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The Effect of NAA, BAP and NAA, BAP Combinations on the Growth of *In-vitro* Culturally Promoted Leaves of *Sansevieria ehrenbergii*

Pengaruh NAA, BAP serta Kombinasi NAA dan BAP terhadap Pertumbuhan Eksplan Daun Sansevieria ehrenbergii yang Diperbanyak secara In-vitro

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ABSTRACT

Sansevieria is an ornamental succulent leaf plant of the family *Agavaceae*, having a beautiful and diverse shape and color. Propagation of *Sansevieria* can be done by tissue culture method with the addition of cytokinins and auxins to MS media. Complete randomized design consisting of 8 treatments, 3 tests, there were 24 experimental units. The variables that were recorded are the timing of callus formation, the color and texture of the callus, its weight and diameter, and the success rate of its growth. Data analysis used variance analysis and tested using 5% DNMRT. The data is processed using *a Statistical Analysis System* (SAS). According to the findings, the application of NAA, BAP, and the combination of NAA and BAP had an impact on the explant per plant of *Sansevieria ehrenbergii* leaves on all observation parameters. The treatment of NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1} gave effect tending to be faster when callus appeared with an average of 75.75 HST, NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} and tends to be larger in callus diameter with an average of 0.9033 g and 19.133 mm, NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1} gives a higher influence on the percentage of callus growth by an average of 100 %.

Keywords: NAA, BAP, tissue culture, Sansevieria, *in-vitro*

ABSTRAK

Sansevieria adalah tanaman hias daun sukulen dari keluarga Agavaceae, memiliki bentuk dan warna yang indah dan beragam. Perbanyakan Sansevieria dapat dilakukan dengan metode kultur jaringan dengan penambahan sitokinin dan auksin ke media MS. Pada penelitian ini, desain eksperimen acak lengkap digunakan yang terdiri dari 8 perlakuan, 3 kali ulangan, dan 24 unit percobaan. Parameter yang diobservasi yaitu waktu munculnya kalus, warna dan tekstur kalus, massa dan diameter kalus, serta tingkat keberhasilan tumbuh. Analisis data menggunakan analisis varian dan diuji lanjut menggunakan DNMRT 5%. Data tersebut diolah menggunakan Statistical Analysis System (SAS). Hasil penelitian menunjukan bahwasanya pemberian NAA, BAP dan kombinasi NAA dan BAP mempengaruhi eksplan per tanaman daun Sansevieria ehrenbergii pada semua parameter pengamatan. Pemberian NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1} memberikan pengaruh cenderung lebih cepat ketika kalus muncul dengan rata-rata 75,75 HST, NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} memberi pengaruh cenderung lebih besar pada berat kalus dan diameter kalus dengan rata-rata 0,9033 g dan 19,133 mm dan NAA 1 mg,l^{-1} + BAP 1 mg.l^{-1} memberi pengaruh yang lebih tinggi pada persentase pertumbuhan kalus dengan rata-rata 100 %.

Kata kunci : *NAA, BAP, kultur jaringan, Sansevieria, in-vitro*

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INTRODUCTION

Sansevieria or tongue-in-law is an ornamental succulent leaf plant of the family *Agavaceae*, having its beautiful and diverse shape and color. Sansevieria became popular because it was able to absorb air pollutants. The results of research by the Space Agency NASA *(National Aeronautics and Space Application)* of the United States and released in 1999, showed that Sansevieria is able to absorb more than 107 unsur of harmful pollutants present in the air (Purwanto, 2006). Sansevieria absorbable compounds such as chloroform, formaldehyde, trichloroethylene, benzene and xylene. Sansevieria plants have the greatest ability to reduce carbon monoxide levels compared to lilies or other plants (Adita *et al*., 2011). The Sansevieria plant also contains saponin compounds, phenols, and flavonoids that serve as antimicrobials (Lombogia *et al*., 2016).

Sansevieria is capable of both generative propagation through seeds and vegetative propagation by taking cuttings of young plants or buds, leaves, shoots, rhizomes, and tissue cultures (Sulistiana, 2013). According to Triharyanto dan Sutrisno (2007), ripening seeds from Sansevieria after 2 to 5 months of age, depending on the variety. Sansevieria seeds have two embryos, thus allowing the production of two different types over a long time. Generative propagation is less effective in meeting market needs because growth and budding are slow, vegetative propagation by cutting saplings or buds, leaf cuttings, cuttings of shoots and cutting rhizomes requires a lot of mother plants, so vegetative propagation is carried out by tissue culture.

According to Yusnita (2003), technic tissue culture through callus is expected to obtain a relatively large number of planlets that grow as adventitious buds. Tissue culture contains two principles, namely plant material that has *totipotency* and controlled cultivation. One of the most commonly used media is *Murashige and Skoog* (MS) which are distinguished by their substantial concentration of inorganic salts. The application of ZPT to tissue culture techniques can produce shoots and shoots in a relatively fast time, the most frequently used ZPT is auxins and cytokinins. Auxins that are often used are *naphthalene acetic acid* (NAA) because they are effective for callus induction, while the cytokinins used are *benzil amino purines* (BAP) effective for the formation of buds and shoots.

Several researchers have conveyed the potential of cultivating Sansevieria species through *in-vitro* propagation. The result of Muliati *et al.* (2017) search, a combination NAA 1 $mg.l^{-1}$ + BAP 1 $mg.l^{-1}$ gives the best effect at the time of emergence of shoots in *Sansevieria macrophylla* but still has not found the right concentration to regenerate Sansevieria plants in other species and the concentration varies for each Sansevieria cultivar.

Based on the description above, the author has carried out a study entitled "The effect of NAA, BAP, and the combination of BAP and NAA on the growth of explants *Sansevieria ehrenbergii* leaves propagated *in-vitro*".

The objective of this study was to investigate the impact and determine the optimal concentration of NAA, BAP, and combinations of BAP and NAA on the growth of explants *Sansevieria ehrenbergii* leaves propagated *in-vitro.*

MATERIALS AND METHODS

Materials used in the study were young leaves of *the Sansevieria ehrenbergii* plant which will be used as explants, MS M159 media, NAA (naphtalene acetic acid), BAP (benzyl amino purine), sucrose, agar, aquades, KOH 1 N and HCl. The sterilization materials used are liquid soap, Dithane M-45, NaOCl 5%, 2%, and 1%, and alcohol 70% and 96%.

The equipment employed in this study consisted of an LAFC (laminar airflow cabinet), autoclave, digital pH meter, bunsen burner, analytical scales, hot-magnetic stirrer, Erlenmeyer flask, measuring cup, pipette, petri dish, culture bottle, aluminum foil, scalpel, plastic wrapping, culture racks, pots, gas stoves, cotton, calipers, cameras, and stationery.

From August 2020 to April 2021, the research was carried out at the Plant Biotechnology Laboratory of Riau University's Faculty of Agriculture, located in Simpang Baru Village, Tampan District, Pekanbaru, at the Binawidya Campus KM 12.5.

A completely randomized design (CRD) with eight treatments was employed in this study. The treatment were; P_1 : NAA 1 mg.l^{-1} P_2 : NAA 2 mg.l^{-1}, P_3 : BAP 1 mg.l^{-1}, P_4 : BAP 2 mg.l^{-1}, P_5 : NAA 1mg.l^{-1}+ BAP 1 mg.l^{-1}, P_6: NAA 1mg.l^{-1}+ BAP 2 mg.l^{-1}, P_7 : NAA 2mg.l^{-1}+ BAP 1 mg.l^{-1}, P_8 : NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}

The above performance was repeated 3 times so that 24 experimental units were obtained. The experimental unit ap seti consists of 4 expslan, for a total of 96 explan.

Research Implementation

Sterilization of the working environment

Sterilization of the working environment can be maintained by limiting the number of people entering the room. Planting Sansevieria is carried out in LAFC. Before that, during and after use, the surface of LAFC is cleaned with a cotton swab yang has been dipped in alcohol 70 %. Tools and materials that will be used at the time of planting such as Petri dish, dissection tools, beker cups, bunsen, planting media, NaOCl 1% and 2%, sterile aquades and wipes are put into LAFC to be sterilized with a UV lamp, then lamp UV is turned on for 30 minutes and then turned off. The blower on the LAFC is turned on during the planting. At work, workers must be clean and wear head coverings and masks.

Sterilization of materials and tools

The tools used in the study should always be in a sterile state. Glass tools (Petri dish, empty bottles, pipettes, and others), metal tools (tweezers, scissors, scalpel handles and others) wrapped in rice paper or stencil paper and aquadest that are inserted into the bottle. Everything is sterilized using an autoclave.

Sterilization using an *autoclave* was performed for 15 minutes at a pressure of 17.5 psi and a temperature of 121 °C. During work at LAFC, planting tools such as scalpel blades and tweezers were sterilized by burning on a bunsen fire, after which a pre-emptive dip in 96% alcohol.

Manufacture of Murashige and Skoog

The media used in this study was the basic of MS M159 with the addition of BAP and NAA according to treatment. Rare early media manufacture weighed 4.5 g of M159 MS media, 3% sucrose (30 g), and 8 g of gelatin. Sucrose 30 g is dissolved with 800 ml of aquades, then MS is added and homogenized. Furthermore, the MS solution is divided into 4 each as much as 200 ml on the Bekker glass and then NAA and BAP are added according to the provisions, and in each medium, aquades are added until the volume is close to 250 ml, after which the pH me is measuredata which is fixed at 5.8. If the medium is acidic (pH<5.8) is added KOH while when it is alkaline (pH>5.8) HCl is added.

The media solution that has been given ZPT according to the treatment and has been measured pH is added gelatinously at 2 g and heated until it is completely dissolved. The finished treatment medium is poured into a culture bottle and labeled according to the treatment, and covered with aluminum foil. The bottles were then plastered using autoclave at pressures between 17.5 psi and a temperature of 121 °C for 15 minutes and incubated for 24 hours before use.

Sterilization, insulation, and planting of explain

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Plant material (explants) to be planted, previously in the sterilized. The sterilization stage for sansevieria young leaf explants consists of 2 stages, namely stage 1 sterilization which is carried out in the preparation room, and stage 2, namely sterilization carried out in LAFC.

Stage 1 sterilization includes: the explants of Sansevieria leaves are washed with anti-bacterial liquid soap and rinsed under running water until clean. A Dithane M-45% solution was used to soak the Sansevieria leaves for a period of 15 minutes, followed by three rinses with aquades. Subsequently, the leaves were soaked in a 5% NaCl solution for a duration of 15 minutes and subsequently rinsed thrice with aquades. Stage 2 sterilization includes: Sansevieria leaves soaked NaOCl 2 % for 15 minutes, after itu rinsed with aquades 3 times, then soaked into NaOCl 1 % for 5 minutes, after which rinsed with aquades 3 cali, then the explant is isolated and cut in a petri dish then planted on the substrate and labeled according to the treatment and planting date.

Observation

Observations are made by observing the development of an explant every week as follows: When callus appears (days after planting / HST), callus color (green, yellow, white or brown), callus texture (crumbly or compact), callus weight (g), callus diameter (mm), percentage of success growing (%)

Analysis Data

Analysis data was carried out statistically using a variety of fingerprints (analysis of variants or ANOVA), to determine the influence of each treatment

The collected data underwent a subsequent analysis to determine any differences in treatment means through the implementation of DNMRT (Duncan's new multiple range test) with a significance level of 5%. The statistical analysis was conducted using the SAS software application.

RESULTS AND DISCUSSION

When Callus Appears

The variance analysis revealed that the application of the NAA, BAP, and NAA-BAP combination had a notable impact on the emergence of callus in the explanted leaves of *Sansevieria ehrenbergii*. The average when callus appears after further tests with the results of the DNMRT at a significance level of 5% are presented in Table 1.

Table 1. The average when the callus appears on the leaves of *Sansevieria ehrenbergii* with the addition of NAA, BAP as well as a combination of BAP and NAA *in-vitro*. Data transformed using $\sqrt{y} + 0{,}5$.

Treatment	When Callus Appears (HST)
NAA 1 mg.l^{-1}	137,33 ab
NAA 2 mg.l^{-1}	173,83 b
BAP 1 mg.l^{-1}	0,00 c
BAP 2 mg.l^{-1}	0,00 c
NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}	109,33 ab
NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}	114,83 ab
NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}	75,75 a
NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}	175,08 b

The numbers indicated by the same lowercase letters on a line were not significantly different from each other according to the DNMRT test at a level of 5%.

Table 1 shows that NAA 2 mg.l^{-1} blended with BAP 1 mg.l^{-1} may accelerate the appearance of the Callus *Sansevieria ehrenbergii* markedly compared to other treatments, except application NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}, NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} dan

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NAA 1 mg.l^{-1}. The application of NAA treatment of 2 mg.l^{-1} + BAP 1 mg.l^{-1} showed that when callus appeared *Sansevieria ehrenbergii* tended to be faster with an average of 75.75 HST, during the NAA treatments of 2 mg.l^{-1} + BAP 2 mg.l^{-1} showed that callus growth tended to be slower with an average of 175.08 HST.

The treatments of NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1} show that when a callus appears tends to be faster suspected because the application of auxins with higher concentrations than cytokinins is quite effective in stimulating cell growth and division, thereby accelerating the formation of callus. The treatment of NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1} indicates when the callus appears tends to be slower suspected because if NAA 2 mg.l^{-1} which is blended with BAP 2 mg.l^{-1} will cause NAA function to be hampered in accelerating callus formation because the concentration of cytokinins administered is too high. According to Marlin *et al.* (2012), callus formation requires a relatively high amount of auxin that explants need to trigger callus growth, while cytokinins in relatively high amounts that explants need to trigger the growth of shoots.

Auxin application combined with cytokinins will cause a callus to form faster than auxins given singly. The initiation of callus development commences with the enlargement of the explant which is a response to the application of the given ZPT. Figure 2 displays when the callus appears.

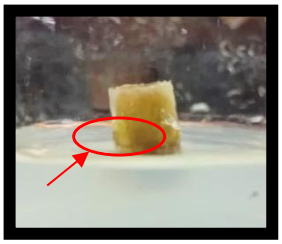

Figure 2. When Callus Appears

Callus formation occurs at different times. Cytokinin activation can affect cell division, causing cell differentiation, while auxins in cells stimulate the working power of cytokinins, therefore if auxins combined with cytokinins with smaller concentrations are quite effective in accelerating when callus appears. According to Hadipoentyani *et al.* (2008), the combination and equilibrium of the appropriately given ZPT will affect the speed at which the callus is formed. The speed of callus formation is determined by the working power of a given ZPT and the phytohormones present in the explant.

Callus is not formed on a single treatment of BAP, however almost all explants at the beginning of incubation look bloated. There are several explants of stopped responses due to contamination by fungi and bacteria. Non-growing explants are characterized by browning on the explants which indicates the cells are unable to survive. This response shows that the application of cytokinins with a small concentration has not been able to stimulate cell growth and division so it has not been able to form the callus *sansevieria ehrenbergii*. According to Ajijah *et al.* (2010), physiological changes in explants are characterized by explants losing chlorophyll or responses to death. According to Fitrianti (2006), the cessation of the explant response is due to the concentration of cytokinins that are small so that they are unable to induce callus, or the concentration of cytokinins that are too high so that they are toxic to the explant which eventually causes the death of the explant.

Callus Color

The observations showed the application of NAA, BAP and a combination of BAP and NAA on the explant *Sansevieria ehrenbergii* almost all treatments produced a green color compared to a green tobrass color. The color of the callus *Sansevieria ehrenbergii* can be seen in Table 2.

Table 2 shows that the resulting callus colors are green and yellowish-green. The Kalus produced in almost all treatments is green, the formation of green on the callus is currently a callus that already contains chlorophyll. The

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creation of callus color is also influenced by ZPT added to the culture medium.

Table 2. Influence of some concentrations of NAA, BAP as well as the combination of BAP and NAA on the callus color of the explant *Sansevieria ehrenbergii*

Treatment	Callus Color
NAA 1 mg.l^{-1}	Green yellowish
NAA 2 mg.l^{-1}	Green
BAP 1 mg.l^{-1}	-
BAP 2 mg.l^{-1}	-
NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}	Green
NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}	Green
NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}	Green
NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}	Yellowish-green

The NAA treatment combined with BAP of almost all explants produces a green callus color, it is suspected that the concentration of auxins combined with cytokinins will increase in a notable augmentation of chlorophyll synthesis in the callus, but on the contrary, if the auxin given singly whose concentration is low or too high in the blend of cytokinins is not able to increase in a notable augmentation of chlorophyll synthesis in the callus so that the callus changes color to yellowish-green. According to Rahayu *et al.* (2003), the higher auxin added to the medium will affect the decrease in chlorophyll and carotene content. Chlorophyll-containing callus is formed due to a combination of NAA and a given BAP. According to Ariati *et al.* (2012), the formation of the green part of the callus is the beginning of morphogenesis. The green color that occurs indicates that the callus contains chlorophyll. The callus is green and the callus is yellowish-green can be seen in Figure 3.

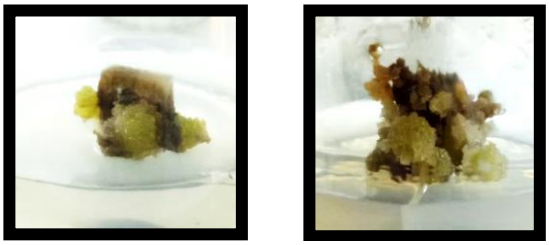

Figure 3. Callus is green and callus is yellowish-green

The research showed that yellowish-green callus formed on the treatment of NAA 1 mg.l^{-1} and a combination of NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}. A yellowish-green callus is formed is a process of maturation of tissues characterized by a change in the callus color. According to Trimulyono *et al.* (2004), the callus is yellow in color because it indicates the more mature the callus age. According to Fauzy *et al.* (2016), the diminishing capacity for callus regeneration can be identified by the progressively lax alterations in its morphology, the color of the callus that changes from greenish-yellow to brownish-yellow and to brown.

The colors resulting from the development of callus are white, green, and yellow or brown. A young callus will be white and then will turn green, then it will turn yellow or brown with age (Pisesha, 2008). According to Rasud and Bustaman (2020), the white callus is the mass of cells that are actively dividing, while the yellowish-white callus is the cell that goes to the end of active division and the brownish cells are the cells that go to the aging phase (*senescence)*.

The change in callus color that occurs is influenced by nutrition in culture media and environmental factors such as light (Evans *et al*., 2003). According to Yuniardi (2019), white light can stimulate the formation of callus and organogenesis in plant tissue culture. According to Sugiharto *et al.* (2007), although the energy needs for *in-vitro* growth are already met by the nutrients contained in the medium to produce

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green planlets with normal leaves light is needed.

Callus Texture

The observations showed the application of NAA, BAP, and a combination of BAP and NAA on the explant *Sansevieria ehrenbergii* almost all treatments resulted in a crumb texture compared to a compact texture. The texture of the callus *Sansevieria ehrenbergii* can be seen in Table 3.

Table 3 displays two types of callus textures; crumb and compact, which indicate either active cell division or cell death in the explant cultured in vitro as a callus. According to Sugiyarto and Kuswandi (2014), callus can be categorized as either compact or crumbly based on the texture and cellular composition. The former has a rigid and compact texture comprising small, densely-packed cells, while the latter has a soft texture and comprises non-dense cells.

Table 3. The impact of various concentrations of NAA, BAP, and their combination on the texture of *Sansevieria ehrenbergii* explant callus was examined.

Treatment	Callus Texture
NAA 1 mg.l^{-1}	Crumb
NAA 2 mg.l^{-1}	Crumb
BAP 1 mg.l^{-1}	-
BAP 2 mg.l^{-1}	-
NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}	Crumb
NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}	Crumb
NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}	Compact
NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}	Crumb

Figure 4. Crumbly Callus Texture

The most commonly obtained callus texture was the crumb texture in almost all treatments, except in NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}. The application of auxin in all treatments produces callus with a crumb texture, it is suspected that to get callus with a crumbly texture requires auxin or auxin combined with cytokinins. The results of yelnititis (2010) research, the formation of crumbly callus from the remin leaf explant (*Gonystylus bancanus* (Miq) Kurz) obtained from the addition of ZPT 2,4-D combined with thiodiazuron has a crumb texture and the white color shows that the crumb callus requires auxin with a higher concentration or auxin combined with cytokinins.

The treatment of NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1} has a compact textured callus texture. It is suspected that obtaining a compact callus requires higher auxin than cytokinins. According to Nisak *et al.* (2012), the compact callus structure has a dense, dense, and difficult-to-separate arrangement of cells. The compact texture of the callus is the effect of auxin and cytokinin application that affects the water potential in the cell. According to Dhaliwal *et al.* (2003), auksin can loosen the fibers of the cell wall so that cells are more flexible and nutrients will enter diffusional. This will continue until the water and osmotic potential is balanced and the cell becomes turgid, then turgid cells with the addition of cytokinins will affect the division and lengthening of cells hance cell divides faster and the callus becomes compact. Andaryani (2010), stated that kalus that has a compact texture can accumulate more secondary metabolites while the crumbly callus can be used for suspension culture in aim is to increase the

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number of callus. Whereas the Figure 5 depicts the compact callus texture.

Figure 5. Compact Callus Texture

Callus Weight

The outcomes of the variance analysis demonstrated that the application of NAA, BAP, and the combination of NAA and BAP had a marked impact on the callus weight of the Explant *Sansevieria ehrenbergii*. Table 4 displays the mean weight of the explant callus following the DNMRT test at a significance level of 5%.

Table 4. The average callus weight of the *explant Sansevieria ehrenbergii* with the application of NAA, BAP and a combination of BAP and NAA *in-vitro*. Data transformed using $\sqrt{y} + 0{,}5$

Treatment	Callus Weight (g)
NAA 1 mg.l^{-1}	0,2500 de
NAA 2 mg.l^{-1}	0,3733 cd
BAP 1 mg.l^{-1}	0,0000 e
BAP 2 mg.l^{-1}	0,0000 e
NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}	0,6000 bc
NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}	0,9033 a
NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}	0,7533 ab
NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}	0,4300 cd

The numbers in the lane followed by the same lowercase letters differed unreal according to the DNMRT test at the level of 5 %.

Table 4 shows that the application of NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} may increase the callus weight of *Sansevieria ehrenbergii* markedly compared to other treatments, except for treatment of NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}. The application of NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} showed that the average callus weight tended to be higher 0.9033 g, while the NAA 1 mg.l^{-1} showed that the average callus weight tended to be lower with an average of 0.2500 g

The application of NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} resulted in a callus weight tending to be higher presumed because the treatment was appropriate in encouraging the increase in callus weight, NAA played a role in cell shouting and BAP played an optimal role in cell division which could lead to an augmentation in the weight of the callus. However, in the singular application of NAA has not been able to increase the weight of the callus.

A callus with a greater weight caused by auxins and the cytokinins given can encourage the formation of a callus faster which is followed by increasing cell growth thereby increasing the wet weight of the callus. Auxins and cytokinins given as well as those found in explants affect the growth of explants (Wulandari *et al.*, 2004). The weight of the callus is primarily determined by the rate of cellular division, proliferation, and enlargement (Andaryani, 2010).

According to Indah and Ermavitalini (2013), the weight of the callus has a content of water and carbohydrates. The provision of nutrients will ensure the availability of energy sources for cells to be able to grow. The weighing of the heat of the callus of the explant *Sansevieria ehrenbergii* can be seen in Figure 6.

Figure 6. Weighing the weight of the callus

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Treatment of NAA 1 mg.l^{-1} resulted in a lower tendentious callus weight with an average of 0.2500 g. This proves that the singular application of NAA has not been able to add to the weight of the callus.

Callus Diameter

The results of the variance show that the application of NAA, BAP and the combination of NAA and BAP have a marked effect on the diameter of the callus on the explant *Sansevieria ehrenbergii*. The average diameter per explant after the DNMRT test at the level of 5% can be seen in Table 5.

Table 5. The average callus diameter of the Explant *Sansevieria ehrenbergii* with the application of NAA, BAP and a combination of NAA and BAP *in-vitro*. Data transformed using $\sqrt{y} + 0{,}5$.

Treatment	Callus Diameter (mm)
NAA 1 mg.l^{-1}	6,533 c
NAA 2 mg.l^{-1}	11,967 b
BAP 1 mg.l^{-1}	0,000 d
BAP 2 mg.l^{-1}	0,000 d
NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}	14,033 b
NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}	19,133 a
NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}	16,433 ab
NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}	12,133 b

The numbers in the lane followed by the same lowercase letters differed unreal according to the DNMRT test at the level of 5 %.

Table 5 shows that the application of NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} may increase diameter of the kalus *Sansevieria ehrenbergii* markedly compared to other treatments, except for the application of NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}. The application of NAA treatment of 1 mg.l^{-1} + BAP 2 mg.l^{-1} showed that the average callus diameter tended to be greater, namely 1 9.133 g, while the average diameter of the smallest callus was found in the NAA treatment of 1 mg.l$^{-1,}$ which was 6,533 mm. The application of NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} resulted in a callus diameter tending to be larger presumably because the treatment was able to increase callus growth so as to increase the callus diameter.

Administering a higher BAP than NAA triggers cell division, thereby increasing the callus diameter. The application of NAA singularly has not been able to increase the diameter of the callus because in increasing cell division requires more cytokinins than auxins. According to Waryastuti *et al.* (2017), the application of BAP in the right concentration can be stimulating in the growth of callus and buds because it has a significant function in natural organogenesis, and Figure 7 shows the callus diameter measurement.

Figure 7. Callus diameter measurement

The lowest callus diameter was found in the application of NAA 1 mg.l^{-1} which was 6,533 mm compared to other treatments. This is believed to be due to auxin treatment with a low concentration and also with the absence of cytokinin addition has not been able to meet the development and growth of callus. Auxin plays a role in cell shading and can stimulate cytokinin activity while cytokinin plays an optimal role in cell division, so the application of auxin combined with higher cytokinins stimulates callus cell division so that the callus diameter is greater than a single auxin application.

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According to Karjadi and Buchory (2008), the addition of auxin and cytokinin hormones given can stimulate cell division, but the growth of each planlet varies depending on the concentration added to the growing medium. Accordingto Dewi (2008), it is generally the concentration balance of several ZPT that will control the growth of the explant.

Growth Success Percentage

The variance analysis revealed that NAA, BAP, and their combination had a significant impact on the success rate of growing Explant *Sansevieria ehrenbergii*. The average percentage of success growth after the DNMRT test at the level of 5% can be seen in Table 6.

Table 6. The average percentage of success in growing explants *sansevieria ehrenbergii* with the application of a combination of BAP and NAA *in-vitro*. Data transformed using $\sqrt{\%} + 0{,}5$

Treatment	Success Percentage(%)
NAA 1 mg.l^{-1}	25,00 cd
NAA 2 mg.l^{-1}	58,33 bc
BAP 1 mg.l^{-1}	0,00 d
BAP 2 mg.l^{-1}	0,00 d
NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1}	100,00 a
NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}	91,67 ab
NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}	75,00 ab
NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}	83,33 ab

The DNMRT test at a 5% significance level showed that the figures with the same lowercase letters were not significantly different.
Ket: The number zero (0.0000) indicates the non-formation of callus on the explant

Table 6 shows that the treatment of NAA 1 mg.l^{-1} application combined with BAP 1 mg.l^{-1} may increase the percentage of growth success of *Sansevieria ehrenbergii* markedly compared to other treatments, except in the application of NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1}, NAA 2 mg.l^{-1} + BAP 2 mg.l^{-1}, and NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1}. The application of NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1} showed that the percentage of success in growing *Sansevieria ehrenbergii* tended to be higher with an average of 100%, while the NAA treatment of 1 mg.l^{-1} showed that the percentage of success tended to be lower with an average of 25 %. The application of NAA 1 mg.l-1 + BAP 1 mg.l-1 showed a higher percentage of success presumably because the treatment given was appropriate in encouraging the formation of callus so as to increase the percentage of growth. Auxin application combined with cytokinins gained a high percentage of success, in contrast to the NAA and BAP treatments given singularly. It is suspected that callus requires auxin combined with cytokinins to trigger callus to stay alive. According to Fitrianti (2006), auxins and cytokinins exogenous given are thought to be the triggers more initially explants undergoing dedifferentation making cells more quickly become meristematic again so that callus is formed.

The callus formed on the surface of the explant is caused by the stimulation of the wound which causes the equilibrium of the cell wall to change direction, part of the protoplast flows out so that the callus begins to form. The generation of callus tissue on explants is affected by various plant-related factors as well as growth-modulating agents (Zulkarnain and Lizawati, 2011).

The high success rate of growing in this study was also influenced by the sterile *plant material of Sansevieria ehrenbergii* and the proper technique of taking explants. The use of plant parts used as explants is young tissue that is still actively growing, the cells are still actively dividing themselves and have high regeneration power, besides that environmental factors also affect callus growth such as cleanliness and sterilization at the time of

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planting. Yuwono (2006), states that one of the conditions in *the in-vitro* tissue culture technique is the cleanliness and sterilization of the tool and the place of planting. This is to prevent bacterial or fungal contamination that grows faster than explant growth.

The NAA 1 mg.l^{-1} resulted in the lowest percentage of callus growth success with an average of 25 %, it is suspected that the application of ZPT singularly has not been able to encourage the formation of callus besides contamination and browning leading to the death of explants quite high causing explants to be unable to survive. In a single treatment bap, there is no growth, this is suspected because the BAP given has not been able to help explants in forming callus because BAP itself plays an active role in forming shoots and shoots besides it is also caused by fungal and bacterial contamination. Contamination can be caused by fungi and bacteria, this canoccur at any time even though previously all tools and materials including media have been sterilized. This is because it can come from the incubation room, the closure of the bottle is not tight and is supported by a suitable medium for the place where fungi and bacteria grow. Explant contamination by fungi and bacteria can be seen in Figure 8.

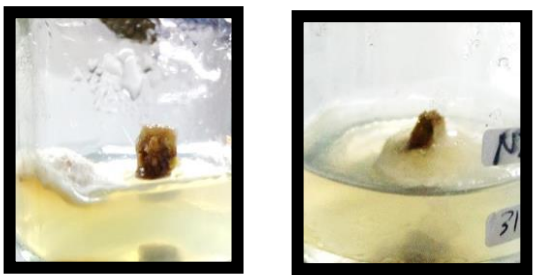

Figure 8. Explanted contaminants by fungi and bacteria

According to Astutik (2002), contamination can occur due to 3 sources, namely in planting media, explants, and implementation. Contamination caused by the implementation occurs due to the technique of making planting media and when the subculture is less aseptic, thus providing an opportunity for the entry of microorganisms such as bacteria and fungi to grow in the media.

Reducing contamination by fungi and bacteria can be done by selecting healthy plant material (free from pests and diseases), keeping the culture room sterile, closing the culture bottle after planting tightly, and careful work procedures, so that contamination by bacteria can be prevented by adding antimicrobials such as antibiotics. The *browning explant* can be seen in Figure 9.

Figure 9. *Explants browning*

According to Hutami (2008), the browning of tissues (*browning*) is caused by the presence of phenolic compounds that are oxidized through the activity of oxidation polyphenol enzymes which causes the tissues to turn brown which then results in death in explants. According to Santosa and Nursandi (2003), *browning* can be caused by the use of non-meristematic planting material or adult tissue, excessive sterilization measures, unsuitable media, or a non-supporting environment.

CONCLUSION

The application of growth regulators NAA, BAP and the combination of NAA and BAP affects the growth of the leaf explant of *sansevieria ehrenbergii* leaves, namely when callus appears, callus weight, callus texture, callus diameter, callus color and growth success percentage.

The treatment of NAA 2 mg.l^{-1} + BAP 1 mg.l^{-1} gave an influence standing to be faster when callus appeared wirh an average 75.75 HST, the application of NAA 1 mg.l^{-1} + BAP 2

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mg.l^{-1} gave an influence tending higher on the weight of the callus and tended to be larger in callus diameter with an average of 0.9033 g and 19.133 mm and the application of NAA 1 mg.l^{-1} + BAP 1 mg.l^{-1} gives a higher influence on the percentage of callus growth with an average of 100 %.

Based on the results of this study, for explant propagation *Sansevieria ehrenbergii* it is recommended to use MS media with the combination of a growth regulator NAA 1 mg.l^{-1} + BAP 2 mg.l^{-1} because it gives a good response to all observations parameters.

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