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Shoots Multiplication of Vanilla (*Vanilla planifolia*) With Benzyl Amino Purine and Kinetin Modification

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Abstract. The ability of vanilla explants to regenerate and differentiate to form multiplication of shoots is still limited. Therefore it is necessary to regulate the addition of Benzyl Amino Purine and Kinetin into the media for optimal shoot multiplication so that the quality of the vanilla culture seedlings can be improved. The objectives of this study were: 1) to analyse the multiplication response of vanilla explant shoots with the addition of BAP, 2) to analyse the multiplication response of vanilla explant shoots with the addition of Kinetin and 3) to analyse the interaction of adding BAP and Kinetin to the multiplication response of vanilla explant shoots. The study was conducted at Tissue Culture Laboratory of Politeknik Negeri Jember using a factorial Complete Randomized Design. Factor 1 is BAP concentration which is 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/liter and factor 2 is Kinetin concentration which is 0.0, 1.0, 2.0 mg/liter. Further data analysis used Duncan's average difference test. The research result show that the addition of BAP affected the germination rate of vanilla explants with the fastest mean rate of 9 days after inoculation with the addition of 2.5 mgL⁻¹ and 67-100% sprouting ability 14 days after inoculation. The average number of shoots was highest in the addition of 0.5 mg L⁻¹ BAP and 2.0 mgL⁻¹ Kinetin at 56 days after inoculation with a mean of 6.00 shoots / explant.

2 Introduction

Vanilla (*Vanilla planifolia* Andrews) is one of the agricultural commodities that generates foreign exchange for Indonesia. Indonesia's position is in second place after Madagascar with vanilla production of 2,304 tons in 2016. The export value of vanilla reached US \$ 72,511 thousand in 2017 but decreased in 2018 with an export value of US \$ 63,062 thousand [1].

Conventional vegetative propagation of vanilla using stem cuttings [2]. However, the development of vanilla is faced with the constraints of Fusarium wilt disease (*Fusarium oxysporum*) and limited planting material because it has to be taken from parent plant tendrils that have never produced fruit [3]. Micro propagation techniques for vegetative propagation of vanilla have been developed to overcome these obstacles.

The success of the micro propagation technique on vanilla has been widely reported. The use of 9.5 µM BA (Benzyl Adenine) on MS base medium resulted in a mean of 18.5 shoots / explant after 5 weeks. The 2-3 cm high plantlets were able to form a root system on ½ MS medium with the addition of 4.5 µM NAA [4]. Therefore [5] suggested that the addition of BAP (Benzyl Amino Purine) 2 mg / L and



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NAA (Naphthalene Acetic Acid) 0.5 mg / L produced the most shoots with an average of 5.33 and shoot height of 4.9 cm. Different results were reported by [6] which revealed that 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] also reported that the multiplication of vanilla shoots was only affected by BAP with the highest average yield of 3 shoots / explant at 28 days after inoculation.

The ability of vanilla explants to regenerate and differentiate to form shoots and shoots multiplication in vitro needs to be controlled by regulating cytokines. Therefore, this study aims to analyse the multiplication response of vanilla explant shoots with the addition of BAP or Kinetin and to analyse the interaction of adding BAP and Kinetin to the multiplication response of vanilla explant shoots.

2. Material and Methods

The research was conducted at Tissue Culture Laboratory Politeknik Negeri Jember of East Java, Indonesia at latitude 8 ° 09'35.1 "S 113 ° 43'27.2" E in June - November 2020. The study was based on factorial Completely Randomized Design with 3 replicates. The follow-up test used the Duncan Multiple Range Test at the 5% level. The treatments were the addition of cytokines in the basic medium of Murashige-Skoog (MS) with 100 mg of L-1 inositol, 30 g of L-1 sucrose, determining the pH of 5.7 - 5.8 and 8 g of L-1 agar. Factor 1 was a BAP 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/liter and factor 2 is Kinetin concentration which is 0.0, 1.0, 2.0 mg/liter.

Explant preparation begins with selecting normal healthy vanilla vines and then washing them with running water until they are clean. Chemical sterilization [5] carried out in Laminar Air Flow (LAF). Vanilla tendrils were washed with 20% Tween solution for 5 minutes and then rinsed with sterile distilled water. Explant sterilized by immersing in a solution of fungicide and bactericide 1.5% for 60 minutes and then immersed in 96% alcohol for 5 seconds. The explants were immersed in 10% bleach [5] solution (commercial bleach with 5.25% sodium hypochlorite) for 5 minutes and the explants were rinsed three times with sterile distilled water [5]. Vanilla nodes were cut about 1.5 cm long as explants [6].

The incubation period was maintained at 26°C ± 2°C with a relative humidity of 60-70% under a cycle of 16 hours of light and 8 hours of darkness with a light intensity of 40.5 µmol provided by fluorescence lamps.

3. Result and Discussion

3.1 The Sprouting Speed (days)

Observation of sprouting present is carried out every day and aims to determine the speed at which explants produce shoots. Based on Figure 1, it can be seen that the average speed of explants to form shoots is between 9-12 days after inoculation. The addition of Benzyl Amino Purine (BAP) affect the speed of the explants in forming shoots, presumably because the explants used came from stem segments where each explant had a candidate bud on each segment. [8] also reported that vanilla explants derived from stem internodes were able to germinate within 2 weeks compared to explants derived from young vanilla leaves.

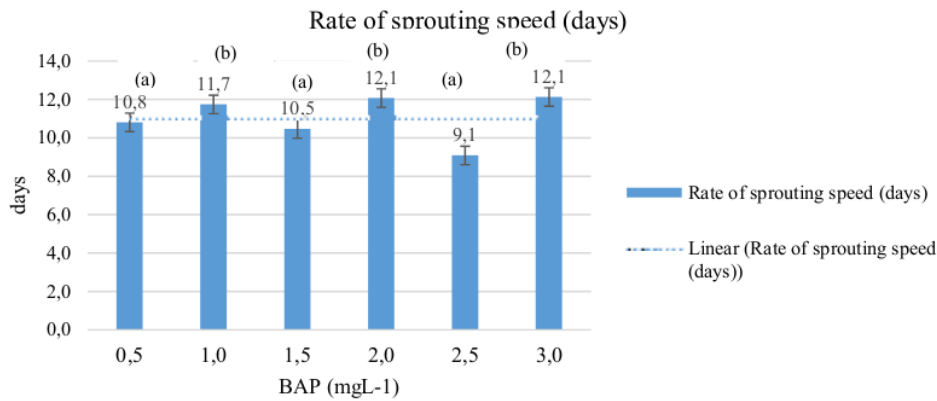


Figure 1. The average sprouting rate of vanilla explants with BAP stimulation

The speed of vanilla explants in forming shoots in about 2 weeks without going through the callus formation phase will minimize the risk of somaclonal variations. This is in accordance with the opinion [9] which states that the ability of explants to form shoots directly or without going through the callus phase will ensure genetic stability.

3.2 The Sprouting Ability (%)

The ability to sprout indicates the ability of vanilla explants to form shoots as a sign of successful vegetative propagation through stem cuttings. The success of explants in forming shoots was 67-100% on the 14th day after inoculation.

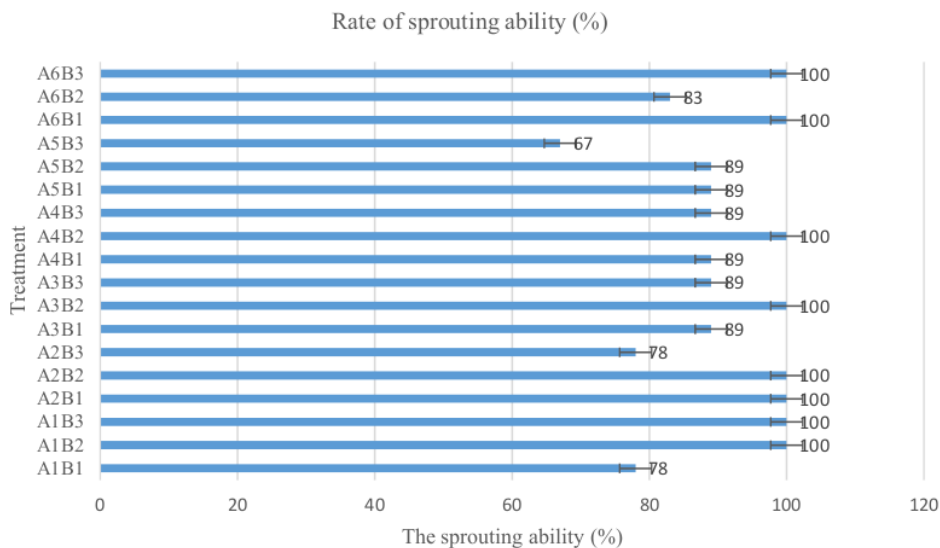


Figure 2. Average ability of vanilla explants to sprout with BAP and Kinetin stimulation

Figure 2 shows that the ability of explants to germinate does not depend on the addition of an exogenous stimulator. Food reserves in explants and media nutrition are thought to be able to meet the needs of explants to form shoots.[7]revealed the same result that shoot formation on vanilla explants was not affected by the addition of exogenous growth regulators. Different results were reported by [10] that the ability of vanilla explants to form 95% of shoots was influenced by the BAP and Kinetin ratio of 2: 1.

3.3 Shoots Number (shoots)

The addition of BAP and Kinetin stimulator intended to be able to double the explant vanilla buds in the propagation of vanilla through micro propagation techniques. Table 1 shows the mean number of shoots produced by explant vanilla influenced by the addition of BAP and Kinetin.

Table 1. Average number of shoots on vanilla explants with the addition of BAP and Kinetin

BAP (mgL-1)	Kinetin (mgL-1)	Average shoots number (shoots)
0.5	0.0	1.37 a
0.5	1.0	4.10 d
0.5	2.0	6.00 f
1.0	0.0	2.44 a
1.0	1.0	1.78 a
1.0	2.0	2.39 a
1.5	0.0	3.00 b
1.5	1.0	2.40 a
1.5	2.0	2.39 a
2.0	0.0	4.44 e
2.0	1.0	2.83 a
2.0	2.0	2.56 a
2.5	0.0	1.43 a
2.5	1.0	3.00 b
2.5	2.0	2.60 a
3.0	0.0	3.28 c
3.0	1.0	3.11 c
5 3.0	2.0	4.00 d

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

Based on table 1, it can be seen that the average number of shoots was highest in the addition of 0.5 mg L-1 BAP and 2.0 mgL-1 Kinetin at 56 days after inoculation with a mean of 6.00 shoots / explant and different with the other treatment. BAP is a growth regulator substance from the cytokine class which is needed to stimulate shoot growth. Cytokines are able to stimulate shoot cell division, causing the growth of meristem cells that will divide and develop into shoots. Giving BAP can stimulate protein synthesis so that it encourages cell division which induces the formation of shoots. [9]stated that for shoot multiplication requires the addition of cytokines. [4]also stated that the multiplication of vanilla shoots was only affected by the addition of [5] 9.55 μ M. The same thing was also conveyed by [11] that the best multiplication of vanilla shoots in MS medium enriched with BAP 1 mg.L-1.

3.4 Growth and Development of Explants

Efforts vanilla vegetative propagation through micro propagation techniques have the advantage of a faster, more efficient planting material, do not damage the mother plant and the nature of the same

ancestry with the parent. But the use of stem segment explants 1 vanilla require additional synthetic growth regulator of the cytokine group that explants are able to form shoots more.

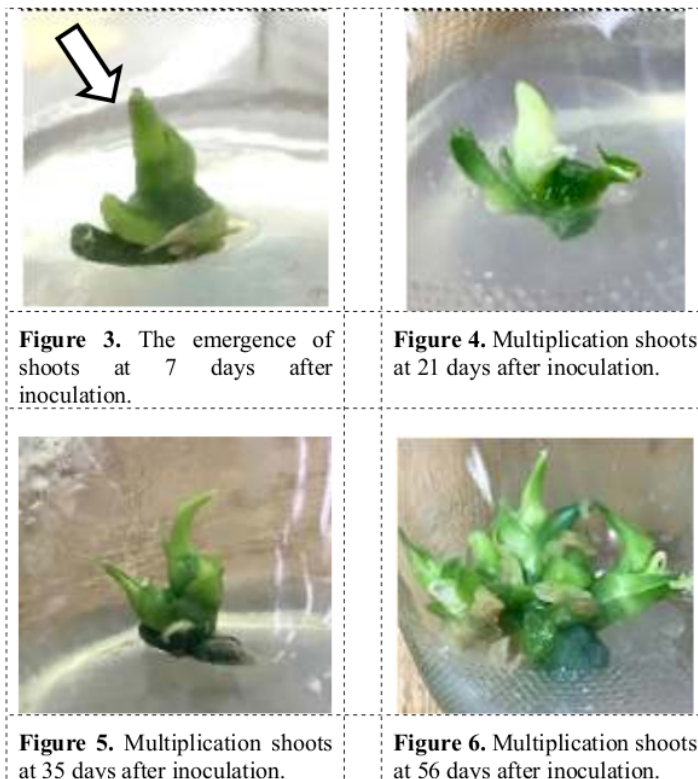


Figure 3. The emergence of shoots at 7 days after inoculation.

Figure 4. Multiplication shoots at 21 days after inoculation.

Figure 5. Multiplication shoots at 35 days after inoculation.

Figure 6. Multiplication shoots at 56 days after inoculation.

Explants sprouted at 7-10 days after inoculation (Figure 3) with the ability to form buds 70-100% at 14 days after inoculation. Multiplication of shoots have started to occur at 21 days after inoculation (Figure 4 and Figure 5) with the highest average number of shoots of 4-6 shoots / explant at 56 days after inoculation (Figure 6). This can happen because the administration of the dossier PGR influence on shoot multiplication of the explants vanilla. BAP Award stimulate synthesis of proteins that encourage cell division which induces the formation of shoots [12].

4. Conclusion

The addition of BAP affected the germination rate of vanilla explants with the fastest mean rate of 9 days after inoculation with the addition of 2.5 mgL⁻¹ and 67-100% sprouting ability 14 days after inoculation. The average number of shoots was highest in the addition of 0.5 mg L⁻¹ BAP and 2.0 mgL⁻¹ Kinetin at 56 days after inoculation with a mean of 6.00 shoots / explant

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