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IN VITRO MICRO-CUTTING OF VANILLA (*Vanilla planifolia* Andrews.) IN DIFFERENT NAA AND BAP

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ABSTRACT

Vanilla is one of the spice plant that has a high selling value. The problem with conventional propagation of vanilla by stem cuttings is the attack of stem rot disease caused by Fusarium oxisporum f sp. and limited planting material, therefore can be overcome by tissue culture techniques. This study aims to examine the interaction between the various concentrations of NAA and BAP, obtain the most appropriate concentrations of NAA and BAP for vanilla's micro-cutting. The study used a Completely Randomized Design (CRD) method. The first factor is the concentration of NAA consisting of N1: 0,5 ppm, N2: 1 ppm, and N3: 1,5 ppm. The second factor is the concentration of BAP consisting of B1: 1 ppm, B2: 2 ppm, and B3: 3 ppm. Observation data were analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level. The results showed that there was no interaction between the treatment of NAA and BAP concentrations on vanilla micro-cutting. The use of 1 ppm NAA gave the best results on root length. BAP at all concentrations gave the same response on parameters of time to grow buds, live percentage, number of shoots, number of roots, number of leaves, root length, and dry weight of plantlets.
Keywords: *Vanilla micro-cutting, NAA, BAP, In Vitro*

INTRODUCTION

Vanilla is one of the spice plants that has a high selling value. Vanilla is widely used in the food, beverage, pharmaceutical, and cosmetic industries because the fruit contains vanillin (C8H8O3) which gives off a distinctive aroma. Indonesia's position ranks second after Madagascar with vanilla production of 2,304 tons in 2016. The export value of vanilla reached US$ 72,511,000 in 2017, but decreased in 2018 with an export value of US$ 63,062,000 (Loedji, 2019 *cit.* Erawati , *et al.,* 2020).

The development of vanilla acreag in Indonesia also shows an increase. In 2012 the area was 19,920 ha, has increased to 31,379 ha in 2017 (Dirjenbun, 2018). This shows that the vanilla commodity has considerable appeal, due to its high economic value.

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Tissue culture techniques with micropropagation for vegetative propagation of vanilla have been widely developed. Tissue culture technique is a method or technique of taking plant parts such as protoplasts, cells, groups of cells, tissues, and organs that are grown in a suitable artificial environment and medium under sterile or aseptic conditions. The main use of tissue culture is to produce a large number of new plants that have physiological and morphological characteristics that are uniform and identical to the parent (Anitasari *et al.,* 2018). The success of tissue culture is influenced by the composition of the growth media and appropriate growth regulators. Growth regulators commonly used in tissue culture are auxins and cytokinins. According to Erawati *et al.,* (2020) the addition of BAP and NAA stimulators functioned so that the explants were able to multiply shoots and produce proportional roots.

METHODS

The research was carried out at the Agricultural Biotechnology Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta from September to November 2020. The research method used laboratory experiments arranged in a Two-Factoral Completely Randomized Design (CRD). The first factor is the concentration of NAA which consists of N1: 0.5 ppm, N2: 1 ppm, and N3: 1.5 ppm. The second factor is the concentration of BAP which consists of B1: 1 ppm, B2: 2 ppm, and B3: 3 ppm. There were 9 treatment combinations which were repeated 3 times. Each treatment consisted of 6 bottles of culture, each bottle contained one explant, so the total number of explants was 162 bottles. The number of sample plants is 3 plants. Observational data were analyzed for diversity using Analysis of Variance (ANOVA) at the level of = 5% and further tested with Duncan's Multiple Range Test (DMRT) at the level of = 5%.

The implementation stage begins with the sterilization of the tools, washed thoroughly with soap and then dried. After drying, they were wrapped in paper (except culture bottles) and then put in an autoclave at a pressure of 15 psi at 121°C

for 45 minutes. Making planting media, namely Murashige and Skoog (MS) media for each treatment as much as 200 ml. Into 50 ml of distilled water, added 100 ml of macro nutrient stock solution, 0.8 ml micro nutrient stock solution, 1 ml iodine stock solution, 1 ml vitamin stock solution, 0.25 ml EDTA stock solution, 1 ml iron stock solution, 80 mg myoinositol, 6 g sucrose. Adjust the pH to reach 5.85 with the addition of 1 N NaOH or 1 N HCL. MS media was added with NAA and BAP with concentrations according to treatment. The vial containing the media was then sterilized using an autoclave at a temperature of 121oC with a pressure of 15 psi for 30 minutes. Explants were derived from 3 months old vanilla microstek. Planting is done in LAF, in 1 bottle there is 1 microstek 2 cm long. Furthermore, the explants were kept in an incubation room at a temperature of 25-26 0C with continuous lighting. Maintenance of explants includes sterilization of shelves by spraying once every three days with 70% alcohol to avoid bacteria and fungi. Observations were made every week from week 0 to week 12. The growth parameters observed were the percentage of plantlet life, day of shoot emergence, number of shoots, number of roots, number of leaves, root length, plantlet color, and plantlet dry weight.

RESULT AND DISCUSSION

Time Growing Shoots

Observations of the time of shoot growth were analyzed for diversity using the Analysis of Variance at the 5% real level. The NAA concentration treatment showed a significant effect, but the BAP concentration treatment showed no significant effect and there was no interaction between the two treatments. The average value when growing shoots can be seen in table 1.

Table 1. Average Growth of Vanilla Microscopy Shoots at Treatments of Various Concentrations of NAA and BAP (days)

Concentration of NAA	Concentration oof BAP			Average
	1 ppm (B1)	2 ppm (B2)	3 ppm (B3)	
0,5 ppm (N1)	25, 67	21, 56	28, 57	25, 30 b
1 ppm (N2)	23, 11	25, 00	25, 00	24, 37 b
1,5 ppm (N3)	36, 67	33, 33	30, 00	33, 33 a
Average	28, 48 p	26, 63 p	27, 89 p	(-)

Note: The mean followed by the same letter in one column, shows that there is no significant difference in DMRT at the 5% level

Table 1 shows that the 1 ppm (N2) NAA concentration treatment was significantly faster in shoot growth than the 1.5 ppm NAA (N3) treatment, but it was not significantly different from N1. This is in line with Santoso (2016) that giving

PGR in low concentrations allows it to cause meristematic cell division, but at high concentrations it allows inhibiting cell division. The right addition of NAA together with cytokinins can stimulate apical shoot growth by stimulating cell enlargement and elongation in the meristem area of the plant tip, such as stem tips, leaf tips, flower tips, and root tips.

BAP treatment at each concentration had no effect on the growth of vanilla microstek shoots. This is due to the administration of BAP concentration which is still too low so that it has not been able to affect the growth of vanilla shoots. According to Santoso (2016) cytokinins play a role in shoot growth. The higher the concentration of cytokinins given to the plant, the higher the number of shoots will be. Cytokinins translocated from the bottom of the explants can stimulate shoot growth, so that the administration of high concentrations of cytokinins can increase shoot growth.

Percentage of life

Observations on the percentage of life were analyzed for diversity using the Analysis of Variance at the 5% real level. The treatment of NAA and BAP concentrations showed no significant effect and there was no interaction between the two treatments. The average value of the percentage of life at the age of 12 MST can be seen in table 2.

Table 2. Average Percentage of Life of Vanilla Microscopy in Treatment of Various Concentrations of NAA and BAP at 12 WAP (%)

Concentrations of NAA	Concentration of BAP			Average
	1 ppm (B1)	2 ppm (B2)	3 ppm (B3)	
0,5 ppm (N1)	88, 89	77, 78	88, 89	85, 18 a
1 ppm (N2)	77, 78	100, 00	100, 00	92, 59 a
1,5 ppm (N3)	94, 44	72, 22	94, 44	87, 03 a
Average	87, 04 p	83, 33 p	94, 44 p	(-)

Note: The mean followed by the same letter in one column, shows that there is no significant difference in DMRT at the 5% level

Table 2 shows that the NAA and BAP treatments had no significant effect on the percentage of viability of vanilla microcuts. In the treatment of various concentrations of NAA and BAP, both high and low concentrations showed the same percentage of survival. This is because the endogenous auxins and cytokinins contained in the explants and the nutrient media were sufficient to meet the needs of the explants during the culture period. In some treatments there were dead plantlets caused by fungal and bacterial contamination. The fungus grows on the surface of the media and then forms hyphae and covers the surface of the media and explants so that the explants die, while the bacterial contamination is characterized

by the presence of mucus in the media. There are many factors that affect the contamination of explants, including from explant sources (both internal and external), small organisms that enter the media such as ants or other insects, culture bottles or tools that are not sterile, as well as the work environment and culture room. less sterile.

Number of Shoots, Number of Roots, and Number of Leaves

Observations on the number of shoots, number of roots, and number of leaves were analyzed for diversity using the Analysis of Variance at a significant level of 5%. The treatment of NAA and BAP concentrations showed no significant effect and there was no interaction between the two treatments. The average value of the number of shoots, number of roots, and number of leaves at the age of 12 WAP can be seen in table 3.

Table 3. Average Number of Shoots, Number of Roots, and Number of Leaves of Vanilla Microscopy at Treatment of Various Concentrations of NAA and BAP at 12 WAP (fruit)

Treatments	Number of shoots	Anumber of roots	Number of leaves
NAA 0,5 ppm	1, 11 a	3, 37 a	2, 59 a
NAA 1 ppm	1, 15 a	2, 15 a	3, 33 a
NAA 1,5 ppm	1, 15 a	2, 59 a	3, 37 a
BAP 1 ppm	1, 07 p	2, 22 p	3, 04 p
BAP 2 ppm	1, 18 p	2, 26 p	2, 85 p
BAP 3 ppm	1, 15 p	2, 63 p	3, 41 p

Note: The mean followed by the same letter in one column, shows that there is no significant difference in DMRT at the 5% level

Table 3 on the number of shoots showed that there was no significant difference between the NAA and BAP treatments. This result was caused by auxin which was produced endogenously in the shoots of the plant which would be distributed in a polar manner which was able to inhibit the growth of lateral shoots. Apical dominance is caused by auxin which is translocated from shoots downwards (polar) and deposited on lateral shoots, this will inhibit lateral shoot growth because its concentration is still too high. A high concentration of auxin will inhibit the growth of lateral shoots close to the shoot (Wiraatmaja, 2017).

Auxins and cytokinins work antagonistically in regulating the growth of axillary buds. Axillary buds located at the bottom of the crown (the area adjacent to the roots) will usually grow longer than axillary buds located close to the terminal buds. Cytokinins that enter from the roots into the plant canopy system, will fight the action of auxin, by signaling the axillary buds to start growing. So the ratio of

auxin and cytokinin is a critical factor in controlling the growth of axillary shoots (Campbell and Reece, 2002 *cit.* Wiraatmaja, 2017).

Root formation is related to the emergence of shoots, this is because shoots that have grown are a source of auxin and will initiate root formation. Giving exogenous auxin will stimulate endogenous auxin activity in the formation of shoots first and then for root growth using auxin produced by shoots and young leaves that are starting to grow. Generally, explants that have sprouted will form roots. Working together with auxins, cytokinins stimulate cell division and differentiation and regulate plant growth and development.

Based on the results of the study the number of leaves of all treatments showed no significant difference. This shows the explants can synthesize nutrients from the media well. In addition, the ability of explants to absorb exogenous auxin and cytokinins was also good so that the explants gave a good leaf growth response as well. Auxins and cytokinins given trigger the formation of leaf primordia in the apical meristem which will then develop into new leaf blades (Talanca and Haris, 2010 *cit.* Haryuni *et al.,* 2015). According to Budiana (2007) *cit.* Isnaini and Asmawati (2017) administering small amounts of regulatory substances, but can stimulate, inhibit or change various plant physiological processes. The number of leaves is affected by the number of shoots that grow. In this study, the number of shoots that grew was not significantly different so that the number of leaves produced was also not significantly different. The comparison of the growth of the number of shoots, the number of roots, and the number of leaves of vanilla plantlets with various treatments can be seen in Figure 1.

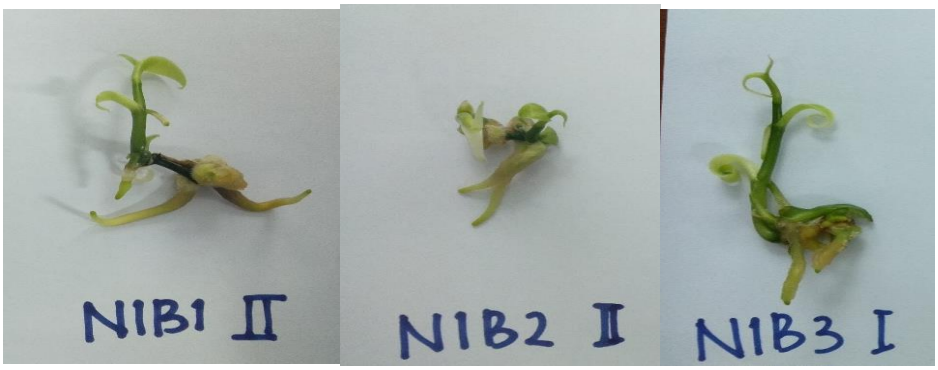

Figure 1. Comparison of Vanilla Plantlet Growth between Treatments of 0.5 ppm NAA (N1) and Various Concentrations of BAP at the Age of 12 WAP

Root of Length

Observations of root length were analyzed for diversity using the Analysis of Variance at the 5% real level. The NAA concentration treatment showed a significant effect, but the BAP concentration treatment showed no significant effect and there

was no interaction between the two treatments. The mean value of root length at the age of 12 WAP can be seen in table 4.

Table 4. Average Root Length of Vanilla Microscopy at Treatment of Various Concentrations of NAA and BAP at 12 WAP (cm)

Concentration of NAA	Concentration of BAP			Average
	1 ppm (B1)	2 ppm (B2)	3 ppm (B3)	
0,5 ppm (N1)	2, 22	2, 35	1, 89	2, 15 b
1 ppm (N2)	5, 07	5, 87	4, 21	5, 05 a
1,5 ppm (N3)	3, 74	2, 08	2, 89	2, 90 b
Average	3, 68 p	3, 43 p	2, 99 p	(-)

Note: The mean followed by the same letter in one column, shows that there is no significant difference in DMRT at the 5% level

Figure 2. .Gowth of Vanilla Plantlet Roots with 1 ppm NAA and 2 ppm BAP Treatment at 8 WAP

The root length showed that the 1 ppm NAA concentration treatment (Figure 2) had the longest root length compared to other treatments. This is consistent with the results of research by Tan et al., (2011) that 88.33% of vanilla explants formed roots with the addition of 1 mg/l NAA with an average root length of 4.4 cm after 4 weeks. This shows that the given concentration has been able to stimulate the process of cell division and elongation in the roots. The increase in root length is caused by the process of cell division in the root tip meristem, followed by the process of cell elongation and enlargement. Auxin causes the expansion of the cortex, phloem, and cambium tissue so that the sclerenchyma cells become damaged, spurring the roots out and causing the root cells to elongate.

One of the functions of the auxin hormone is to stimulate root growth. The process of root formation is related to the ratio between the hormone auxin and cytokinin. When the auxin ratio is higher than cytokinin then organogenesis will tend to lead to root formation so that the addition of exogenous auxin can make the auxin ratio higher and accelerate the root initiation process.

Planlet of Color

Plantlet color was observed using the Munsell Color Chart for Plant Tissue. The color of the plantlets can be seen in Table 5.

Table 5. Color of Vanilla Microscopy Plantlets in Treatment of Various Concentrations of NAA and BAP

Treatment Combination	Color of code	Noted	Color
N1B1	7,5 GY 5/2	Dark Yellowish Green	
N1B2	7,5 GY 5/4	slightly dark yellowish green	
N1B3	7,5 GY 5/2	Dark Yellowish Green	
N2B1	7,5 GY 5/2	Dark Yellowish Green	
N2B2	2,5 GY 7/8	Bright Yellowish Green	
N2B3	7,5 GY 5/2	Dark Yellowish Green	
N3B1	7,5 GY 4/4	Slightly Light Yellowish Green	
N3B2	5 GY 5/8	Light green	
N3B3	5 GY 5/6	bright light green	

Noted : V/C = Value/Chroma
GY = Green yellow (hijau kekuningan)

Plantlet dry weight

Observations of root length were analyzed for diversity using the Analysis of Variance at the 5% real level. The NAA concentration treatment showed a significant effect, but the BAP concentration treatment showed no significant effect and there was no interaction between the two treatments. The average value of plantlet dry weight at the age of 12 WAP can be seen in table 6.

Table 6. Average Dry Weight of Vanilla Microscopy Plantlets in Treatment of Various Concentrations of NAA and BAP at 12 WAP (mg)

Konsentrasi NAA	Konsentrasi BAP			Rerata
	1 ppm (B1)	2 ppm (B2)	3 ppm (B3)	
0,5 ppm (N1)	81, 90	70, 60	96, 87	83, 12 b
1 ppm (N2)	110, 63	117, 63	113, 03	113, 77 a
1,5 ppm (N3)	113, 70	101, 37	128, 33	114, 47 a
Rerata	102, 08 p	96, 53 p	112, 74 p	(-)

Note: The mean followed by the same letter in one column, shows that there is no significant difference in DMRT at the 5% level

Vanilla plantlets cultured at 1 ppm NAA and 1.5 ppm NAA had a heavier dry weight than vanilla plantlets cultured at 0.5 ppm NAA because they had longer roots so as to increase plantlet dry weight. Dry weight is a description of the accumulation of organic materials and minerals that play an important role in plant growth. Dry

weight is the weight of the material after drying and is the accumulation of photosynthate (Mengesha *et al.,* 2012). According to Nurdin (2011) the number of leaves can affect the increase in plant dry weight because the leaves are a place for the accumulation of plant photosynthetic results. Photosynthesis results in an increase in plant dry weight due to the uptake of carbon dioxide.

According to Nurdin (2011), the number of leaves can affect the increase in plant dry weight because the leaves are a place for the accumulation of plant photosynthetic results. Photosynthesis results in an increase in plant dry weight due to the uptake of carbon dioxide. Measurement of plant biomass can also be done using plant dry weight. The increase in the size or dry weight of the plant reflects the increase in the size and number of cells.

CONCLUSION

The results showed that there was no interaction between the concentration of NAA and BAP on vanilla microscopy. The use of 1 ppm NAA gave the best results on root length parameters. Giving BAP at all concentrations gave the same response for all parameters

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