between these compounds (Lestari, 2011). Paulos, M., V.R. Joshi & S.R. Pawar (2015) reported that the average number of shoots in-vitro of Banana (*Musa paradisiaca* cv Grand Naine on MS medium with BAP concentration of 5 mg/L produced 2.6 shoots at 30 days after induction (Maretta *et al*., 2016). In addition, a research conducted by Wulansari, A., Martin AF, Rantau DE, Ermayanti TM (2014) reported that BAP and vitamins including thiamine and adenine can trigger shoot growth in taro which can support the multiplication of *in vitro* taro shoots.

Based on the description above, it is necessary to conduct research on the regeneration of various Japanese taro mutants at various concentrations of BAP *In-Vitro*.

B. Methology

1. Research Design and Procedures

The research was conducted at the Plant Tissue Culture Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi. The study was conducted in May-November 2022. The method used was complete randomized design with 2 factors as the environmental design with BAP as the first factor and mutant plants as the second factor. While the levels of BAP (Z) used were concentrations of 0.0 mg/L (z0), 1.0 mg/L (z1), 1.5 mg/L (z2), 2.0 mg/L (z3) and 2.5 mg/L (z4). While the treatment of mutant plants (M) was diploid control (mo), 1 c1t1 putative-Tetraploid mutant, (m1), 2 c2t1 mixoploid mutants (m2), 3 c3t1 mixoploid mutants (m3), 4 c2t2 putative-Tetraploid mutants (m4). Thus, the treatment consisted of 25 treatment combinations. Each treatment was repeated 3 times with each replication using 3 shoot heads so that there were 225 experimental units. All media used were added with 0.2 mg/L IBA.

The research procedure begins with sterilizing bottles and equipment using an autoclave at a temperature of 121 ^{0}C with a pressure of 17.5 psi for one hour. Furthermore, the preparation of regeneration media was carried out by dissolving MS (according to the composition) + 30 g/L sucrose +0.2 mg/L IBA + BAP (according to the treatment concentration) which was then made up to 1000 ml using distilled water. Furthermore, the pH was adjusted to 5.8 by adding NaOH or HCl. Subsequently, the solution was put into a pot and 7 grams of agar was added. The media was heated on an electric stove while stirring until it was close to the boiling point of 98°C. After the media boiled, the media was removed and poured into culture bottles of 25 ml each. Culture bottles were covered with aluminum foil and tied with rubber bands. The media was sterilized by autoclaving at a pressure of 10 psi for 15 minutes. Culture bottles containing the media were placed on the culture rack for 1 week to determine the sterility of the media. In the planting process, Japanese taro mutants were taken from culture bottles and then cut into pieces and planted into regeneration media. The regeneration medium used was MS media with the addition of a combination of BAP + IBA according to treatment. The shoots were kept in an incubation room at a temperature of 25-26°C with 16 hours/day irradiation and an irradiation intensity of ±1,000-2,000 lux. Shoots were incubated for 4 months and observed every week.

Parameters observed were number of roots (cm), root length, number of shoots (fruit), shoot height (cm), number of leaves (pieces) and plantlet formation rate (days). In plants with the best growth, polyploidy level analysis was performed using Flowcytometry (CyFlow (R) Space, Partec, Germany). Samples were taken from the leaves of ± 25 mm^2 of the treated plants. The sample was then ground using a razor blade in a petri dish with 0.2 ml of extraction buffer (solution A from Partec Kit), then incubated for 10 minutes at room temperature. The supernatant from the sample was then filtered using 30 µm nylon mesh and placed in a cuvette, then was added with 1 ml of DAPI solution. The sample was then incubated for 1 minute. The sample was then inserted into a tool called Flowcytometry. The tool will read the light intensity from the sample to show the number of ploidy changes that occur which are shown through the graph displayed in the tool.

2. Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) and further tested with the DMRT (Duncan's Multiple Range Test) test at a significance level of 5% using STAR Software (Statistical Tool for Agricultural Research). Furthermore, the treatments with the best results were analyzed for chromosomes using Flowcytometry.

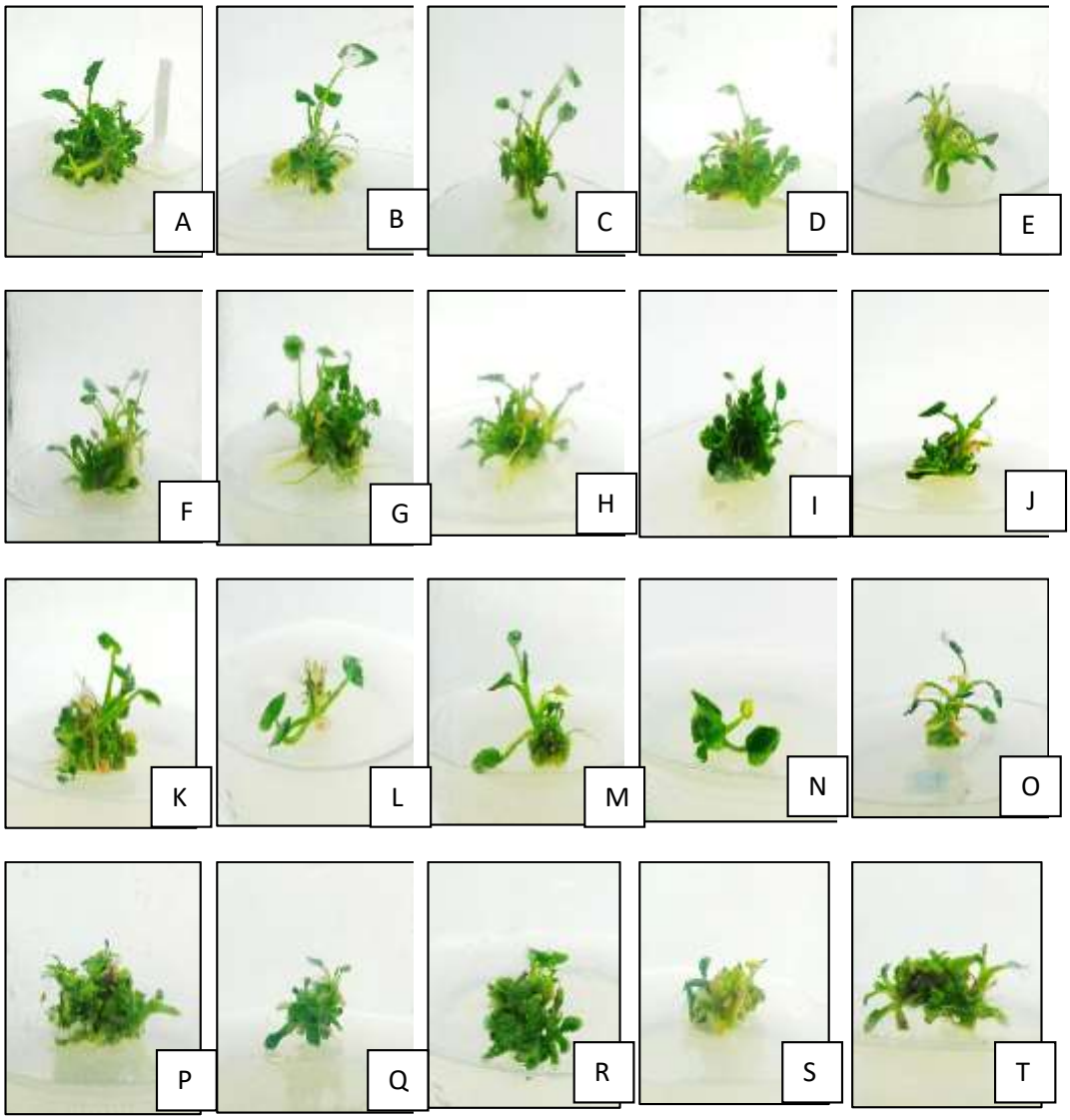
C. Result and Discussion

The results of the mid-square analysis of variance in the mutant taro regeneration experiment using BAP *in vitro* are shown in Table 2. The results of the analysis showed that the concentration of BAP and mutant plants significantly affected all parameters including root number, root length, number of shoots, shoot height, number of leaves, and plantlet formation rate.

Table 2. Mid-Square Analysis of Varian on Several Observation Parameters in Regeneration Experiments of Mutant Taro Using BAP *In Vitro*

SK	DB	Middle Square					
		JT	TT	JA	PA	JD	KMP
M	1	209.963**	0.525**	23.638**	1.108**	84.219**	474.560**
Z	3	76.77**	0.194**	37.233**	1.627**	69.566**	3277468**
MxZ	3	81.57**	0.108**	12.530**	0.332**	30.905**	34.015**
Error	16	3.051	0.010	0.123	0.005	0.692	14.002
KK		10.35%	8.97%	12.85%	9.31%	11.79%	12.28%

Description: **: very significant effect, *: significant effect, tn: no significant effect, SK: source of diversity, DB: degrees of freedom, KK: coefficient of diversity, M: mutant plant, Z: concentration of BAP, JT: number of shoots, TT: shoot height, JA: number of roots, PA: root length, JD: number of leaves, KMP: plantlet formation rate.



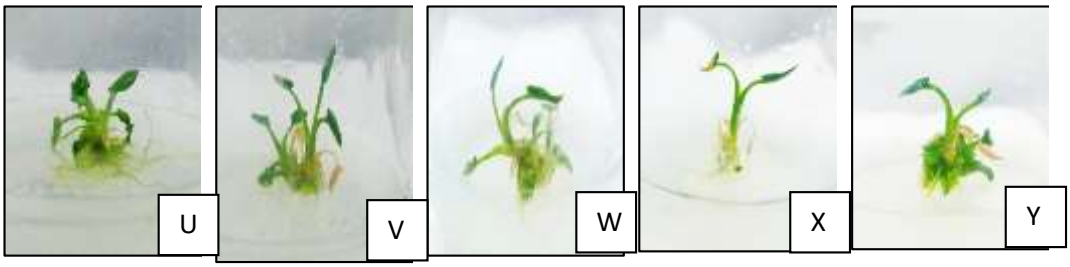


Figure 1. Appearance of Various Taro Plants with Various Concentrations of BAP *In-Vitro* after 16 MST

Description: (A) z0m0 (BAP mg/L control plant); (B) z1m0 (BAP 1 mg/L, control plant); (C) z2m0 (BAP 1.5 mg/L, control plant); (D) z3m0 (BAP 2 mg/L, control plant); (E) z4m0 (BAP 2.5 mg/L, control plant); (F) z0m1 (BAP 0 mg/L, mutant plant 1); (G) z1m1 (BAP 1 mg/L, mutant plant 1)); (H) z2m1 (BAP 1.5 mg/L, mutant plant 1); (I) z3m1 (BAP 2 mg/L, mutant plant 1); (J) z4m1 (BAP 2.5 mg/L, mutant plant 1) ; (K) z0m2 (BAP 0 mg/L, mutant plant 2); (L) z1m2 (BAP 1 mg/L, mutant plant 2); (M) z2m2 (BAP 1.5 mg/L, mutant plant 2); (N) z3m2 (BAP 2 mg/L, mutant plant 2); (O) z4m2 (BAP 2.5 mg/L, mutant plant 2); (P) z0m3 (BAP 0 mg/L, mutant plant 3); (Q) z1m3 (BAP 1 mg/L, mutant plant 3); (R) z2m3 (BAP 1.5 mg/L, mutant plant 3); (S) z3m3 (BAP 2 mg/L, mutant plant 3); (T) z4m3 (BAP 2.5 mg/L, mutant plant 3); (U) z0m4 (BAP 0 mg/L, mutant plant 4); (V) z1m4 (BAP 1 mg/L, mutant plant 4); (W) z2m4 (BAP 1.5 mg/L, mutant plant 4); (X) z3m4 (BAP 2 mg/L, mutant plant 4); (Y) z4m4 (BAP 2.5 mg/L, mutant plant 4).

The difference in morphology of various mutant taro plants to the level of BAP concentration can be seen in Figure 1.

The treatment of BAP concentration and mutant plants was found to affect the morphological characteristics of the regenerated plants under in vitro conditions. The results of ANNOVA analysis from observations to cultures aged 16 WAP showed that the interaction of BAP concentration treatment and mutant plants had a very significant effect on the morphology of taro *Safira*.

Table 3. The Effect of BAP Concentration on Root Amount of *In Vitro* Mutant Taro *Safira*

Mutant Plant	BAP concentration				
	z0 (0 mg/L)	z1 (1.0 mg/L)	z2 (1.5 mg/L)	z3 (2.0 mg/L)	z4 (2.5 mg/L)
m0 (Control)	11.66_{p}^{a}	4.33_{q}^{b}	2.16_{r}^{a}	1.50_{s}^{b}	2.00_{rs}^{a}
m1 (Mutant 1)	3.50_{q}^{c}	8.66_{p}^{a}	2.00_{r}^{a}	3.00_{q}^{a}	1.16_{s}^{b}
m2 (Mutant 2)	2.55_{p}^{e}	2.66_{p}^{c}	1.66_{q}^{ab}	1.16_{q}^{a}	1.27_{q}^{b}
m3 (Mutant 3)	1.66_{p}^{d}	1.27_{pq}^{d}	1.33_{pq}^{b}	1.00_{q}^{a}	1.16_{pq}^{b}
m4 (Mutant 4)	4.33_{p}^{b}	3.78_{p}^{b}	1.66_{q}^{ab}	1.50_{q}^{b}	1.44_{p}^{ab}

Description: numbers followed by the same letter in rows (abcd) and columns (pqrs) means that they are not significantly different in the DMRT test with a level of = 0.05

The DMRT test = 0.05 in Table 3 shows that the interaction of treatment media without BAP (z0) with control plants (mo) had the highest average number of roots, namely 11.66 pieces, which was significantly different from all treatments of mutant plant species (m1, m2, m3, m4). Control plants (mo) without BAP treatment (z0) had a significantly different average root length with all BAP concentration treatments (z1, z2, z3, z4). The difference in the number of roots was due to the different levels of BAP or cytokinin content in the regeneration medium, thus affecting the number of shoots formed. According to Hattu, W., Parera Dj, F., dan Raharjo S. H. T., 2018), the use of BAP may inhibit root initiation, elongation, and development. In addition, the type of explant affects the number of roots formed. Sinaga (2020) reported that in taro *Kaliurang,* root growth in diploid clones was better than that of tetraploid clones.

Table 4. The Effect of BAP Concentration on Root Length (cm) of *In Vitro* Mutant Taro *Safira*

Mutant Plant	BAP concentration				
	z0 (0 mg/L)	z1 (1.0 mg/L)	z2 (1.5 mg/L)	z3 (2.0 mg/L)	z4 (2.5 mg/L)
m0 (Control)	2.41^{a}_{p}	1.16^{a}_{q}	0.98^{a}_{r}	0.63^{b}_{s}	0.56^{a}_{s}
m1 (Mutant 1)	1.16^{c}_{pq}	1.10^{a}_{qr}	1.00^{a}_{r}	1.25^{a}_{p}	0.33^{b}_{s}
m2 (Mutant 2)	0.60^{e}_{p}	0.72^{b}_{p}	0.70^{b}_{p}	0.33^{a}_{q}	0.38^{b}_{q}
m3 (Mutant 3)	0.76^{d}_{p}	0.55^{c}_{q}	0.40^{c}_{r}	0.55^{a}_{q}	0.33^{b}_{r}
m4 (Mutant 4)	1.66^{b}_{p}	0.66^{bc}_{q}	0.63^{b}_{qr}	0.58^{b}_{qr}	0.53^{a}_{r}

Description: numbers followed by the same letter in row (abcde) and column (pqrs) mean not significantly different in DMRT test with level α = 0.05

The DMRT test at α = 0.05 in Table 4 shows that the interaction of treatment media without BAP (z0) on control plants (mo) showed the best average root length of 2.41 cm which was significantly different from all mutant plant treatments (m1, m2, m3, m4). Control plant (mo) without BAP treatment (z0) had an average root length that was significantly different from all BAP concentration treatments (z1, z2, z3, z4). It is suspected that this is due to the different ZPT content in the growth medium of taro plants and also to differences in endogenous auxin in mutant plant tissues. Widiastoety (2014) states that the formation of explant roots is related to the levels of endogenous cytokinins and auxins in plant tissues, followed by the process of elongation and division of meristematic cells. According to Pamungkas (2015), higher BAP concentrations will reduce root length in explants.

Table 5. The Effect of BAP Concentration on Number of Shoots of *In Vitro* Mutant Taro *Safira*

Mutant Plant	BAP concentration				
	z0 (0 mg/L)	z1 (1.0 mg/L)	z2 (1.5 mg/L)	z3 (2.0 mg/L)	z4 (2.5 mg/L)
m0 (Control)	24.16^{a}_{p}	22.50^{b}_{p}	11.66^{a}_{r}	10.33^{c}_{r}	16.05^{b}_{q}
m1 (Mutant 1)	10.33^{c}_{q}	26.00^{a}_{p}	12.66^{a}_{q}	24.16^{a}_{p}	11.83^{c}_{q}
m2 (Mutant 2)	22.83^{a}_{pqr}	20.22^{b}_{r}	21.11^{a}_{qr}	24.50^{a}_{p}	23.89^{a}_{pq}
m3 (Mutant 3)	13.00^{c}_{p}	7.66^{d}_{s}	11.33^{a}_{qr}	19.83^{cb}_{p}	8.50^{d}_{rs}
m4 (Mutant 4)	16.00^{b}_{qr}	11.61^{c}_{s}	13.50^{a}_{rs}	21.16^{c}_{p}	16.89^{b}_{q}

Description: numbers followed by the same letter in row (abcde) and column (pqrs) mean not significantly different in DMRT test with level α = 0.05

The DMRT test at α = 0.05 in Table 5 shows that the interaction of media with BAP treatment at a concentration of 1.0 mg/L (z1) on mutant plants 1 (m1) showed the highest average number of shoots, which was 26 fruit which was significantly different from the treatment on mutant plants (m0, m2, m3, m4). In the treatment of mutant plant 1 (m4) without BAP treatment of 1.0 mg/L (z1), the average shoot height was not significantly different from the BAP concentration of 2.0 mg/L (z3) which was 24.16 pieces and significantly different from the BAP concentration treatments (z0, z2, z3, z4). It indicated that the mutant plants had more shoots than the control plants. This is in line with Sinaga (2020), the difference in the characters of diploid and tetraploid taro *Kaliurang* is seen in the higher number of tetraploid shoots compared to the diploid. Furthermore, the different BAP concentrations also affected the number of shoots formed. According to Lestari (2011), BAP functions to encourage the formation, multiplication, and growth of shoots in various plants so as to increase their effectiveness in plant propagation.

Moreover, Gowen (1995) states that *in vitro* shoot formation is influenced by the presence of high levels of cytokinins in culture media, and the most effective type of cytokinin is BAP.

Table 6. The Effect of BAP Concentration on Shoot Height (cm) of *In Vitro* Mutant Taro *Safira*

Mutant Plant	BAP concentration				
	z0 (0 mg/L)	z1 (1.0 mg/L)	z2 (1.5 mg/L)	z3 (2.0 mg/L)	z4 (2.5 mg/L)
m0 (Control)	1.00^{a}_{ps}	1.33^{a}_{pq}	1.50^{a}_{p}	1.25^{a}_{qr}	1.11^{a}_{rs}
m1 (Mutant 1)	1.16^{a}_{pq}	1.33^{bc}_{p}	1.00^{bc}_{q}	1.00^{bc}_{q}	1.08^{a}_{q}
m2 (Mutant 2)	1.02^{a}_{q}	1.50^{b}_{p}	1.02^{b}_{q}	1.00^{bc}_{q}	1.08^{a}_{q}
m3 (Mutant 3)	0.66^{b}_{r}	1.01^{dc}_{p}	0.83^{c}_{qr}	0.90^{c}_{pq}	0.80^{b}_{qr}
m4 (Mutant 4)	1.83^{a}_{p}	1.44^{b}_{q}	1.16^{b}_{r}	1.16^{ab}_{r}	1.11^{a}_{r}

Description : numbers followed by the same letter in row (abcde) and column (pqrs) mean not significantly different in DMRT test with level α = 0.05

The DMRT test at α = 0.05 in Table 6 shows that the interaction of media without BAP treatment (z0) on mutant plant 4 (m4) showed the highest average shoot height of 1.83 cm which was significantly different from all treatments of mutant plant species (m0, m1 , m2, m3). In the treatment of mutant plants 4 (m4) without BAP treatment (z0), it showed a significantly different average shoot height with all BAP concentration treatments (z1, z2, z3, z4). The presence of ZPT cytokinin content in the media may cause cell elongation and enlargement which tends to lead to the formation of shoots. According to Lestari (2011), the use of BAP and several other compounds in culture media may stimulate shoot proliferation due to the synergism between these compounds. The difference in shoot growth at the same concentration is thought to be caused by differences in the content of endogenous hormones in the explant tissue, which will give different organ growth. Furthermore, the composition of the media for each type of plant is not the same or specific due to different genotypes and plant physiological conditions (Lestari, 2011).

Table 7. The Effect of BAP Concentration on Number of Leaves of *In Vitro* Mutant Taro *Safira*

Mutant Plant	BAP concentration				
	z0 (0 mg/L)	z1 (1.0 mg/L)	z2 (1.5 mg/L)	z3 (2.0 mg/L)	z4 (2.5 mg/L)
m0 (Control)	9.05^{b}_{qr}	12.00^{b}_{p}	8.16^{a}_{rs}	6.83^{a}_{s}	9.94^{b}_{q}
m1 (Mutant 1)	6.66^{c}_{q}	16.00^{a}_{p}	3.50^{c}_{r}	4.00^{bc}_{r}	4.00^{c}_{r}
m2 (Mutant 2)	15.72^{a}_{p}	7.33^{c}_{r}	5.94^{b}_{s}	5.33^{b}_{s}	14.22^{a}_{q}
m3 (Mutant 3)	2.00^{d}_{s}	7.16^{c}_{p}	5.83^{b}_{p}	3.33^{c}_{qr}	3.50^{c}_{q}
m4 (Mutant 4)	7.66^{c}_{p}	7.33^{c}_{p}	3.16^{c}_{q}	3.83^{c}_{q}	3.89^{c}_{q}

Description : numbers followed by the same letter in row (abcde) and column (pqrs) mean not significantly different in DMRT test with level α = 0.05

The DMRT test at α = 0.05 in Table 7 shows that the interaction of media treatment with BAP concentration of 1.0 mg/L (z1) on mutant plant 1 (m1) showed the highest average number of leaves, which was 16 pieces which were significantly different from all treatments in mutant plants (m0, m2, m3, m4). In the treatment of mutant plant 1 (m1) at the concentration of BAP of 1.0 mg/L (z1), the average number of leaves was significantly different from all treatments with BAP concentration (z0, z2, z3, z4). All types of mutants were able to produce leaves although grown in media without cytokinins. This condition occurred due to the use of MS media which had met sufficient nutrient intake for the explants to form leaves. According to Joni Y., Efendi, D., dan Roostika, I. (2014), MS media is known to have a high nitrogen content. The high nitrogen content may increase the cytokinin content in plants. The difference in the number of leaves is thought to be caused by the type of mutant plant that contains different endogenous cytokinins so that the addition of the same BAP will produce a different number of leaves. According to Hartmann and Kester (1983), different plants may respond to cytokinin and auxin hormones in different concentrations. This is caused by differences in the concentration of the endogenous

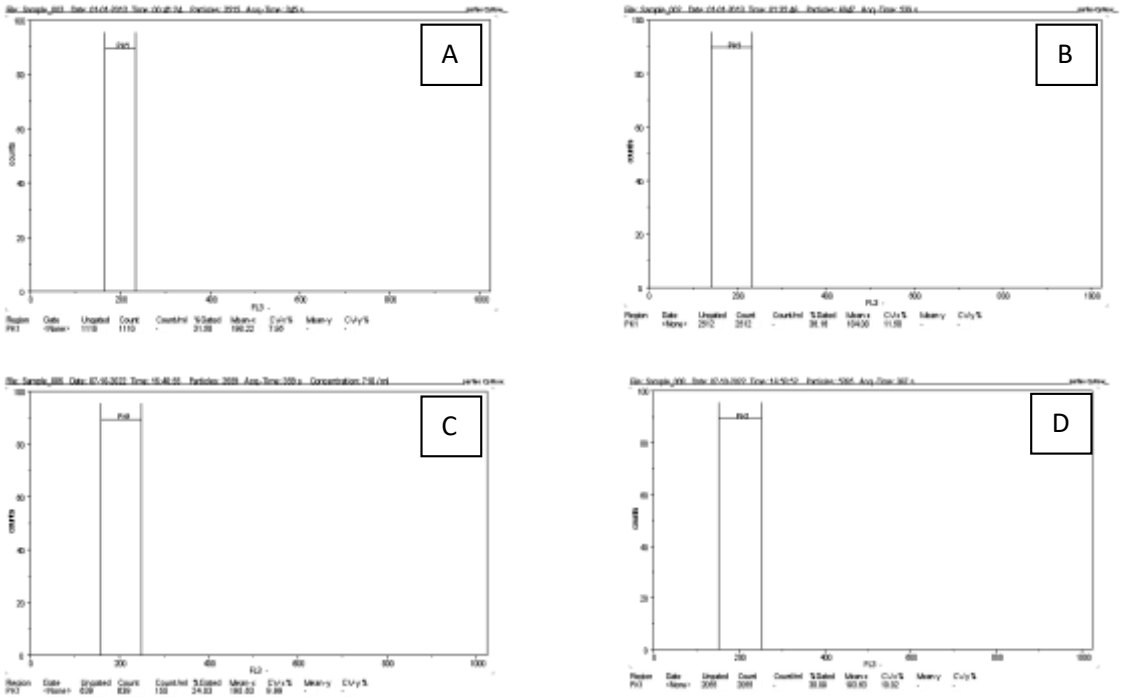
hormone content of the plant itself. Cytokinins first accumulate in the roots, then in the xylem fluid, and finally in the leaves.

Table 8. The Effect of BAP Concentration on the plantlet formation rate (HST) of *In Vitro* Mutant Taro *Safira*

Mutant Plant	BAP Concentration				
	z0 (0 mg/L)	z1 (1.0 mg/L)	z2 (1.5 mg/L)	z3 (2.0 mg/L)	z4 (2.5 mg/L)
m0 (Control)	13.27_{r}^{b}	15.33_{r}^{b}	18.83_{r}^{c}	30.00_{q}^{c}	39.33_{p}^{d}
m1 (Mutant 1)	16.16_{r}^{ab}	16.00_{r}^{b}	20.33_{r}^{c}	33.33_{q}^{c}	47.00_{r}^{c}
m2 (Mutant 2)	19.55_{r}^{ab}	17.66_{r}^{ab}	22.66_{sr}^{bc}	41.33_{q}^{b}	5366_{p}^{b}
m3 (Mutant 3)	21.00_{s}^{a}	23.66_{rs}^{a}	29.33_{r}^{a}	52.33_{q}^{a}	60.0_{r}^{a}
m4 (Mutant 4)	19.66_{r}^{ab}	20.00_{r}^{ab}	27.66_{q}^{ab}	50.66_{p}^{a}	53.22_{p}^{b}

Description : numbers followed by the same letter in row (abcde) and column (pqrs) mean not significantly different in DMRT test with level α = 0.05

The DMRT test = 0.05 in Table 8 shows that the interaction of media concentrations without BAP treatment (z0) on control plants (mo) showed the average plantlet formation rate of 13.27 days which was significantly different from all treatments in mutant plants (m1, m2, m3, m4). In control plants (mo) without BAP treatment (z0), it had an average plantlet formation rate that was not significantly different with BAP concentration of 1.0 mg/L (z1) which was significantly different from other BAP concentrations (z2, z3, z4). It is presumably due to control that a plant with diploid cells has a faster cell division orientation compared to polyploid mutant plants. Comai (2005) states that polyploid plants have a larger nucleus size which causes the cell division process to be more complicated and take longer. This condition is also associated with the activation and suppression of gene expression. In addition, Gantait S., N. Mandal, S. Bhattacharyya, & PK Das (2011) reported that the growth of tetraploid plants was considered slower than diploid plants which also occurred in ornamental plants *Gerbera jamesonii* Bolus cv.



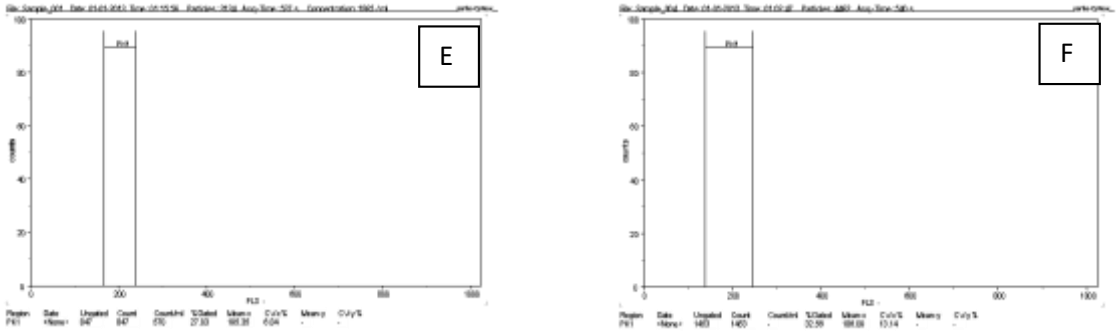


Figure 2. Graph of Ploidy Degrees with Flow Cytometry of mutant 4 taro resulted of regeneration of various BAP concentrations *In Vitro*

Description: (A) z0m0 (BAP 0 mg/L, control mutant plant); (B) z0m4 (BAP 0 mg/L, mutant plant 4); (C) z1m4 (BAP 1 mg/L, mutant plant 4); (D) z2m4 (BAP 1.5 mg/L, mutant plant 4); (E) z3m4 (BAP 2 mg/L, mutant plant 4); (F) z4m4 (BAP 2.5 mg/L, mutant plant 4).

Flow cytometry analysis is a modern method for determining the ploidy level in plants. Flow cytometry has proven to be a fast and efficient method for estimating the ploidy level in almost all plant tissue regeneration, and is particularly suitable for studies involving large sample sizes (De, K.K., Saha, A., Tamang, R., Sharma, B., 2010; Eeckhaut T. Leus, L. Van Huylenbroeck, J., 2005). Jones, J.R. G.T. Ranney, & T.A. Eaker (2008) used flow cytometry to determine the ploidy level and genome size of 200 diverse species and Rhododendron cultivars which are now a valuable database for plant breeders. Flow cytometry was also used to determine the ploidy level of polyploid adventitious shoots induced from three *Rhododendron* hybrids.

According to the ploidy analysis using flow cytometry on samples of mutant taro plants with putative-tetraploid parental traits at various BAP concentrations (figure 2), it shows that all samples regenerating at various BAP concentrations did not produce putative-tetraploid traits like its parent type yet produced plants with a detectable diploid chromosome. The percentage (%) gated at 0 mg/L (z0) BAP concentration in control plants (m0) was 31.58%, BAP concentration of 0 mg/L (z0) in mutant 4 (m4) was 36.16%, BAP concentration (z1) of 1 mg/L in mutant 4 (m4) was 24.03%, BAP concentration of 1.5 mg/L (z2) in mutant 4 (m4) was 38.09%, BAP concentration of 2 mg/L (z3) in mutant 4 (m4) was 27.03%, BAP concentration of 2.5 mg/L (z4) in mutant 4 (m4) was 32.56%. Chromosomal differences detected after regeneration were due to the putative-tetraploid parental trait which showed the dominant chromosome, namely 4n (tetraploid) with a percentage of 35.50% but did not show much difference with chromosome 2n (diploid) with a percentage of 29.94% and 3n (triploid) with a percentage of 15.39 % so that this allows the occurrence of ploidy selection. Variations found in regenerated plants tend to be caused by variations that are inherited by the plant of parent type. This is supported by Esfahani *et al.* (2020) that polyploidy induction and high percentage of mixoploid yields are generally considered to be drawbacks of the procedure because the unstable polyploidy state often returns partially or completely to the diploid state after successive cell divisions of two different developmental cycles of cells in the mixoploid shoots leading to competition during its growth. According to Kainth & Grosser (2010), diploid cells have a faster division rate than autotetraploid cells, allowing the resulting mixoploid shoots to turn back into diploids.

D. Conclusion

MS media containing different concentrations of BAP caused different growth in mutant taro. Mutant plant parent types (diploid, mixoploid, putative-tetraploid) at the same media concentration also produced different growth. Diploid cells have a faster division rate than polyploid cells. The interaction of BAP treatment that showed the best regeneration was the concentration of 0 mg/L in the control plants with the parameters of root length (2.41 cm), number of roots (11.66 pieces) and plantlet formation rate (13 days). Concentration of BAP of 1 mg/L in mutant plant 1 showed the parameters of the number of shoots (26 pieces) and number of leaves (16 pieces). Concentration of BAP of 0 mg/L in mutant plant 4 had a shoot height of 1.83 cm.

E. References

Amelia, D. D., & Yumiati (2016). Analisis Usahatani Talas Satoimo (*Colocasia esculenta* var. antiqourum) (Studi Kasus : di Desa Suka Sari Kecamatan Kabawetan Kabipaten Kepahiang). Jurnal *Agritepa*, 2(2): 188-198.

Comai, L. (2005). The Advantages and Disadvantages of Being Polyploid. Nature Reviews Genetics. 6:836-846.

De, K.K., Saha, A., Tamang, R., Sharma, B. (2010). Investigation on relative genome sizes and ploidy levels of Darjeeling-Himalayan Rhododendron species using flow cytometer. Indian J. Biotechnol. Vol. 9: 64-68.

Eeckhaut, T. Leus, L. Van Huylenbroeck, J. (2005). Exploitation of Flow Cytometry for Plant Breeding. Acta Physiologiae Plantarum Vol. 27: 743-750.

Esfahani, S.T., Karimzadeh, G., Naghavi, M.R. (2020). In Vitro Polyploidy Induction in Persian Poppy (Papaver bracteatum Lindl.). International Journal of Cytology, Cytosystematics and Cytogenetics Vol. 73(1): 133-144.

Gantait, S., N. Mandal, S. Bhattacharyya, & PK Das. (2011). Induction and identification of tetraploid using in vitro colchicine treatment of Gerbera Jamesonii Bolus cv. Sciella. Plant Cell Tissue Organ Culture.106: 485 – 493.

Gowen, S. (1995). Bananas and Plantains. Chapman and Hall. London, UK.

Hartmann, H.T & D.E. Kester. (1983). Plant Propagation-Principle and Practices. Prentice Hall International Inc. New York : p. 238.

Hattu, W., Parera Dj, F., dan Raharjo S. H. T. (2018). Penggunaan Adenin Sulfat pada Perbanyakan Mikro Talas Jepang. Jurnal *Agrologia*, 7(2): 59-70.

Ilham, M., Sugiyono dan Prayoga, L. (2019). Pengaruh Interaksi BAP dan IAA Terhadap Multiplikasi Tunas Talas Satoimo *Colocasia esculenta* (L.) var. Antiquorum Secara *In Vitro*. Bioeksakta: Jurnal *Ilmiah Biologi* Unsoed. 1(2): 48-55.

Jones, J.R. G.T. Ranney, & T.A. Eaker (2008). A novel method for inducing polyploidy in *Rhododendron* seedlings. *J. Am. Rhododendron Soc. 62*, 130–135.

Joni, Y., Efendi, D., dan Roostika, I. (2014). Morfogenesis Ekspla Keping Biji Dari Tiga Klon Manggis Pada Tiga Jenis Media Dasar. *Horticultura*, Vol 24(2).

Kainth, D., & J.W. Grosser (2010). Induction of Autotetraploids in Pummelo (*Citrus grandis* L. Osbeck) through Colchicine Treatment of Maristematically Active Seeds In Vitro. *Proc. Fla. State Hort. Soc.* 123:44-48.

Lestari, E. G. (2011). Peranan Zat Pengatur Tumbuh dalam Perbanyakan Tanaman melalui Kultur Jaringan. Jurnal AgroBiogen, 7(1), 63-68.

Maretta, D., Handayani, D. P., Rosdayani, H., dan Tanjumg A. (2016). Multiplikasi Tunas dan Induksi Umbi Mikro Satoimo (*Colocasia esculenta* (L.) Schoot) pada Beberapa Konsentrasi Sukrosa dan Benzilaminpurin. Jurnal *Bioteknologi dan Bio Sains Indonesia*, 3(2): 81-88.

Pamungkas, Tri, Sigit, S. (2015). Pengaruh Konsentrasi Naa Dan Bap Terhadap Pertumbuhan Tunas Eksplan Tanaman Pisang Cavendish (Musa paradisiaca L.) Melalui Kultur In Vitro. *Gontor AGROTECH Science Journal*, Vol 2(1).

Paulos, M., V.R. Joshi & S.R. Pawar (2015). Effect of BAP and NAA on *In Vitro* Shoot Establishment and Proliferation of Banana (*Musa paradisiaca*) Cv. Grand naine. *International Journal Of Science and Research* 4(5):318-323.

Sinaga, Aini, K., Wulandari, Retno, D., Ratnadewi, D. (2020). Optimasi Medium Perbanyakan In Vitro Tunas Talas Kaliurang (Colocasia esculenta L.) Diploid dan Tetraploid. *J Bioteknol Biosains Indonesia.* Vol 7 (2).

SEAMEO (2013). Talas Jepang (Satoimo) Tissue Culture-Service Laboratory SEAMEO BIOTROP, Bogor, Indonesia.

Widiastoety, D. (2014). Pengaruh Auksin dan Sitokinin Terhadap Pertumbuhan Planlet Anggrek Mokara. J. Hortikultura., 24(3), 230-238.

Wulansari A, Martin AF, Rantau DE, Ermayanti TM. (2013). Perbanyakan beberapa aksesi talas (Colocasia esculenta L.) diploid secara kultur jaringan dan konservasinya mendukung diversifikasi pangan. Prosiding Seminar Nasional Riset Pangan, Obat-obatan dan Lingkungan untuk Kesehatan. Bogor 27-28 Juni 2013. hal 11-19. ISBN: 978-602-14503-1-4.