

CONTRIBUTIONS OF IAA (INDOLE ACETIC ACID) AND 2-IP (DIMETHYL ALLYL AMINO PURINE) ON MULTIPLICATION OF RED PLANT BANANA EXPLANTS (*MUSA PARADISIACA*) IN MS MEDIA BY IN VITRO

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ABSTRACT

This study aims to obtain the concentration of IAA (Indole Acetic Acid) and 2-IP (Dimethyl Allyl Amino Purin) which are suitable for the growth of Red Barangan Banana Explants (*Musa Paradisiaca* L) on MS Media In Vitro. This study uses factorial completely randomized design (RAL) with 2 factors, namely the first factor IAA (Indole Acetic Acid) treatment symbol I consisting of 3 levels, namely: I1 (2 ppm), I2 (3 ppm), I3 (4 ppm). The second factor is the treatment of 2-IP (Dimethyl Allyl Amino Purin) with the symbol P consisting of 3 levels, namely: P1 (3 ppm), P2 (5 ppm), P3 (7 ppm). The results showed that administration of 2-IP on MS media significantly affected callus diameter at age 8 MST with an average of 12.03 mm / explants at 7.0 ppm treatment, number of shoots aged 7 MST with an average of 6.00 shoots / explants at 3 ppm treatment, and 5 ppm treatment with an average of 6.33 buds / explants, 8 MST wet age with an average of 17.48 grams / explants at 5 ppm treatment and 7 ppm treatment with an average of an average of 16.91 grams / explants, and the height of barangan banana shoots aged 8 MST with an average of 6.53 cm / explants at a treatment of 7 ppm. Furthermore, the provision of IAA in MS media significantly affected the number of roots aged 8 MST at 3 ppm treatment with an average of 5.67 roots / explants. The interaction of IAA and 2-IP showed a significant effect on the number of barangan banana shoots aged 8 MST with an average of 10.00 shoots / explants in the administration of 2 ppm IAA and 5 ppm 2-IP.

Keywords : Indole acetic acid, Dimethyl allyl amino purine, Red Plant Banana Explants, In Vitro

A. INTRODUCTION

Banana (*Musa paradisiaca* L.) is a plant from Southeast Asia which is now widespread throughout the world. Banana has long been a commodity of tropical fruit which is very popular in the world. This is because the taste is delicious, the nutrition is high, and the price is relatively cheap (Sunarjono, 2002). In Indonesia, one type of banana plant that has high potential value and has the opportunity to be developed is barangan banana (*Musa Acuminata* L). Because fruit flesh is soft and has a high flavor, no watery, distinctive aroma, good skin appearance, and good aesthetic value. High as a table fruit this banana contains higher carbohydrate levels compared to banana kepok or other bananas, which is 22.05%, while the banana kepok and banana mas respectively 20.53% and 21.30% (Supriyadi and Satuhu, 2002).

Growth regulators (PGR) also influence seedling growth. Auxin as ZPT can accelerate root growth. The hormones in the auxin group are IAA (Indoleacetic Acid), NAA (Naphthaleneacetic Acid), and IBA (Indolebutyric Acid), which are rhizalines. Generally this ZPT contains complete hormones such as Rootone Up which has the composition of naphthalene acetamide 0.067%, metal-1-naphthalene acetamide 0.13%, metal-1-naphthalene acetate 0.033%, indol-3-butyrate 0.057%, and thiram 4% (Rahardja and Wiryanta, 2003).

Cytokines are compounds that can increase cell division in plant tissue and regulate plant growth and development. The role of auxins and cytokines is

very evident in the regulation of cell division, cell lengthening, cell differentiation and organ formation. Cytokinin interactions with Auxin also occurs in determining the formation of stem or root in tissue culture. If the ratio between auxin is higher than cytokinin, differentiation of callus cells will become root. If cytokinin levels are higher than auxin, callus cells differentiate into shoot bud meristems (Zulkarnain, 2009). For this reason, a study was conducted to determine the effect of giving IAA (Indole Acetic Acid) and 2-IP (Dimethyl Allyl Amino Purin) on multiplication red barangan banana explant (*Musa Paradisiaca*) on MS media in vitro.

B. MATERIALS AND METHODS

The study was conducted at UPT. Horticultural Seed Center Hall, North Sumatra Province Agriculture Office, Jl. Abdul Haris Nasution No.20, Medan Johor from July to September 2016. The materials used in this study are explants of barangan banana sprout, agar, glycine, water, sucrose, MS solid cotton medium, IAA, 2-IP, aquades, 70% alcohol, Mankozeb 80% (Dithane M-45 80 WP), Clorox, Streptomycin Sulfate 20% (Agrept 20 WP), HgCl₂, detergents, aluminum foil, and label paper.

The tools used are laminar air flow cabinet, shaker, autoclave, analytical balance, petridish, culture bottle, pH meter, oven, tube rack, measuring cup, stirring glass rod, tweezers, scapel knife, scissors, handsprayer, erlenmeyer, funnel, and

stationery. This study used factorial completely randomized design (RAL) with 2 factors: IAA treatment factors consist of 3 levels, namely: I₁ : 2 ppm, I₂ : 3ppm and I₃ : 4 ppm. The 2-*Ip* treatment factor consists of 3 levels, i.e.: P₁ : 3 ppm, P₂ : 5 ppm dan P₃ : 7ppm. To see the difference in each treatment, a DMRT test was performed at 1% level (Gomez and Gomez, 1995).

Maintenance So that the inoculated plants are not contaminated, the culture room is sterilized every week by spraying formalin 1% around the culture shelves or by spraying 96% alcohol every day. Contaminated culture bottles were immediately removed from the culture room.

C. RESULTS AND DISCUSSION

Callus Diameter (mm)

The results of data analysis on observing the diameter of barangan ballus kaleus aged 8 MST (weeks after planting) showed a significant effect on 2-*ip* administration while IAA administration and interactions showed no significant effect. The difference can be seen in the table below.

Table 1. Average Diameter of Callus (mm) for Barangan Banana Age 8 MST

Treatment	P1	P2	P3	Average
I ₁	8,99	11,16	13,41	11,19
I ₂	8,59	10,19	11,45	10,08
I ₃	10,10	10,12	11,22	10,48
Average	9,23c	10,49b	12,03a	

Note: Numbers followed by unequal letters on the same line show marked differences in

Duncan Multiple Distance (DMRT)

Based on table 1, it can be seen that the diameter of the tested kallus at the age of 8 MST on 2-*ip* administration is in the treatment of 7 ppm 2-*ip* (P₃) which is 12.03 mm / explant and the lowest in treatment (P₂) 5 ppm 2-*ip* is 10.49 mm / explants and followed (P₁) 3 ppm 2-*ip* (9.23 mm / explants). This is assumed at the administration of 7 ppm, can stimulate cell division so that cell growth occurs. According to Smith (1992), administration of cytokines to tissue culture media is important to induce the growth and development of explants such as increasing cell division, cell poliferation, and cell morphogenesis. The same thing with Rainiyanti et al., (2005) that the administration of high cytokinin *zpt* causes the formation of shoots in a long time, only forming kallus, and so explants do not develop. Furthermore, the results of Arimarstiowati's research (2011) show that administration of 5µM 2,4-D and 20µM 2-*ip* can induce the formation of kallus Arabica coffee leaves in US 2K clones.

The barangan kallus diameter chart in the 2-*Ip* treatment shows a positive linear relationship. Giving 2-*Ip* shows a real influence on diameter of barangan banana kallus. The administration of 2-*ip* significantly influences the diameter of barangan banana callus. Giving 2-*ip* with a higher concentration can increase the diameter of the callus, it can be seen in the picture that there is an increase in callus diameter from 3 mg / l with 5 mg / l increasing by 1.26 cm and with 7 mg / l increasing by 1.54 cm. Auxin and cytokinin hormones are hormones that are often used in regulating the growth and development of explants / plantlets in vitro. But these hormones respond differently to explants or plantlets on different plants. According to Gunawan (1987) in Sihotang (2016) states that the administration of cytokinin ZPT has a significant effect on the process of cell division, callus poliferation and morphogenesis. Furthermore IAA administration did not show a positive response in the formation of callus. According to Rainiyanti et al., (2005) auxin influences cell lengthening and organ formation. Furthermore Zulkarnain (2009) states that the administration of IBA is very effective for inducing roots. Marlin (2008) results of the embryogenetic callus initiation in "cured" banana heart culture in the combination treatment of BAP and 2,4-D encourage accelerated callus formation.

Number of Buds

The results of data analysis on observing the number of barangan banana shoots aged 1 MST (weeks after planting), 2 MST, 3 MST, 4 MST, 5 MST and 6 MST showed no significant effect on IAA and 2-*ip* administration while age 7 MST showed no effect The effect on 2-*ip* administration and at the age of 8 MST shows a significant influence on the interaction of IAA and 2-*ip* administration, can be seen in table 2 below.

Table 2. Average Number of Barangan Banana Buds Age 7 MST

Treatment	P1	P2	P3	Average
I ₁	6,00	8,00	5,00	6,33
I ₂	5,00	5,00	5,00	5,00
I ₃	7,00	6,00	3,00	5,33
Average	6,00a	6,33a	4,33b	

Note: Numbers followed by unequal letters on the same line show marked differences in

Duncan Multiple Distance (DMRT)

Based on table 2 it can be seen that the parameter number of 7 MST buds shows that 2-*ip* administration gives a positive response. P₂: 5 ppm 2-*ip* is 6,33 shoots / explants is the best treatment in responding bud formation on Barangan banana plants, but it is not significantly different from P₁: 3 ppm 2-*ip* is 6.00 shoots / explants and is significantly different by giving P₃: 7 ppm 2-*ip*

which is 4.33 shoots / explants. Furthermore, the formation of shoots in each treatment varies, in this study shoots began to appear at the age of 3 MST. This shows that the success in tissue culture techniques is very dependent on the media and growth regulators. According to Zulkarnain (2009) cytokines are compounds that can increase cell division in plant tissue and regulate plant growth and development. Rubbyyanto (1992) research results of giving BAP 5 mg / l + IBA 1 mg / l produced 5.2 buds within 5 MST. Furthermore according to Wijayanti (1995) in Marlin (2008) giving 10 mg / l BAP + 5 mg / l IBA produced 4.4 ambon banana shoots within 8 MST. However, according to Marlin (2008) BAP administration at high concentrations able to inhibit the growth of callus. Furthermore, according to Rainiyanti et al., (2005) administration of zpt with high concentrations causes the formation of shoots in a long time, only form kallus, and cause explants do not develop. Giving 5 ppm 2-ip is not significantly different from giving 3 ppm 2-ip, but it is significantly different from giving 7 ppm 2-ip. Giving 2-ip with various concentrations showed different responses in the formation of shoots, it can be seen on the graph that an increase in the number of shoots from 3 mg / l with 5 mg / l increased by 0.33 buds and with 7 mg / l decreased by 2.00 buds. It is thought that the high concentration takes longer to respond to the formation of shoots. The results of Sihotang's research (2016) giving 1.5 mg / l BA without auxin is the best concentration in the formation of barangan banana shoots with an average of 4.00 shoots / explants. Furthermore according to Hartono (2010) 0.5 ppm BA is the best concentration in doubling the number of lateral buds in lowland longan. The same thing was stated by Butar-butur (2010), a combination of 0.5 BA is the best combination in producing the highest number of shoots in Dendrobium orchid. Meanwhile, according to Rainiyanti et al., (2005) administration of high concentrations of cytokinins will cause buds to form in a very long time, only form kalus, and cause explants to not develop. Furthermore George and Sherington, (1984), Wattimena (1998) in Marlin (2008) said that the use of cytokinins at high concentrations would inhibit the formation of shoots.

Table 3. Average Number of Barangan Banana Bananas Age 8 MST

Treatment	P1	P2	P3	Average
I ₁	6,00b	10,00a	6,00b	7,33
I ₂	6,00b	5,00bc	5,00bc	5,33
I ₃	7,00b	6,00b	3,00c	5,33
Average	6,33	7,00	4,67	

Note: Numbers followed by unequal letters on the same line show marked differences in

Duncan Multiple Distance (DMRT)

Based on table 3 it can be seen that the parameter number of 8 MST shoots shows that the provision of IAA and 2-ip gave a positive response. IIP2 treatment is the best treatment in response to bud formation with an average of 10.00 shoots / explants in the administration of 2 ppm IAA and 5 ppm 2-ip. The provision of IAA significantly affected the age of 8 MST. It is suspected that explants have been able to change zpt to become more active. According to Wattimena (1992), plants have the ability to change zpt to be more active or less active as well as the metabolic ability of plants themselves. In this study the provision of IAA with low concentrations ie 2 ppm combined with 5 ppm 2-ip significantly affected the formation of barangan banana shoots. according to Pierick (1997) in Marlin (2008) suggested that the formation of shoots in plant propagation in vitro requires low concentrations of auxin and high concentrations of cytokinins. Yelnititis research results (2014) that the concentration of 0.5 mg / l BA is the best concentration in inducing shoots from explants in the pocket book *Gyrinops nersteegii*. Furthermore, Marlin's research (2008) showed that by giving 0.4 ppm IAA and 4 ppm kinetin produced the most shoots, 3 shoots, the longest shoot growth was 10.36 cm and the highest shoot weight was 0.2789 g.

The provision of IAA and 2-ip shows a significant effect on the formation of barangan banana shoots. The administration of various ZPT concentrations that we give influences the formation of shoots. The fastest shoot formation was seen at the age of 3 MST in IIP2 and IIP3 treatments. Administration of IAA and 2-ip with various concentrations showed different responses to the formation of barangan banana shoots, shown in the graph that the treatment of IIP1 (2 mg / l IAA and 3 mg / l 2-ip) with I2P1 (3 mg / l IAA and 3 mg / l 2-ip) did not increase the number of shoots and with I3P1 (4 mg / l IAA and 3 mg / l 2-ip) increased by 1.00 shoots, IIP2 treatment (2 mg / l IAA and 5 mg / l 2 -ip) with I2P2 (3 mg / l IAA and 5 mg / l 2-ip) decreased in the number of shoots 5.00 buds and with I3P2 (4 mg / l IAA and 5 mg / l 2-ip) increased 1.00 shoots, then IIP3 (2 mg / l IAA and 7 mg / l 2-ip) with I2P2 (3 mg / l IAA and 7 mg / l 2-ip) decreased the number of 1,00 shoots and with I3P2 (4 mg / l IAA and 7 mg / l 2-ip) decreased 2.00 shoots It is presumed that the treatment that we provide meets the needs of explants of Barangan bananas. According to Ruswaningsih (2008) in Marlin (2008) BAP administration of 1 mg / l combined with 30 ml of

ammonium nitrate can increase the number of shoots of *Artemisia annua*. But in this study the I3P1 treatment responded to root formation. In accordance with the results of Sihotang's research (2016) giving IBA with or without BA is more dominant in forming roots. Root formation is thought to be due to high IAA concentrations. According to Sandra (2012) auxin groups such as NAA, IBA, IAA, 2,4-D and NOA are able influences plant physiology such as inducing callus occurrence, encouraging callus morphological processes, forming roots, encouraging embryogenesis, and influencing plant genetic stability.

Shoot Height (cm)

The results of data analysis on the observation of baranganese explant shoot height of 8 MST showed a significant effect on 2-ip administration, while the age of 1 MST, 2 MST, 3 MST, 4 MST, 5 MST, 6 MST and 7 MST showed a significant effect on IAA administration and IAA and 2-ip interactions, can be seen in table 4 below.

Table 4. Mean Height of Barangan Banana Buds Age 8 MST

Treatment	P ₁	P ₂	P ₃	Average
I ₁	4,50	7,10	7,80	6,43
I ₂	5,40	5,50	6,20	5,70
I ₃	4,60	4,20	5,60	4,80
Average	4,83b	5,60b	6,53a	

Note: Numbers followed by unequal letters on the same line show significantly different levels = 0.05 (lowercase) on the Duncan Multiple Range Test (DMRT)

Based on table 4 it can be seen that the administration (P3): 7 ppm 2-ip which is 6.53 cm / explant has a significant effect on the height of the banana shoots, significantly different from the administration (P1): 3 ppm 2 -ip which is 4.83 cm / explants and administration (P2): 5 ppm 2-ip that is 4.83 cm / explant. It is suspected that explants have been able to change zpt given to be more active. According to Wattimena (1992), plants have the ability to change ZPT to become more active. Gunawan (1998) in Sihotang (2016) also states that the plant growth regulator is not sensitive, the plant growth regulator will not respond. Marlin (2008) research results in giving kinetin in ambon curry banana in vitro can increase the height of ambon banana. The picture above shows the administration of 2-ip significantly affects the height of barangan banana shoots. Provision of 2-ip with various concentrations showed different responses in shoot height, shown in the graph that an increase in shoot height of 3 mg / l with 5 mg / l increased by 0.77 cm and with 7 mg / l increased by 0.93 cm. In accordance with the opinion of Zulkarnain (2009)

cytokinins play a role in increasing cell division in plants as well as growth and development. Furthermore according to Marlin (2008) cytokines also play a role in the growth, development and multiplication of red barangan banana explants. The same thing was stated by Bhojwani and Radjan (1998) in Marlin (2008) that kinetin is a plant hormone that plays a role in cell division, cell differentiation and budding.

Number of Roots

The results of data analysis on the observation of the number of barangan bananas aged 8 MST showed a significant effect on IAA administration while 2-ip administration and IAA and 2-ip interactions showed no significant effect, can be seen in table 4 below.

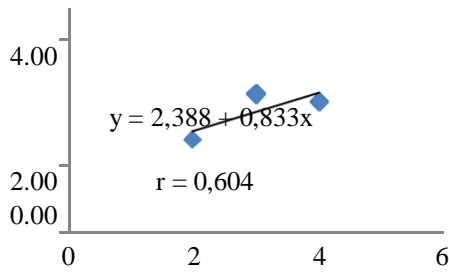
Table 5. Average Number of Barangan Banana Roots Age 8 MST

Treatment	P ₁	P ₂	P ₃	Average
I ₁	3,00	4,00	4,00	3,67b
I ₂	6,00	6,00	5,00	5,67a
I ₃	8,00	4,00	4,00	5,33a
Average	5,67	4,67	4,33	

Note: Numbers followed by unequal letters on the same line show significantly different levels $\alpha = 0.05$ (lowercase) on Duncan's Multiple Range Test (DMRT)

Based on the table above, giving IAA has a significant effect on root formation. Giving I2: 3 ppm IAA that is 5.67 roots / explants is the best treatment in responding to the number of roots, not significantly different from giving I3: 4 ppm IAA which is 5.33 roots / explants and I1: 2 ppm IAA which is 3.67 roots / explants. This shows that the formation of barangan banana roots in vitro requires zpt auxin. According to Rukmana (2009) auxin zpt stimulates growth which is very influential in root formation and root lengthening. Based on Marlin's research (2008) the administration of 1 ppm IBA combined with 0.0 ppm kinetin is the best combination in stimulating the formation of curly ambon banana roots with the number of roots formed, 11 roots / explants. Furthermore 2-ip administration did not show any real effect on root formation. According to Karjadi (2007) that high levels of auxin will induce root growth and whereas if high cytokinin levels will induce shoots. Furthermore, the results of Triatminingsih's research (2001) in Marlin (2008) to obtain mangosteen plantlet rooting obtained treatment of 10 ppm and 20 ppm IBA incubated in the dark for 14 days thus giving the best root percentage of 83.5% and the highest number of roots 2, 40 roots.

ah Akar
 8.00
 6.00



IAA (Indole Acetic Acid)

Figure 5. Relationship between the number of barangan banana roots and the provision of IAA

The picture above shows the provision of IAA significantly affected the formation of barangan banana roots. Giving IAA with various concentrations showed different responses in root formation, it can be seen in the graph that an increase in the number of roots from 2 mg / l with 3 mg / l increased by 2.00 roots and with 4 mg / l decreased by 0.34 roots. The higher the concentration of IAA given the more roots formed otherwise the lower the concentration of IAA given causes longer roots. The same thing was said by Marlin (2008) that by increasing the concentration of IBA it actually decreases the length of the roots produced. According to Nurhafni (2009) the provision of NAA can stimulate better root growth, because NAA stimulates roots and NAA contains macro and micro elements that are very influential on root formation. Nasution research results (2016) formation of pineapple roots require low auxin without cytokinins or a combination of high auxin with low cytokinin.

Wet Wet Weight (gram)

The results of data analysis on observing the wet weight of barangan banana explants aged 8 MST showed a significant effect on 2-ip administration, whereas IAA administration showed no significant effect, it can be seen in table 6 below.

Table 6. Average Weight of Barangan Banana Explants Age 8 MST

Treatment	P1	P2	P3	Average
I ₁	14,86	19,40	16,72	16,99
I ₂	15,55	16,40	16,20	16,05
I ₃	15,77	16,64	17,80	16,74
Average	15,39b	17,48a	16,91a	

Note: Numbers followed by unequal letters on the same line show significantly different levels 0.05 (huruf kecil) pada Uji Jarak Berganda Duncan (DMRT)

Based on table 6 it can be seen that the administration (P2): 5 ppm 2-ip that is 17.48 grams / explant has a significant effect on the

explant wet weight not significantly different from the administration (P3): 7 ppm 2-ip which is 16.91 grams / explant and significantly different from the administration (P1): 3 ppm 2-ip that is 15.39 grams / explant. It is suspected that there is an enlargement of cells in plant organs so that plant parts such as shoots and kallus experience swelling due to cell enlargement due to water and 2-ip. According to Salisbury and Ross (1992) in Marlin (2008), wet weight will increase if there is sufficient water intake, so cells can develop properly. Furthermore Haryanto (1993) in Marlin (2008) suggested that the weight of Gladiolus plants was significantly affected by the addition of 2,4-D and kinetin. Swelling or enlargement of cells is an explanatory physiological response due to the presence of water and cytokinins which consequently encourage cell division, morphogenesis, budding, cell differentiation, and chloroplast formation (Wattimena, 1992; Smith, 1992 in Marlin, 2008).

Provision of 2-ip is very real on explants wet weight. Giving 2-ip with a higher concentration gives a different response to the wet weight explants of barangan bananas, it can be seen on the graph that there is an increase in wet weight from 3 mg / l with 5 mg / l increasing by 2.09 grams and with 7 mg / l decreasing 0.57 gram. According to Marlin (2008) that the total wet weight of banana plants is affected by the cytokinin zpt. Furthermore, according to Zulkarnain (2009), cytokines are compounds that can increase cell division in plant tissue and regulate plant growth and development. The same thing was expressed by Wattimena (1992) in Marlin (2008) the physiological role of cytokinins is to encourage cell division, morphogenesis, budding, stomata formation, flowering, and parthenocarp fruit formation. Furthermore according to Davies (1995) in Sihotang (2016) the physiological role of cytokinins is to encourage cell division, morphogenesis, lateral shoot growth, leaf enlargement, stomata opening and chloroplast formation.

D. CONCLUSIONS

1. Giving a concentration of 3 ppm IAA on MS Media has a significant effect on the parameters of the number of roots, which is an average of 6.33 roots / explants at the age of 8 MST.
2. Giving a concentration of 7 ppm 2-ip on MS Media has a significant effect on the

diameter of the callus diameter, which is an average of 12.03 mm / explant age of 8 MST, the number of shoots aged 7 MST with an average of 6.00 shoots / explant in treatment 3 ppm, and treatment of 5 ppm with an average of 6.33 shoots / explant buds, wet weight age of 8 MST with an average of 17.96 grams / explants at 5 ppm treatment and 7 ppm treatment with an average of 16.91 gram / explant, and the height of barangan banana shoots aged 8 MST with an average of 6.53 cm / explants at 7 ppm treatment.

3. The interaction of IAA and 2-ip shows a significant effect on the number of barangan banana shoots aged 8 MST with an average of 10.00 shoots / explants in the administration of 2 ppm IAA and 5 ppm 2-ip.

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