Micropropagation of Vanilla (Vanilla planifolia Andrews) with Modification of Cytokinins

by Dyah Nuning Erawati

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Abstract. The constraints of vanilla development in conventional vegetative propagation is the limitations of the parent plant as stem cuttings. The ability of the explants to regenerate and differentiate to form buds in vitro is excessive need to be controlled through cytokinin regulation to multiplication of vanilla seedlings as a parent, healthy and uniform. The objectives of this research is the effect of BAP and Kinetin to shoot multiplication vanilla. The study was conducted in the Tissue-Culture Politeknik Negeri Jember using a Completely Randomized Design (CRD). Treatment in the form of the addition of cytokinins in MS basal medium. Factor 1 is BAP concentration 0 mg.L^{-1}, 1 mg.L^{-1}, 2 mg.L^{-1} and 3 mg.L^{-1}. Factor 2 is Kinetin concentration 0 mg.L^{-1}, 1 mg.L^{-1}and 2 mg.L^{-1}. Data analysis used the DMRT test of 5%. The result showed the emergence of vanilla shoots is not influenced by exogenous growth regulators and addition of BAP 3 mg.L^{-1} gave the most multiplication results of 3-4 shoots with shoot lengths of 2-2.5 cm at 28 days after inoculation

1. Introduction

The obstacle to developing Indonesian vanilla is the lack of a large-scale supply of vanilla seeds in a short time. Propagation of vanilla seeds using conventional methods, namely stem cuttings. Stem cuttings conventional method has the disadvantage of a low rate of multiplication and requires a lot of time and energy making it difficult to meet the needs of many seeds in a short time [1], [2]. One method of vegetative propagation that can be done to overcome this obstacle is in vitro propagation through tissue culture techniques [3], [4], [5], [6].

Based on research, the provision of growth regulators BAP and Kinetin in a ratio of 2 : 1 ppm can grow maximum shoots on vanilla explant segments by 95% (9 shoots/explants) [7]. Whereas in the other study showed the maximum induction of shoots in the vanilla segment was obtained by combining BAP growth regulators as much as 1 ppm and 15% coconut water producing maximum shoot induction of 97% [8]. The multiplication results of vanilla shoots can be multiplied 6 times more by the treatment of MS basal medium with the addition of BAP [9].

Micropropagation constraint occurs when the explant's ability to regenerate and differentiate to form buds in vitro excessive need to be controlled by regulating cytokinins. Therefore, it needs to be implemented vanilla seed multiplication and development through micro propagation techniques with BAP and Kinetin modification. To reduce the risk of soma clonal variation is necessary to research the

development of vanilla seeds with modifications cytokinins to stimulate shoot multiplication to get a vanilla seed in sufficient quantities, healthy and uniform through tissue culture. Thus, interest in the study was the effect of BAP and Kinetin to shoot multiplication vanilla.

2. Material and Methods

This research was conducted at Tissue Culture Laboratory at the Politeknik Negeri Jember East Java Indonesia (8 ° 09'35.1 "S 113 ° 43'27.2" E), from June to December 2019. This study is based on a Completely Randomized Design (CRD) with 5 replication. Treatment in the form of the addition of cytokinins in MS basal medium. Treatment consists of two factors. Factor 1 is the concentration of BAP 0 $mg.L^{-1}$, 1 $mg.L^{-1}$, 2 $mg.L^{-1}$ and 3 $mg.L^{-1}$. Factor 2 is the concentration of Kinetin 0 $mg.L^{-1}$, 1 $mg.L^{-1}$ and 2 $mg.L^{-1}$.

2.1 Preparation of Planting Material

Planting material to be used as an explant vanilla plant is a result of acclimatization and maintenance in the Greenhouse laboratory tissue culture Politeknik Negeri Jember use paranet as a 50% shade which is gradually opened during the acclimation process. The acclimatized plants used as planting material. The acclimatized plant were treated with watering, fertilizing and spraying fungicide. Plants used as planting materials selected in advance to obtain superior seedlings. Plants that are taken are plants that are fresh, normal, healthy, and free from pests and diseases [10].

2.2 Vanilla Shoot Multiplication Medium

Murashige and Skoog (MS) basal medium consists of macro nutrient, micro nutrients, 100 $mg.L^{-1}$ inositol, amino acids, 30 $g.L^{-1}$ sucrose, Benzyl Amino Purine (BAP) and Kinetin according to the treatment and determination of pH 5.7 - 5.8 then 8 $g.L^{-1}$ agar-agar was added. The medium was sterilized in an autoclave at 121^0C for 20 minutes.

2.3 Sterilization and Culture of Explant

Vanilla vines that have been prepared and then washed with running water until clean. Sterilization performed in a laminar air flow. Furthermore, vanilla vines washed with Tween 20% solution for 5 minutes and then rinsed with sterile distilled water. The explants were sterilized by soaking in a solution of fungicide and bactericide 1.5% for 60 minutes and then soaked in 96% alcohol for 5 seconds. Then, the explants were immersed in 10% Bayclean solution (commercial bleach with 5.25% sodium hypochlorite) for 5 minutes and explants rinsed three times with sterile distilled water [8]. Vine shoots are cut and vanilla nodal of approximately 1.5 cm length as explants [6].

2.4 Incubation Period

Each bottle containing 1 explant vanilla in the incubation chamber maintained at a temperature of 26^0C $\pm 2^0$C with a relative humidity of 60-70% under the cycle of 16 hours of light and 8 hours dark with a light intensity of 40,5 µmol provided by white fluorescent lamps.

2.5 Analysis of Experimental Design

The experimental variables into a simple classification variance analysis purposes procedures using ANOVA (Analysis of Variance). The average difference test continued using the DMRT test at 5% [11]. The parameters observed in this study are (1) the ability of explants to form shoots (%), (2) number of shoots (shoots) and (3) length of shoots (cm).

3. Result and Discussion

3.1 The Ability of Explants to Form Buds (%)

Observation about the ability of explants to form shoots is intended to determine the potential of explants in producing shoots in units of percent. The observations showed that all explants gave positive responses to shoot formation by an average of 30% at 7 days after inoculation and 80 -100% at 14 days after inoculation (table 1).

The addition of plant growth regulator cytokinins group of Benzyl Amino Purine and Kinetin at various concentration levels do not affect the ability to explant to form buds. The success of 80-100% explants forming shoots within 14 days after inoculation on all treatments indicates explants ability to germinate is not dependent on the addition of an exogenous plant growth regulator. The explants were used in this study comes from the buds on each segment of vanilla vines (fig.1).

Table 1.The Ability of explants form to shoots on micropropagation vanilla with modified cytokinins

Cytokinins (mg.L^{-1})		The ability of explant forming shoot (%)	
BAP	Kinetin	7 dai	14 dai
0	0	0.30	0.80
0	1	0.30	0.80
0	2	0.30	0.80
1	0	0.30	0.80
1	1	0.30	0.90
1	2	0.30	1.00
2	0	0.30	0.80
2	1	0.30	0.80
2	2	0.30	0.80
3	0	0.30	0.80
3	1	0.30	0.80
3	2	0.30	0.80
		ns	ns

dai: days after inoculation

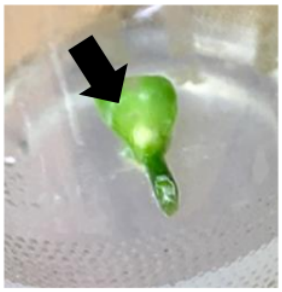

Figure 1. Regeneration explant marked by the emergence of shoots at 7 days after inoculation.

Figure 2. Shoots about 0.3 cm tall at 14 days after inoculation.

Buds are meristematic tissues that are easier to regenerate because they have cells that are actively dividing (fig. 2). This is similar to the results of studies using the two types of explants as tissue culture planting material in vanilla. Explants derived from the buds successfully grown in 2 weeks, while the explants derived from leaf tissue mostly dead or inactive [9]. Explants derived from buds on the vanilla segment gave a better response to the average number of shoots by 15 shoots compared to explants originating from shoot tip which only formed as many as 7 shoots [12].

3.2 Number of Shoots (shoots)

One purpose of this study was to evaluate the response of explants vanilla shoot multiplication to the addition of BAP and Kinetin in MS media. The number of shoot will give a picture of the effect of a single BAP, a single Kinetin or there is an interaction between the two plant growth regulators with variations in concentration.

Table 2. The number of shoots on micropropagation vanilla with modified cytokinin

BAP (mg.L-1)	Number of shoots (shoots)							
	7 dai		14 dai		21 dai		28 dai	
0	0.33	a	0.50	a	0.83	a	1.00	a
1	0.47	b	1.00	b	1.37	b	1.90	b
2	0.36	a	1.07	b	1.37	b	1.77	b
3	0.90	c	1.50	b	1.90	c	2.30	c

Means followed by the same letter in the line do not differ significantly according to the DMRT ($\alpha = 0.05$)

The multiplication of vanilla shoots is only influenced by the addition of BAP singly and there is no interaction with the addition of Kinetin (fig. 3). Based on Table 3 known that the addition of BAP gave the best response to the shoot multiplication. Without the addition of BAP on MS basic medium then shoots vanilla incapable duplicated. Several studies have reported that the addition of BA singly affect vanilla shoot induction [3], [4], [13]. Plant Growth Regulator BA (Benzyl Adenin) has a stronger activity in stimulating the ability of explants to form buds because it has a benzyl group [14].

Figure 3. Micropropagation vanilla on MS with BAP 3 mg.L^{-1} doubling shoots at 21 days after inoculation.

Figure 4. Multiplication of shoots in the 28 days after inoculation.

The addition of BAP with higher concentrations will increase the potential for explants to multiply vanilla shoots (fig. 4). The number of shoots added along with the amount of BA concentration

increases [15], [16]. However, increasing the concentration of BAP into the media must also be limited to a certain level that is still responded well by explants because it has the potential to inhibit the multiplication of shoots [17].

3.3 Length of Shoots (cm)

The success of explants forming shoots followed by the growth and development of shoots. One of the parameters to determine the pattern of explants reaction on vegetative growth to the addition of cytokines demonstrated by the activity of the length of shoot.

Based on fig. 5 showed that the length shoots highest in the treatment without the addition of growth regulators and the explant with Kinetin addition of 1 mg.L^{-1}, which are 3.0 cm and 2.7 cm. The pattern of the length of shoots is positive, especially in explants grown on medium without plant growth regulator BAP. This happens because, in the treatment, the explants are not experiencing shoot multiplication so that the buds continue to grow higher than other treatments. However, an increase in explant length in media added by BAP of 3 mg.L^{-1} also gave a positive response with shoot length ranging from 2.0 cm to 2.5 cm followed by successful multiplication of shoots. The number of shoots is negatively correlated with the length of the shoots, where the more shoots formed will affect the shorter shoot length. The energy needed for shoot height is more used for multiplication of shoots [18].

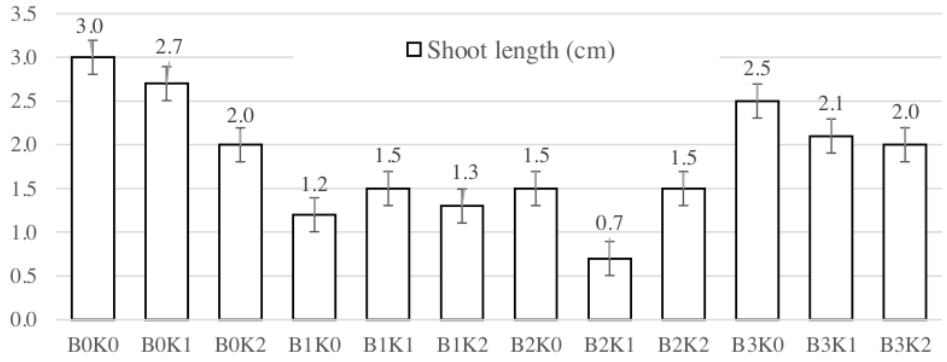

Figure 5. Length shoots in vanilla micropropagation with cytokinin modification at 28 days after inoculation

4. Conclusion

The emergence of vanilla shoots is not influenced by exogenous growth regulators because they already have axillary buds in each explant nodal and addition of BAP 3 mg.L^{-1} singly gave the most multiplication results of 3 to 4 shoots with shoot lengths of 2 to 2.5 cm at 28 days after inoculation.

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