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Research Article
In vitro Callus Induction of Sipahutar Pineapple (*Ananas comosus* L.) from North Sumatra Indonesia

[1]Fauziyah Harahap, [1]Diky Setya Diningrat, [2]Roedhy Poerwanto, [3]Nanda Eska Anugrah Nasution and [4]Rifa Fadhilah Munifah Hasibuan

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Jln. Willem Iskandar, Psr V Medan Estate, 20221 North Sumatra, Indonesia
[2]Center for Tropical Horticulture Studies, Bogor Agricultural University, IPB Baranangsiang Campus, Jalan Raya Pajajaran, 16143 Bogor, Indonesia
[3]Departement of Biology Education, Faculty of Education and Teacher Training, Institut Agama Islam Negeri Jember, East Java, Indonesia
[4]Agronomy and Horticulture, Undergraduate, Bogor Agricultural University, IPB Dramaga Campus, Jalan Raya Dramaga, 16680 Bogor, Indonesia

Abstract

Background and Objective: Sipahutar pineapple (*Ananas comosus* L.) is a indigenous of pineapple grown in Sipahutar district, North Sumatra, Indonesia. Propagation of Sipahutar pineapple that being done traditionally is less effective, because the number of seeds that produced is very limited and requires a long time. Propagation through *in vitro* culture is an alternative solution to solve this problem. It is necessary to add plant growth regulator (PGR) to stimulate callus formation in Sipahutar pineapple explants (*Ananas comosus* L.). Callus induction of pineapple from Sipahutar was carried out by PGR treatment on MS medium. The purpose of this study was to determine the effect MS medium treatment with added dichlorophenoxyacetic acid (2,4-D) and benzyl amino purin (BAP) PGR on Sipahutar pineapple callus formation (*Ananas comosus* L.) with light and dark treatment. **Materials and Methods:** This callus induction research used a completely randomized design (CRD) with 2 factors, the first factor was treatment 2,4-D (0, 1, 2) ppm. The second factor is BAP (0, 0.5, 1) ppm. **Results:** Nine combinations of treatments are obtained. Each combination of treatments is treated in both light and dark conditions. The parameters of this study were the percentage (%) of explants that formed callus, the time of callus formed, callus texture, callus biomass, callus surface height and callus surface area. Data were analyzed with two-way ANOVA, followed by Duncan Multiple Rate Test (DMRT). **Conclusion:** The study showed that the interaction between 2,4-D and BAP significantly affected the time of callus formed but 2,4-D and BAP did not significantly affect callus biomass, callus surface height and callus surface area. All explants can form callus, except explants without the addition of 2,4-D and BAP. The callus formed on 10 days after induction (DAI) and 12 DAI with the treatment of light and dark. The color of the produced callus were white, yellowish white, greenish white, brown, brownish yellow, brownish white, brownish green, yellowish green and greenish white. The callus formed is generally compact textures, except for explants which by giving 1 ppm 2,4-D produce friable callus.

Key words: 2,4-D, BAP, *Ananas comosus* L., Sipahutar pineapple, callus

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Corresponding Author: Fauziyah Harahap, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Jln. Willem Iskandar, Psr V Medan Estate, 20221 North Sumatra, Indonesia Tel: +6281376817918

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sipahutar pineapple is a indegeneus, local pineapple that is famous for its sweet taste, watery, large and yellow skin color[1]. This pineapple has long been cultivated, has prospects, has the potential to be developed. Sipahutar Pineapple provides good prospects, to help increase agricultural production, especially for food crop needs. Efforts to develop Sipahutar pineapple plant continue to be carried out, especially in the supply of seeds.

Usually farmers grow pineapple traditionally. At present, Sipahutar Pineapple has been planted like a pineapple plantation but the supply of seeds has always been a big problem[1]. In order to achieve large scale development, traditional propagation is not effective, because the number of seedlings produced is measly and takes long time. Propagation through tissue culture is an alternative technique for solving this problem[1-3], specifically using callus culture. Callus is a collection of amorphous cell masses which divide continuously, composed by parenchymal cells which bonds are very tenuous[2,3]. Callus culture aims to obtain callus from grown explants on a culture medium continuously, by *in vitro* technique, one of the methods to develop of reproducible of plantlets through callus because it was the most suitable material used for genetic transformation in plant[4], induction of somatic embryogenesis[5]. Callus culture is important to do with various purposes including to study cell metabolism and differentiation, cell morphogenesis, somaclonal variation, genetic transformation, secondary metabolite production[6]. In this study, callus culture was carried out to obtain the best combination of media and to produce the best callus that could be regenerated, becoming a source of explants that would eventually be produced in large numbers of Sipahutar pineapple plantlets.

In terms of inducing callus, growing media are needed, generally using Murashige and Skoog (MS) media, PGR is combined with basic media. The most commonly used compounds for callus induction is 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetate acid (NAA), indole acetate acid (IAA), indole butyric acid (IBA). Amin *et al.*[7] states that there is an effect of pineapple callus growth of 75% by adding 2,4-D of PGR 2.0 mg L^{-1}, the combination between 2.4-D 2.0 mg L^{-1} and BAP 2.0 mg L^{-1} showed an effect of 95% callus growth.

This study aims to determine the effect of (1) PGR 2,4-D, (2) PGR BAP, (3) Combination of PGR 2,4-D and BAP and (4) Dark and light treatment, on induction of Sipahutar pineapple callus (*Ananas comosus* L.).

MATERIALS AND METHODS

This research was conducted at YAHDI Tissue Culture Laboratory, Perum Pelabuhan Jl. Lambung No. 16 Tanah 600 Medan Marelan, Medan and Universitas Negeri Medan Biology Laboratory, for 8 months from March-October 2018. Tools that being used in this study were standard tissue culture tools. The material used in this study are: *in vitro* Sipahutar pineapple, Murashige and Skoog (MS) media, PGR 2,4-D, PGR BAP, alcohol, 0.1 N HCl, 0.1 N NaOH, sterile aquadest, detergent, chlorox.

Sterilization and making the media: All tools sterilized using an autoclave, at 121°C for 1 h at a pressure of 17.5 psi. Everything is according to the amount listed in the composition of making 1 L MS media, all ingredients are mixed. 2,4-D and BAP were added according to the treatment.

Callus induction: The plant material was used as of this study was 1 cm *in vitro* Sipahutar pineapple bulb. This study was carried out in completely randomized design (CRD) with 9 treatment combinations. This study used MS basic media with added PGR, namely (2,4-dichlorophenoxyacetic acid (0, 1, 2 ppm) and benzyl amino purine (0, 0.5, 1 ppm), with 4 replications, therefore there are 36 experimental units. All combinations of treatment are placed in both dark and light treatment, hence experimental units are 72 bottles.

Callus induction was carried out in a laminar air flow cabinet (LAFC) using *in vitro* Sipahutar pineapple bulb. *In vitro* shoots are taken, placed on petridish, *in vitro* leaves are removed. Buds cut into 1 cm size for each treatment media according to the concentration that has been made.

Maintenance was carried out by placing bottles filled with explants on culture racks at a temperature range of 22°C for 36 bottles of light treatment by application of fluorescent light of 3000-3200 lux in a 16 h photoperiod and 36 bottles closed using black cloth as a dark treatment. These samples were incubated, maintained at 24°C by regulating the room air conditioner in the culture room.

Observation parameters

Percentage of explants that formed callus: Explants forming callus were observed from the 1st day after induction to 35th day of observation. The percentage of explants that formed callus calculated by the equation:

$$\text{Explants (\%) form callus} = \frac{\text{No. of explants that make up the callus}}{\text{Total No. of explants}} \times 100\%$$

Time of the callus formation: The time of the callus formation, characterized by the emergence of irregular amorphous cells, were observed from the 1st day after induction to 35th day of observation.

Callus biomass: Callus biomass measurements in Sipahutar pineapple explants on light and dark treatments were carried out after 35 days after induction (DAI). The callus was removed from the culture bottle and weighed using a digital scale.

Callus color: The color of callus was observed after the formation of callus, 20th day and 35th day. Determination of callus color was based on Andaryani[8] with the researchers modifications, namely: Brown (1), brownish yellow (2), brownish white (3), greenish white (4), brownish green (5), yellowish green (6), whitish green (7) and green (8).

Callus texture: Callus texture was observed 35 days after induction. Characterized by a compact and friable callus texture. Friable callus is marked by the form of callus that is easily separated. While the compact callus is marked by the callus that is not easily separated.

Callus height stack: Callus stack height was measured after 35 days after induction. Callus was removed from the culture bottle and measured using millimeter paper and a ruler.

Callus surface area: The callus surface area was measured after 35 days after induction. Callus was removed from the culture bottle and measured using millimeter paper.

Statistical analysis: This research uses factorial completely randomized design model and analysis with factorial ANOVA, the equation:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+\varepsilon_{ijk}$$

Where:

Y_{ijk} = Observations on the k test, which received 2,4-D treatment the-i and BAP treatment the -j
μ = Middle value
α_i = Effect of 2,4-D concentration on the i level
β_j = Effect of BAP concentration at the j level
$(\alpha\beta)_{ij}$ = Effect of the interaction of 2,4-D treatment at the i-level and the BAP-j treatment
ε_{ijk} = Effect of the error with 2,4-D treatment at the i level and BPA treatment at the j level at the k-replication

If the hypothesis testing obtained significantly different, then proceed with the Duncan Multiple Range Test (DMRT).

RESULT

Percentage of explants forming callus: Both light and dark treatment, all explants (100%) formed callus. Only callus treated with MS media without the addition of PGR formed callus of 75%, the rest of the explants were able to form callus (Table 1). The highest percentage of explants forming callus came from the treatment of MS media with an additional 1 ppm 2,4-D and 0.5 ppm BAP. The treatment of 1 ppm 2,4-D and 1 ppm BAP was also able to induce rapid and good callus formation.

Time of callus formed: From Table 1, it can be obtained that the combination treatment of MS medium with the addition of 1 ppm PGR 2,4-D and 1 ppm BAP was able to induce the fastest callus at 10 days after induction (DAI) in the light treatment and 12 DAI in the dark treatment.

With the same treatment of PGR 2,4-D, which is 1 ppm and addition of the lower concentration of BAP (0.5 ppm) causing delayment to form callus, that is on day 12 in the

Table 1: Percentage of explants forming callus and time of callus formation for light and dark treatment

PGR treatments		Explant forming callus (%)		Callus texture	
2,4-D (ppm)	BAP (ppm)	Light treatment	Dark treatment	Light treatment	Dark treatment
0	0.0	75	75	Compact	Compact
0	0.5	100	100	Compact	Compact
0	1.0	100	100	Compact	Friable
1	0.0	100	100	Compact	Friable
1	0.5	100	100	Friable	Friable
1	1.0	100	100	Friable	Friable
2	0.0	100	100	Compact	Compact
2	0.5	100	100	Friable	Compact
2	1.0	100	100	Compact	Compact

light treatment and day 14 in dark treatment. Increasing 2,4-D concentration to 2 ppm with combination of 0, 0.5 and 1 ppm BAP was not able to accelerate in forming callus, callus emergence was delayed to days 13-16 for light treatment and days 17-20 for dark treatment. While the longest form of callus is without added of PGR, to be exact at 25 DAI in light treatment and 27 DAI in dark treatment (Fig. 1).

Callus color: The treatment of 2,4-D and BAP PGR for light treatment resulted in a variety of callus colors (brownish white, greenish white and others) (Table 2). Observations at 20 days after induction, explants without additional and additional of low-dose 2,4-D single or combined with low-dose BAP (0, 0.5 ppm), both with light and dark treatment did not show callus formation.

Explants without the addition of 2,4-D with the addition of BAP 1 ppm produced white callus in light treatment meanwhile dark treatment did not produce callus. Addition of 1-2 ppm 2,4-D produced variety of callus colors which varied from white, yellowish white, greenish white (Fig. 2).

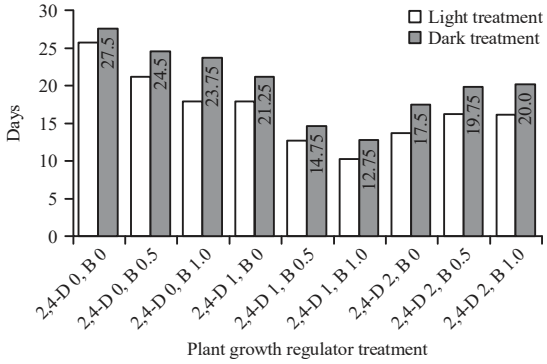

Fig. 1: Average time of callus formed in dark and light treatment
2,4-D: 2,4-dichlorophenoxyacetic acid, B: Benzyl amino purin

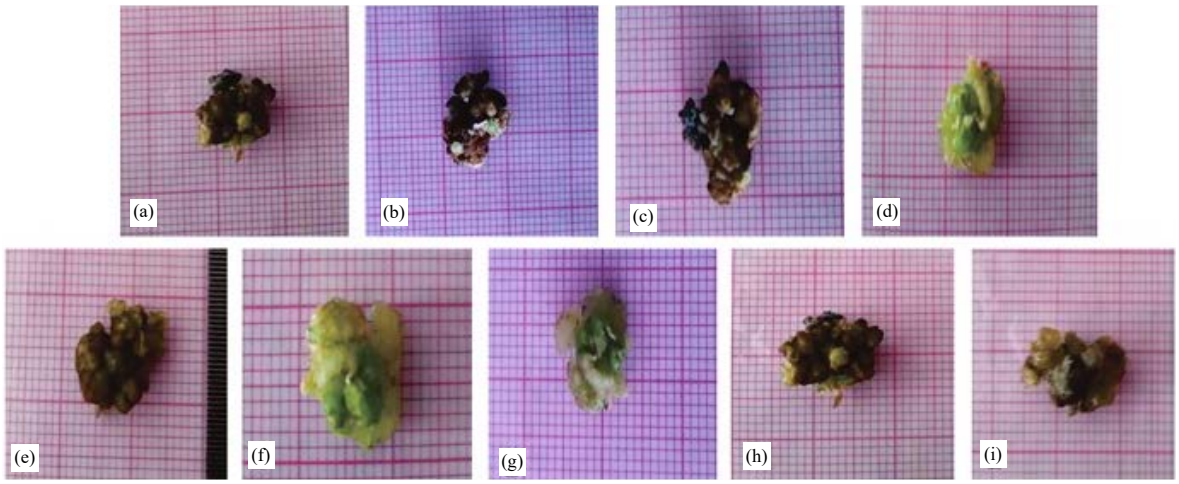

Fig. 2(a-i): Performance of callus at 35 DAI in a row starting from treatment, (a) 2,4-D 0 ppm and BAP 0 ppm, (b) 2,4-D 0 ppm and BAP 0.5 ppm, (c) 2,4-D 0 ppm and BAP 1 ppm, (d) 2,4-D 1 ppm and BAP 0 ppm, (e) 2,4-D-1 ppm and BAP 0.5 ppm, (f) 2,4-D 1 ppm and BAP 1 ppm, (g) 2,4-D 2 ppm and BAP 0 ppm, (h) 2,4-D 2 ppm and BAP 0.5 ppm and (i) 2,4-D 2 ppm and BAP 1 ppm

Table 2: Color of callus age 20 and 35 days after induction for light and dark treatment

PGR treatments		Color of callus (20 DAI)		Color of callus (35 DAI)	
2,4-D (ppm)	BAP (ppm)	Light treatment	Dark treatment	Light treatment	Dark treatment
0	0.0	Callus hasn't appeared yet	Callus hasn't appeared yet	Brown (1)	Brown (1)
0	0.5	Callus hasn't appeared yet	Callus hasn't appeared yet	Brownish yellow (2)	Brownish white (3)
0	1.0	Brownish white (3)	Callus hasn't appeared yet	Brown (1)	Brownish white (3)
1	0.0	Greenish white (7)	Callus hasn't appeared yet	Brownish green (5)	Brownish green (5)
1	0.5	Greenish white (7)	Greenish white (7)	Brownish green (5)	Brownish green (5)
1	1.0	Greenish white (7)	Greenish white (7)	Yellowish green (6)	Yellowish green (6)
2	0.0	Yellowish white (3)	Yellowish white (3)	Brown (1)	Brown (1)
2	0.5	Yellowish white (3)	Yellowish white (3)	Brown (1)	Brownish yellow (2)
2	1.0	Brownish white (3)	Yellowish white (3)	Brown (1)	Greenish white (7)

Callus biomass: Based on the results of the analysis of variance, 2,4-D PGR addition had huge effect on callus biomass for light treatment and dark but BAP in the light and dark treatment and the interaction of 2,4-D and BAP for light and dark treatment have no gave effect. The highest callus biomass was produced from the treatment of 2,4-D 1 ppm and BAP 1 ppm in the light and dark treatment that is 3.32 and 2.94 g. While the lowest callus biomass was 1.67 g (light treatment) and 1.46 g (dark treatment) from the treatment of 2,4-D 0 ppm and BAP 0 ppm (Fig. 3).

The Duncan's Multiple Range Test (DMRT) results showed that the average callus biomass was not different. It is seen that in both treatments (light and dark), the heaviest biomass is 3.32 g (bright) and 2.94 g (dark), the results of 2,4-D 1 ppm treatment and BAP 1 ppm. The lightest callus biomass is the result of 2,4-D 0 ppm and BAP 0 ppm, which is 1.67 g from light and 1.46 from dark treatment (Fig. 3).

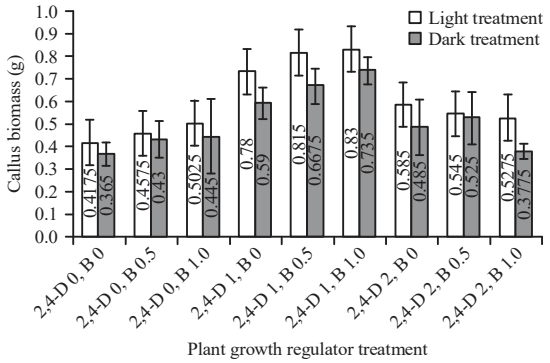

Fig. 3: Average callus biomass (g) for light and dark treatment
2,4-D: 2,4-dichlorophenoxyacetic acid, B: Benzyl amino purin

Callus texture: The formed callus texture is differentiated into callus with friable texture and compact texture callus (Fig. 4). Friable callus is characterized by an easily separated callus texture, compact callus is in the form of a solid lump which is difficult to separate. Based on the observation of PGR 2,4-D and BAP treatment, it produced 2 types of callus texture, namely compact and friable (Fig. 2). Light and dark treatment shows that the most dominant texture is compact callus texture. Friable texture callus is generally found in 2,4-D PGR treatment with a concentration of 1 ppm both in light and dark treatments. The results of observations carried out in this study indicate that 2,4-D added to the media has an effect on the appearance of callus texture (Table 1).

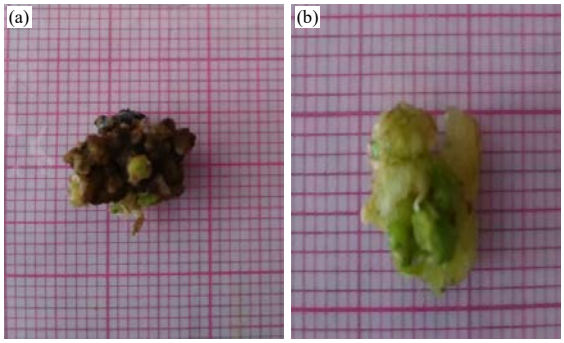

Fig. 4(a-b): Color and texture of callus, (a) Brown (2,4-D 0 ppm+BAP 1 ppm and (b) Yellowish green (2,4-D 1 ppm+BAP 1 ppm) and compact callus texture

Height stack of callus: The treatment of 2,4-D 2 ppm and BAP 0 ppm produced the highest callus stack which was 1.7 cm. The lowest callus stack height is the result of 2,4-D 0 ppm treatment and 0 ppm BAP with a stack height of 1.28 cm. Dark treatment, 2,4-D 0 ppm and 0 ppm BAP produced the highest callus stack height of 1.7 cm. The lowest callus height was treatment of 2,4-D 0 ppm and BAP 0 ppm in the light treatment, with a stack height of 1.28 cm callus (Fig. 5).

Results of analysis of variance, treatment of PGR 2,4-D; BAP; the interaction of 2,4-D and BAP on the height of the callus stack with light and dark treatment did not have effect.

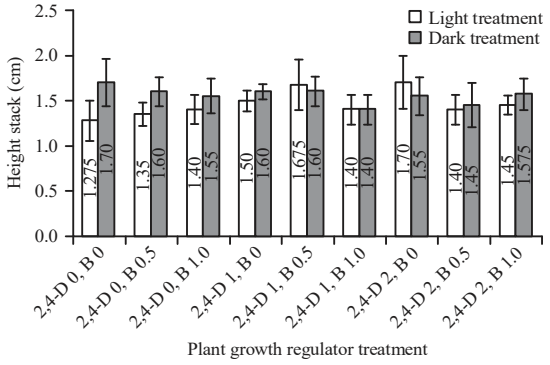

Fig. 5: Average height stack (cm) of callus for light and dark treatment
2,4-D: 2,4-dichlorophenoxyacetic acid, B: Benzyl amino purin

Callus surface area: According to the results of analysis of variance analysis, the addition of 2,4-D affected the surface area of callus both in light and dark treatment (Table 3, 4). Meanwhile, the BAP treatment did not affect the surface area of callus in both light and dark treatment. The interaction 2,4-D and BAP did not affect callus biomass for light or dark treatments (Table 4). The 2,4-D 1 ppm and BAP 0.5 ppm treatment produced the highest callus surface area of 0.95 cm

Table 3: Analysis of variance (ANOVA) effect of interaction between 2,4-D and BAP on the callus surface area of 35 DAI at light treatment

Main effect	Degree of freedom	Sum squared	Middle squared	F-count	F-table (0.05)	F-table (0.01)
Main effect						
2,4-D treatment	2	0.22	0.11	4.23*	3.35	5.49
BAP treatment	2	0.06	0.03	1.15^{ns}	3.35	5.49
Interaction of 2 factors						
2,4-D, BAP	4	0.13	0.032	1.23^{ns}	2.73	4.11
Error	27	0.70	0.026			
Total	35					

2,4-D treatment is significant, while for BAP treatment and interaction between 2,4-D and BAP is not significant, ns: Not significantly different, *Significantly different

Table 4: Analysis of variance (ANOVA) effect of interaction between 2,4-D and BAP towards the surface area of callus of 35 DAI at dark treatment

Variants effect	Degree of freedom	Sum squared	Middle squared	F-count	F-table (0.05)	F-table (0.01)
Main effect						
2,4-D treatment	2	0.11	0.06	3.55*	3.35	5.49
BAP treatment	2	0.03	0.015	0.97^{ns}	3.35	5.49
Interaction of 2 factors						
2,4-D, BAP	4	0.16	0.04	1.29^{ns}	2.73	4.11
Error	27	0.83	0.031			
Total	35					

2,4-D treatment is significant, while for BAP treatment and interaction between 2,4-D and BAP is not significant, ns: Not significantly different, *Significantly different

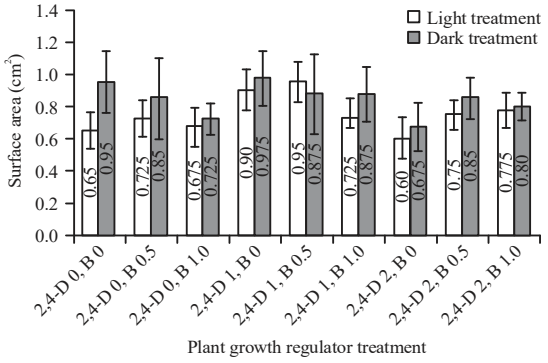

Fig. 6: Average callus surface area (cm^2) in dark and light treatment

2,4-D: 2,4-dichlorophenoxyacetic acid, B: Benzyl amino purin

(light) and PGR 2,4-D ppm and BAP 0 ppm, resulting 0.98 cm (dark) callus surface area. While the lowest surface area of callus is 0.6 cm (light) and 0.68 cm (dark) the results of 2,4-D 2 ppm and BAP 0 ppm (Fig. 6).

DISCUSSION

The data shows that, explants are generally able to form callus, except explants that were not given 2,4-D and BAP. The PGR is absolutely necessary for good callus formation, in this research was 2,4-D and BAP which will effect on increasing the percentage of explants that are able to form callus, callus appearance and acceleration of callus time formed. Callus formed in explants is formed due to the presence of openings on the tissue and response to hormones or growth regulators. The appearance of callus in the injured part is thought to be due to the stimulation of the tissue in the explants to cover the wound. One of the main characteristic of plant cells is having high plasticity for cell differentiation. Ikeuchi *et al.*[9] said, plants produce unorganized cell masses, such as callus or tumors, in response to pressure, such as wounds or pathogenic infections.

Dalila *et al.*[10] said the addition of 2,4-D and kinetin on basic MS medium gave better results of callus compared to using MS medium added with sucrose or phytagel. This is characterized by high frequency of callus induction, the callus produced is friable, beige color and grows intensively. Anitha and Kumari[11] states auxiliary as IAA and IBA were not effective for callus induction in all explants tested but 2,4-D was very effective for inducing callus with sources of petiole, leaf, cotyledon and hypocotyl of *Rauvolfia tetraphylla* L., while cytokines singularly it cannot induce this plant's callus. This statement is in line with the statement of Chakraborty *et al.*[12] which resulted the maximum callus obtained on MS medium with addition of a combination of 2,4-D (0.5 mg L^{-1}) and kinetin 0.2 mg L^{-1}.

Based on the this research, the concentration of 2,4-D, BAP gave an effect on the time of callus formed. 2,4-D is a PGR that is most often used in callus culture because of its stable activity to stimulate cell multiplication, suppress organogenesis and maintain callus growth. This strong and optimal 2,4-D activity is caused by carboxyl groups separated by carbon and oxygen[11]. Each growth regulator has an influence on the induction of pineapple callus. Yifter *et al.*[13]

stated that MS media that supplemented with BAP 2 ppm and NAA 1 ppm in *Sesamum indicum* L. Hirhir variety was the best composition for accelerating time to grow this plant.

In this study, the addition of 2,4-D and BAP which is getting higher, causing the delayment of callus being formed. It appears that the optimum concentration for Sipahutar pineapple callus formation is 1 ppm 2,4-D with 1 ppm BAP. It can be understand that too high the concentration of auxin and cytokine PGR in cells, causing cells to keep on racing to make elongation and stretching. This activity takes place repeatedly without giving the cell a chance to do normal, so in the end it will cause no expression of the normal callus formation process. This study also in line with Tahir *et al*.[14] which explains that the addition of 2,4-D 3.5 mg L^{-1} gives the best effect in the formation of the callus sugarcane then the growth of callus decreases with the addition of 2,4-D above 3.5 mg L^{-1} and Mostafiz and Wagiran[15], the formation of rice callus shows better as the addition of concentration 2,4-D but declining growth if exceeding 3 mg L^{-1}.

The dark treatment did not show a positive effect for accelerating formation of callus. As the latest study known that auxin works maximally on dark situations. Most likely there is another factor that affected the delayment of forming the callus in the dark treatment. Auxin works optimally in dark conditions and will be disturbed if there is light. From the results of this study there may be other factors that affect the formation of callus. Harahap[2] stated the ratio of auxin and cytokinin in the cell will determine the direction of induction in the tissue. If inside the cell, the auxin:cytokinin ratio is 1:1 so the tendency that occurs is callus formation. From this statement, the possibility is not only light factor which inhibits the formation of callus but there are other factors, in this case for example the balance of 2,4-D and BAP in the cell has not reached the desired ratio to form callus in the treated explants. Light in general is not giving strong effect for callus growth[2]. However, light affects the cell metabolism and effectiveness of PGR in the media. Light can damage auxin and can also cause the transfer of auxin in a direction away from light[16]. Tissue culture method in dark conditions is one of the way to make auxin effective in order to accelerate callus formation.

In vitro plant culture growth is not always hampered by the presence of light, whereas light is actually needed for optimal results. George and Sherrington[3] stated that in most cultures, cells will be able to do division in light conditions with the presence of external auxin in the media. In this study 2,4-D PGR was very effective for inducing callus of Sipahutar pineapple. As the literatures stated that IAA and IBA are not effective for inducing callus but 2,4-D is more effective for inducing callus with sources of petiole, leaf, cotyledonary leaf, hypocotyl explants[11].

The speed of growth that occurs in explants is due to the proper interaction between endogenous hormones explants and exogenous hormones given. This is reinforced by Urfiana[17] and Maciel *et al*.[5], stating that the interaction and balance of PGR given to the media and endogenously produced by plants determines the direction of development of a culture, Wahyuni *et al*.[18] say the interaction and the balance between each plant growth regulator which provided to the medium and produced by the plant cells indigenously determinated the direction of the culture development, this also in line with research from Chakraborty *et al*.[12] that stated BAP treatment alone was not all suitable for induction of callus.

The emergence of callus obstructed and also the emergence of the brown callus in treatment without 2,4-D and low dose either in single or combined with BAP, indicating that there is no addition of auxin PGR in the treatment of both light and dark treatments will inhibit callus growth and affect the color of the callus to brown, as well as the addition of sugar. Harahap and Solim[19] stated that the high content of sugar and carbohydrates in the medium can spur the occurrence of browning. The addition and increasement in the dose of 2,4-D and BAP both with light and dark treatment, will delay the change in callus color to brown.

Growth is characterized by one of which is increasing weight, so that measurements of callus biomass can represent variable callus growth originating from explants. According to Wahyuni *et al*.[18] said the fresh weight is an increase in the callus fresh weights is due to an increasing number of cells (cell division) and the increase in the cell size (cell enlargement). In conclusion the result of fresh weight is depend on the speed at which the cells divide, multiply themselves and continue with the enlargement of the callus. Through this study, it showed in order to induce callus maximally, besides 2,4-D, BAP was also needed so that the resulting callus biomass was maximal. This is in line with the statement from Harahap *et al*.[20] and Qosim[21] that BAP is needed to regulate cell division, which is characterized by increased production of number of leaves, number of segments and nodules of mangosteen callus.

2,4-D is a growth regulating agent in the auxin group which functions to boost callus induction and has ability to affect plant genetic stability. This is in accordance with the research results of the Dalila *et al*.[10], indicating that PGR 2,4-D

and kinetin with various combinations in MS Medium produced better callus compared to other basic media. Harahap[2], stated that 2,4-D is effective for forming callus because its strong activity spurred cell dedifferentiation processes, suppressing organogenesis. Tang *et al.*[22] obtained that the highest frequency of callus formation was acquired on MS medium with 0.5 mg L^{-1} BA and 3.0 mg L^{-1} 2, 4-D. The ratio between endogenous hormones explants and exogenous hormones given will determine the direction of the culture development and organ type formation[20].

Auxin affects the division, enlargement and elongation of cells. Auxin is usually applied to stimulate callus growth, cell suspension and organs and root initiation. While cytokines play a role in regulating cell division, tissue and organogenesis[23]. According to Dalila *et al.*[10], Harahap and Solim[19], that the addition of basic media without auxin as growth regulator substances or only given kinetin cannot induce callus growth. Overall showed that 2,4-D was essential for inducing callus, this is in line with the Romeida and Ganefianti[24] study, MS medium that supplemented with 1 mg L^{-1} 2,4-D produced the highest callus diameter, friable callus structure and transparent green callus and the addition of kinetin are very useful for increasing callus growth. Many researchers report that the size of the callus that being transferred to the regeneration medium also determines the success of regeneration. Callus measuring 1-2 mm is the best callus to be transferred to the regeneration medium, while callus measuring less than 1 mm will be difficult to regenerate or die[19,20]. Another study obtained, the addition of 2,-D with kinetin on MS media produced better callus than only by giving MS basic media[10].

Callus texture is one of the indicators used to assess the growth of a callus. Lizawati[25] obtained a yellowish-white, friable callus, which is characteristic of embryogenic callus, obtained from 2.5 ppm of 2,4-D treatment with the addition Tridiazuron (TDZ), this is in accordance with the results of this study. In addition, compact texture callus is a good producer of secondary metabolites. Compact callus texture is considered good because it can accumulate more secondary metabolites[26], the adding of 2,4-D around 0.25-1.00 mg L^{-1} was able to maintain the green color of explants, friable callus quality and transparent green color[23].

According to Harahap[2] friable callus is a callus that composed of long tubular cells where the structure of cells is tenuous, irregular and fragile. Dwi *et al.*[27] stated that the callus induced with cytokinin has a compact texture than the callus compared to callus that is not induced by cytokines. The compact callus texture is the effect of cytokinin and auxin which affect the water potential in cells. This causes the absorption of water from the medium into the cell to increase, so the cell becomes more rigid. 2,4-D concentrations of 1-2 ppm can produce friablely textured callus. This is in accordance with what was revealed by Dalila *et al.*[10], MS medium was added 1.5 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kinetin, produced friable callus textured and Khatak *et al.*[28] get result 2,4-D generate green friable callus and inclusion of BAP as a cytokinin, the days to callus induction decrease and Dharmayanti *et al.*[29] also gets the same results, namely giving a combination 2 ppm BA and 1-4 ppm 2,4-D can induce good callus formation and inhibits shoots and roots growth.

CONCLUSION

This study found that 2,4 D and BAP plant growth regulator is needed to induce callus on Sipahutar pineapple bulb. All explants can form callus, except explants without the addition of 2,4-D and BAP. The concentration of 2,4-D and BAP PGR of 1 ppm gave the best results for callus growth. Increased dose of 2,4-D and BAP causes the delayment of callus being formed. The dark treatment did not accelerated the formation of Sipahutar pineapple. This study will help the researchers to uncover the critical areas of auxin use (2,4-D) in dark and light treatments for callus induction, that many researchers were not able to explore. Thus a new theory on auxin ratio: cytokines in cells for callus induction may be arrived at.

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