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The Effect of Benzyl Amino Purine (BAP) and Coconut Water on the Growth of Vanilla (*Vanilla planifolia* Andrews.) In Vitro

Pengaruh *Benzyl Amino Purine* (BAP) dan Air Kelapa terhadap Pertumbuhan Vanili (*Vanilla planifolia* Andrews.) in Vitro

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ABSTRACT

Vanilla (*Vanilla planifolia* Andrews.) is one of the plantation crops that has a high selling price, namely wet vanilla IDR 200,000-300,000/kg, regular quality dry vanilla around 1-3 million/kg, and export quality reaching 5-7 million/kg. The opportunity for farmers to develop vanilla commodities in Indonesia is very large, but limited planting materials still hamper it. In-vitro propagation is one technique that can be used to overcome the problem of vanilla propagation in Indonesia. This research aimed to determine the interaction of BAP and coconut water on the growth of vanilla nodal explants in vitro. The method used in this research was a factorial Completely Randomized Design (CRD) with a combination of Benzyl Amino Purine (BAP) and coconut water. The BAP concentration consists of 0 mg/L, 1 mg/L, and 2 mg/L. Coconut water has 0%, 15%, and 30% levels. Data were analyzed using ANOVA and further tested with the DMRT test at a confidence level of 95%. The results showed an interaction between BAP and coconut water on the growth of vanilla explants. The combination treatment of 1 mg/L BAP + 15% coconut water showed the best results with the fastest response time of 8.76+1.53 DAP, average shoot length 2.84±0.70 cm/explant and average root length 0.75±0.07 cm/explant.

Keywords: Benzyl Amino Purine, Coconut Water, In-Vitro, Vanilla

ABSTRAK

Vanili (*Vanilla planifolia* Andrews.) merupakan salah satu tanaman perkebunan yang memiliki harga jual tinggi, yaitu vanili basah Rp 200.000-300.000/kg, vanili kering kualitas biasa sekitar 1-3 juta/kg dan kualitas ekspor mencapai 5-7 juta/kg. peluang petani untuk mengembangkan komoditas vanili di Indonesia sangat besar, tetapi masih terkendala oleh terbatasnya bahan tanam. Perbanyakan secara in-vitro merupakan salah satu teknik yang dapat dilakukan untuk mengatasi permasalahan perbanyakan vanili di Indonesia. Tujuan penelitian ini untuk mengetahui interaksi BAP dan air kelapa pada pertumbuhan eksplan nodal vanilla secara in vitro. Metode yang digunakan dalam penelitian ini yaitu Rancangan Acak Lengkap (RAL) faktorial dengan kombinasi Benzyl Amino Purine (BAP) dan air kelapa. Konsentrasi BAP terdiri dari taraf 0 mg/L, 1 mg/L, dan 2 mg/L. Air kelapa terdiri dari taraf 0%, 15%, dan 30%. Data dianalisis menggunakan ANOVA dan diuji lanjut dengan uji DMRT pada taraf kepercayaan 95%. Hasil penelitian menunjukkan terdapat interaksi antara BAP dan air kelapa terhadap pertumbuhan eksplan vanili. Kombinasi perlakuan 1 mg/L BAP + 15% air kelapa menunjukan hasil terbaik dengan waktu respon tercepat 8.76+1.53 HSI, rata-rata panjang tunas 2.84±0.70 cm/eksplan dan rata-rata panjang akar 0.75±0.07 cm/eksplan.

Kata Kunci: Benzyl Amino Purine, Air Kelapa, In-Vitro, Vanili

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INTRODUCTION

The vanilla plant (*Vanilla planifolia* Andrews.) is a plantation and spice commodity with high economic value. This commodity has a relatively high selling price; wet vanilla is around IDR 200,000-300,000/kg, regular dry vanilla is 1-3 million/kg, and export quality reaches 5-7 million/kg (Ditjenbun, 2022). One constraint to developing vanilla in Indonesia is limited planting material and the use of cuttings that are not yet optimal. This is because cuttings require a long time, much labor, low germination ability, and a high risk of being attacked by vanilla stem rot disease caused by the *Fusarium oxysporum* f. sp. vanilla, even if the death rate reaches 50-80% (Ramadhan et al., 2019). Plant propagation using tissue culture can overcome the constraints of limited planting materials because it can produce plants in large quantities quickly, has uniform growth, has the same characteristics as the parent, does not depend on the season, and can avoid pest and disease attacks on seedlings.vanilla (Widyastuti & Deviyanti, 2018).

The combination of natural and chemical PGRs applied to culture media can support the growth and development of vanilla plant explants. Coconut water is liquid endosperm which contains up to 17% Potassium (potassium) and other minerals such as Sodium (Na), Calcium (Ca), Magnesium (Mg), Ferum (Fe), Cuprum (Cu), Phosphorus (P), and Sulfur (S) (Ramadhan et al., 2019). Coconut water contains organic compounds such as 1,3 diphenylurea, zeatin glucoside, zeatin ribose, high levels of K and Cl, protein and carbohydrates (Yunita, 2011). Coconut water contains kinetin of 50.09 mg/L, zeatin of 28.65 mg/L, and IAA of 20.89 mg/L after being heated at 121ºC (Kristina & Syahid, 2012). The proliferation rate of vanilla explants with BAP hormones is higher because it has a benzyl group that is more stable, highly effective, and resistant to heat (Tan et al., 2011). BAP-type cytokinin is more effective than kinetin-type cytokinin for shoot induction (Sharma & Bora, 2017).

Adding 10% coconut water to Murashige Skoog (MS) media significantly affected the early germination of vanilla explants. In comparison, adding 15% coconut water substantially impacts vanilla explants' number of leaves and height (Setyowati, 2022). Giving 1 mg/L Benzyl Amino Purine (BAP) to vanilla plants gave the highest average number of shoots, namely 6.8 shoots/explant (Erawati et al., 2020). Research by Hasnu & Tanti (2022) stated that MS media with BAP 4.44 µM + 15% coconut water produced a maximum shoot length of 6.35 cm and an average number of nodes of 5.27 ± 0.33 per shoot 90 days after inoculation on vanilla plants. This research aimed to determine the interaction of BAP hormones and coconut water on the growth of vanilla nodal explants in vitro.

MATERIALS AND METHOD

Time and place of research

The research was conducted in the Plant Tissue Culture Laboratory, Faculty of Agriculture, University of Jember University, Bondowoso Campus from February 2023 to December 2023.

Instrument and materials

The instruments used in this research include culture bottles, analytical balances, pH meters, pinsets, scalpel handles, micropipettes, magnetic stirrers, autoclaves, ovens, Laminar Air Flow (LAF), binocular microscope, and other supporting tools. The materials used in this research include nodal vanilla explants, a medium of Murashige Skoog (MS), Benzyl Amino Purine (BAP), coconut water, sugar, gel, and other supporting materials.

Experimental design

This study used a factorial Completely Randomized Design (CRD) with combination of BAP concentration and coconut water, namely B0C0 (control), B0C1 (BAP 0 mg/L + 15% coconut water), B0C2 (BAP 0 mg/L + 30% coconut water), namely B1C0 (BAP 1 mg/L + 0% coconut water), B1C1 (BAP 1 mg/L + 15% coconut water), B1C2 (BAP 1 mg/L + 30% coconut water), namely B2C0 (BAP 2 mg/L + 0% coconut water), B2C1 (BAP 2 mg/L + 15% coconut water), B2C2 (BAP 2 mg/L + 30% coconut water). The treatment was repeated in 3 replicates.

Research procedure

Media was made with a composition of Murashige Skoog MS 4.43 g/L, sugar 30 g/L, and gelatin powder seven g/L, which was homogenized. The pH was then measured between 5.6 and 5.8. BAP and coconut water were given according to each treatment and then autoclaved. Nodal explants from the field were washed until clean and soaked in 2g/L fungicide. They were sterilized in the LAF with 1% Clorox and 70% alcohol and rinsed with

distilled water thrice. Explants were cut ± 1 cm and then planted in media. The media was stored in the incubation room at a temperature of 25℃ and illuminated with LED lights for 8 hours/day, with a light intensity of 2000 lx.

Observation

Observation variables include the early emergence of shoots, plant height, number of shoots, number of leaves, and histology. Observations of the early emergence of shoots were carried out from the beginning of inoculation until they showed a response. The early emergence of shoots was observed by recording the time the explants showed a shoot formation response. Shoot length, root length, and percentage of responses were observed at the end of the observation in the 8th week.

Histology

The best treatment of shoot observed by histology. Histological observation by making preparations with stages, including sample fixation using formaldehyde for 12-24 hours at a temperature of 25-30˚C. The samples are dehydrated using acetone with graded concentrations (70%, 80%, and 90%) daily. The samples were cleared using xylol twice for 15 minutes. They embed the sample by placing it in liquid paraffin. Blocking was carried out using paraffin. The sample cut uses a microtome knife and is stained using hematoxylin-eosin. The sample is put down on the glass slide and labeled. Observations used a binocular microscope at 100x magnification (Karabiyik & Sen, 2023).

Statistical analysis

The data obtained were analyzed using Analysis of Variance (ANOVA); if there were significant differences between treatments, it was continued with the DMRT (Duncan Multiple Range Test) further test with a significance level of 95%. Data analysis was carried out using the SPSS 26 statistical application. Values were means ± standard deviation.

RESULT AND DISCUSSION

Explant growth

Vanilla nodal explants grown on MS media with the addition of BAP and coconut water experienced regeneration with a direct organogenesis pathway without going through the callus formation phase (Figure 1). This aligns with Erawati et al. (2021), nodal explants of vanilla forming shoot without going through the callus formation phase. Eksplan nodus berpotensi untuk diregenerasikan membentuk multiplikasi tunas (Pathak & Joshi, 2021). This organogenesis process is characterized by changes in single parenchyma cells or small groups of cells, which then undergo division and produce a meristemoid (globular) mass of cells, which will then develop and form root or shoot primordium (Roostika et al., 2005). This organogenesis pathway is unipolar, which means that the response to growing explants only occurs in one direction of growth (Anis & Ahmad, 2016).

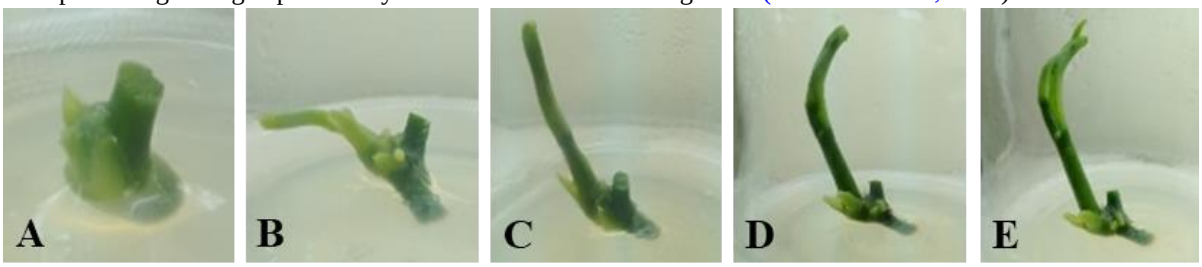

Figure 1. Development of explants with a combination of treatment of 1 mg/L BAP + 15% coconut water (B1C1), (A) 1 week after inoculation of explant, (B) 3rd week, (C) 5th week, (D) 7th week, (E) 8th week.

In this study, the addition combination of BAP 1 mg/L with 15% coconut water showed the best shoot formation response. The nodal explants grow with the fastest early shoot formation. According to Erawati et al. (2020), the right combination of cytokinin and auxin hormones affects the height of vanilla plant shoots. The hormone cytokinin with the right concentration can be a factor in the success of vanilla bud organ multiplication (Erawati et al., 2020). Combining cytokinin BAP 0.5 mg/L with kinetin 2 mg/L resulted in the best bud multiplication with many six buds/explant at 56 days after inoculation (Erawati et al., 2021). Adding coconut water to the culture medium can provide a natural plant growth regulator (PGR) of auxin, which can affect the growth

of vanilla in vitro. According to Ningsih et al. (2021), the natural plant growth regulator (PGR) of auxin was found in coconut water waste. Coconut water contains natural auxin growth regulators that can stimulate the root system (Martana et al., 2020). This aligns with Chutimanukul et al. (2023) that coconut water contains phytohormones such as auxins, cytokinins, and gibberellins, which can increase production, nutritional value, bioactive compounds, and antioxidant activity. The content of IAA in coconut water in the liquid endosperm formation stage was 1.51 µg/mL and fully matured dried stage, namely 1.01 µg/mL (Mintah et al., 2018).

Histology

The histology of the formation of *V. planifolia* shoots using a binocular microscope is shown in Figure 2. The initial response of vanilla nodal explants begins with swelling due to the presence of actively dividing meristem tissue and the appearance of sheath cells (Isda et al., 2020). The sheath cell is then torn naturally so that the color changes from green to white and then develops to form a bud (Eriansyah et al., 2014).

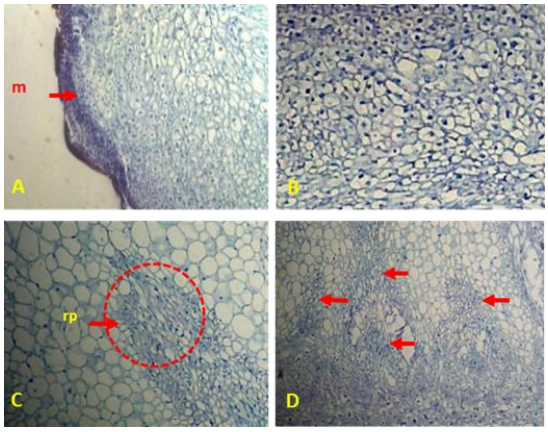

Figure 2. Histology of vanilla plant shoots at magnification. (A) m = meristem tissue 40x magnification. (B) Meristematic cells at 400x magnification. (C) Shoot base, rp =root primordium 100x magnification. (D) Embryogenic or buds with 100x magnification

Meristem cells are also called elongation zones (Crang et al., 2018), have a tighter arrangement, have no spaces between cells, are composed of a lot of protoplasm, thin cell walls, and actively divide (Figure 2 (A) and (B). Meristem cells at the base of the shoot form root primordia (rp), which grow sideways and continue to divide until they form roots (Figure 2 (C)). Meristem cells at the tip are formed embryogenically, which will grow and develop to form axillary buds and leaf primordia, which then gradually develop into perfect plantlets (Figure 2 (D)). The starch content contained in meristem tissue is also very supportive in the organogenesis of vanilla plants (Ramdhini et al., 2021).

Early response

The response to the emergence of shoots is characterized by swelling or green lumps in the area of the bud, while the presence of small white spots on the right or left side of the vanilla shoot indicates the response to the emergence of roots. The treatment combination of 2 mg/L BAP + 15% coconut water showed the best results with an average response time of 8.00±1.00 days after inoculation (DAI) was not significantly different from the treatment combination of 1 mg/L BAP + 15% coconut water with the average 8.76+1.53 days after inoculation (DAI). The interaction between BAP and coconut water on the early response of vanilla plant explants can be seen in Table 1.

Table 1. Results of Duncan's multiple distance test. Effect of interaction between giving BAP and Coconut Water on the early response observation variable

BAP	CW		
	0%	15%	30%
BAP 0 mg/L	10.67±1.15 (a) A	15.67±7.23 (b) B	16.00±1.73 (b) B
BAP 1 mg/L	13.67±0.58 (b) A	8.76+1.53 (a) A	11.33±0.58 (ab) A
BAP 2 mg/L	13.00±1.00 (b) A	8.00±1.00 (a) A	9.67±2.08 (ab) A

Description: Numbers followed by identical lowercase (horizontal) letters indicate no significant difference in the simple effect of CW concentration on the same BAP concentration; Numbers followed by the same uppercase (vertical) letter indicate no significant difference in the simple effect of BAP concentration on the same CW concentration

The interaction between BAP and coconut water affects the early response of vanilla explants because the auxin hormone in coconut water experiences basipetal transport so that at the base of the plant, there is an accumulation of auxin, which encourages root formation (Arhvitasari et al., 2019), while BAP can stimulate protein synthesis and promote cell division to form vanilla shoots (Satriawan et al., 2021). The explant response preceded by the emergence of shoots indicates higher cytokinin levels. In contrast, the explant response preceded by the emergence of roots means that the auxin level is higher than cytokinin. Coconut water influences the hormonal balance to trigger shoot formation because it contains various phenolic compounds and the hormone auxin, which responds to the differentiation of meristem cells to form shoots (Tan et al., 2011).

Shoot length

The length of explant shoots was measured from the base to the tip of the shoot at 8 WAI. The combination treatment of 1 mg/L BAP+15% coconut water gave the best results on average 2.84±0.70 cm/explant. This is in line with Ariyanti et al. (2021), who found that the addition of coconut water 15% affected the bud height of vanilla explants, namely 4.45 cm/explant. A combination of control treatments with an average shoot length of 0.00±0.00 cm/explant shows that in this treatment, no shoot growth occurred until the end of the study. The combination of BAP and coconut water, which contains organic acids, carbohydrates, vitamins, amino acids, mineral phytohormones, and ZPT such as auxin, cytokinin, and gibberellin encourages tissue proliferation, facilitates metabolism and respiration (Molnar et al., 2011). Vitamin C in coconut water can stimulate stem growth by dividing, elongating, and enlarging new plant cells (Kristina & Syahid, 2012). The interaction between BAP and coconut water on the shoot length of vanilla plant explants can be seen in Table 2.

Table 2. Results of Duncan's multiple range test on the effect of giving interaction Benzyl Amino Purine (BAP) and Coconut Water on shoot length observation variables

BAP	CW		
	0%	15%	30%
BAP 0 mg/L	0.00±0.00 (c) C	1.72±0.66 (a) B	1.89±0.48 (a) A
BAP 1 mg/L	1.88±0.20 (b) A	2.84±0.70 (a) A	1.62±0.28 (b) A
BAP 2 mg/L	1.41±0.48 (b) A	2.76±0.86 (a) A	1.46±0.18 (b) A

Description: Numbers followed by identical lowercase (horizontal) letters indicate no significant difference in the simple effect of CW concentration on the same BAP concentration; Numbers followed by the same uppercase (vertical) letter indicate no significant difference in the simple effect of BAP concentration on the same CW concentration

Coconut water also contains nitrogen in the form of amino acids and phytohormones, which can increase the metabolism of the photosynthesis process (Souza et al., 2013). Important phytohormones in coconut water, such as trans-zeatin riboside and kinetin riboside (Lee et al., 2022), can increase cell proliferation without increasing undesirable mutations compared to using synthetic cytokinins which can reduce the quality of the shoots produced due to their toxicity at higher concentrations. Vanilla explants experience continuous elongation because all their energy is used in cell elongation, so shoots do not multiply.

Root length

The length of the explant roots was measured from the base to the tip of the root at eight weeks after inoculation (WAI). The control treatment (0 mg/L BAP + 0% coconut water) B0C0 showed the best results, averaging 1.88±0.27 cm/explant. There is a significant difference with B1C0 (1 mg/L BAP + 0% coconut water) and B2C0 (2 mg/L BAP + 0% coconut water). The treatment combined with BAP tends to grow into shoots, as shown in Table 2. The control treatment showed the best root length because the vitamins and minerals contained in the culture media and endogenous auxin can trigger root growth.

Meanwhile, treatment combined with BAP tends to increase shoot growth. This is in line with Dwiyani (2015) that the combination ratio of auxin and cytokinin can influence the direction of morphogenesis and growth and development of plants in tissue culture. The interaction of BAP and coconut water on the root length of vanilla explants can be seen in Table 3.

The vitamin and mineral content in the explants and media can encourage root growth and elongation without giving BAP and coconut water. This is also due to the Thiamine in MS media, which is assisted by coenzymes in the reaction that can produce energy and carbohydrates that play a role in accelerating root meristem

division (Ariyanti et al., 2021). Providing auxin and cytokinin in high concentrations can inhibit the performance of other PGRs. Providing auxin in high concentrations can inhibit cell elongation by producing inhibitor compounds in ethylene from a reaction that can inhibit cell elongation so that shoot growth will be hampered. Providing cytokinin in high concentrations can reduce the length and number of roots because it acts as an inhibitor in the formation of lateral roots and counteracts the influence of auxin (Noah et al., 2021). Sharma & Bora (2017) state that increasing the cytokinin concentration above the auxin concentration will cause the meristematic zone at the root tip to be inactive to start root organogenesis. Cytokinin encourages the first stage in adventitious root initiation, namely cell proliferation. However, in the second stage, it will inhibit cell reprogramming, which leads to the specification of adventitious root founder cells from the previously formed microcallus (Lakehal et al., 2020).

Table 3. Results of Duncan's multiple range test on the effect of giving interaction Benzyl Amino Purine (BAP) and coconut water on root length observation variables

BAP	CW		
	0%	15%	30%
BAP 0 mg/L	1.88±0.27 (a)	1.28±0.62 (b)	0.89±0.31 (b)
	A	A	A
BAP 1 mg/L	1.20±0.32 (a)	0.75±0.07 (a)	1.21±0.48 (a)
	B	A	A
BAP 2 mg/L	0.97±0.23 (a)	1.20±0.32 (a)	0.71±0.00 (a)
	B	A	A

Description: Numbers followed by identical lowercase (horizontal) letters indicate no significant difference in the simple effect of CW concentration on the same BAP concentration; Numbers followed by the same uppercase (vertical) letter indicate no significant difference in the simple effect of BAP concentration on the same CW concentration

Percentage of responses

The percentage of explant response is the number of explants with each initial response divided by the number of samples in each treatment combination. The difference in BAP and coconut water concentration influenced the initial differences in the response given to each sample. The initial percentage results of vanilla explant response are presented in Figure 3.

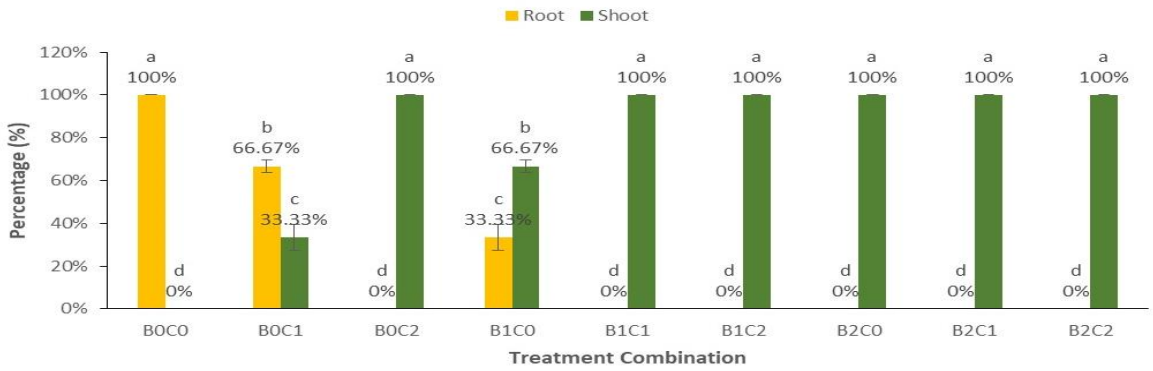

Figure 3. Percentage of giving effect Benzyl Amino Purine (BAP) and Coconut Water on the initial response of vanilla plant explants
Note: Lowercase letters indicate the interaction of BAP and coconut water. Numbers followed by different lowercase letters for each treatment (notations a, b, c, and d) indicate significantly different values.

Figure 3 shows that the concentration of BAP and coconut water influenced the initial response of vanilla explants. Explants without BAP showed an average tendency to start with a root emergence response, while explants with BAP showed an average tendency to start with a shoot emergence response. This is in line with the research conducted by Andriyani et al. (2023), which states that adding cytokinin can interfere with the performance of auxin in root formation by inhibiting anticlinal division in pericycle cells, which initiates lateral root formation. Root growth and development are influenced by auxin, which is transported polarly by the PIN FORMED (PIN) protein. Cytokinin can inhibit the expression of PIN-FORMED (PIN) protein so that it affects the distribution of auxin and disturbs the formation of the root primordium (Jing & Strader, 2019)

CONCLUSION

The addition of Benzyl Amino Purine (BAP) and Coconut Water to MS media affects the growth of vanilla in vitro. The combination of 1 mg/L BAP + 15% coconut water (B1C1) showed the best results for explant growth

with the fastest early response results of 8.76+1.53 DAI, average shoot length 2.84±0.70 cm/explant and root length 0.75±0.07 cm/explant.

REFERENCES

Andriyani, N.H., Anwar, S., Kusmiyati, F., 2023. Kajian penggunaan BAP dan NAA terhadap pertumbuhan kultur in vitro tanaman vanili (*Vanilla planifolia* Andrews.) pada fase akhir subkultur. *Agroeco Science Journal,* 2(1): 25-33.

Anis, M., Ahmad, N., 2016. Plant tissue culture: Propagation, conservation and crop improvement. *Plant Tissue Culture: Propagation, Conservation and Crop Improvement.*

Arhvitasari, A., Muslimin, M., Waeniyanti, W., Wardah, W., 2019. Organogenesis tanaman gaharu (*Aquilaria malaccensis* Lamk) pada berbagai konsentrasi zat pengatur tumbuh *Benzyl Amino Purin* (BAP) - *Indole Butric Acid* (IBA) secara in vitro. *Jurnal Warta Rimba,* 7(3): 88–93.

Ariyanti, N.K., Erawati, D.N., Sarita, R., Belinda, S.J., 2021. Analisis peran air kelapa terhadap pertumbuhan eksplan kultur vanili (*Vanilla planifolia*). *Agropross: National Conference Proceedings of Agriculture*, *5*, 89–97.

Chutimanukul, P., Sukdee, S., Prajuabjinda, O., Thepsilvisut, O., Panthong, S., Ehara, H., Chutimanukul, P., 2023. Exogenous application of coconut water to promote growth and increase the yield, bioactive compounds, and antioxidant activity for *Hericium erinaceus* cultivation. *Horticulturae*, 9(10).

Crang, R., Lyons-Sobaski, S., Wise, R. 2018. *Plant Cell Structure and Ultrastructure: Plant Anatomy*. Springer: Cham.

Direktorat Jenderal Perkebunan., 2022. *Statistik Perkebunan Non-Unggulan Nasional 2020-2022*. Jakarta: Sekretariat Direktorat Jenderal Perkebunan.

Dwiyani, R. 2015. *Kultur jaringan tanaman.* Cetakan Pertama. Denpasar Barat: Pelawa Sari.

Erawati, D.N., Fisdiana, U., Kadafi, M., 2020. Respon eksplan vanili (*Vanilla planifolia*) dengan stimulasi BAP dan NAA Melalui Teknik Mikropropagasi. *Agriprima: Journal of Applied Agricultural Sciences*, 4(2):146–153.

Erawati, D.N., Wardati, I., Humaida, S., Mawadah, Y., Ikanafiah, A., Ryana, W.M., 2021. Shoots multiplication of vanilla (*Vanilla planifolia*) with benzyl amino purine and kinetin modification. *IOP Conf. Series: Earth and Environmental Science. The 3rd International Conference on Food and Agriculture.* 672.

Eriansyah, M., Susiyanti, S., Putra, Y. 2014. Pengaruh Pemotongan eksplan dan pemberian beberapa Konsentrasi air kelapa terhadap pertumbuhan dan perkembangan eksplan pisang ketan (*Musa paradisiaca*) secara in vitro. *Agrologia,* 3(1): 54–61.

Hasnu, H., Sneha, S., Tanti, B., 2022. Multiple shoot induction and regeneration of *Vanilla borneensis* Rolfe - a critically endangered orchid of Assam, India. *Plant Science Today*, 9(1): 96–104.

Isda, M.N., Elvianis, E., Siti, F., 2020. Induksi Tunas pada beberapa tipe pemotongan eksplan bonggol pisang udang (*Musa acuminata* Colla) secara in-vitro. *Jurnal Biologi Andalas*, 8(1): 20-28.

Jing, H., Strader, L.C., 2019. Interplay of auxin and cytokinin in lateral root development. *International Journal of Molecular Sciences*, 20(3): 1-12.

Karabiyik, S., Sen, E.Y., 2023. Using histological techniques for plant tissue culture studies. *MolBiotech*: 151–157.

Kristina, N.N., Syahid, S.F., 2012. Pengaruh Air kelapa terhadap multiplikasi tunas in vitro, produksi rimpang dan kandungan xanthorrhizol temulawak di lapangan. *Jurnal Penelitian Tanaman Industri*, 18(3): 125–134.

Lakehal, A., Dob, A., Rahneshan, Z., Novák, O., Escamez, S., Alallaq, S., Strnad, M., Tuominen, H., Bellini, C., 2020. Ethylene response factor 115 integrates jasmonate and cytokinin signaling machineries to repress adventitious rooting in arabidopsis. *New Phytologist*, 228(5): 1611–1626.

Lee, Y.J., Srikandi, D., Subramaniam, S., Chew, B.L., 2022. The Effects of banana, potato, and coconut water in the regeneration of *Ficus carica* Cv. Japanese Btm 6. *Malaysian Applied Biology*, 51(1): 163-170.

Martana, S.B., Sofyadi, E., Widyastuti, L.S.N., 2020. Pertumbuhan tunas dan akar setek tanaman mawar (*Rosa* sp.) akibat konsentrasi air kelapa. *Paspalum: Jurnal Ilmiah Pertanian*, 8(1): 31–36.

Mintah, L.O., Arhin, L., Anim, J.O., Nkansah, G.O., 2018. Effect of coconut (*Cocos nucifera* L.) water of different fruit maturity stages on axillary bud initiation, growth, and development of plantain (*Musa* AAB.). *Journal of Applied Horticulture*, 20(1): 42–47.

Molnar, Z., Emese, V., Vince, O., 2011. Natural substances in tissue culture media of higher plant. *Acta Biologica*

Szegediensis, 55(1): 123-127.

Ningsih, E.M.N., Sudiyono, S., Anggraeni, F.D., 2021. Plant growth regulator of auxin content in fermented coconut water waste. *Journal of Physics: Conference Series*, 1908(1)

Noah, A.M., Casanova-Sáez, R., Ango, R.E.M., Antoniadi, I., Karady, M., Novák, O., Niemenak, N., Ljung, K., 2021. Dynamics of auxin and cytokinin metabolism during early root and hypocotyl growth in *Theobroma cacao*. *Plants*, 10(5): 1-18.

Pathak, A.R., Joshi, A.G., 2021. Regeneration of *hemidesmus indicus* (L.) r. br. using in vitro nodes: An alternative method for efficient multiplication of shoots. *Notulae Scientia Biologicae*, 13(2): 1–8.

Ramadhan, M.F., Setyorini, E., Rachmawati, N., Andrewsiati, E., 2019. *Ayo berkebun vanili*. Bogor: Pusat Perpustakaan dan Penyebaran Teknologi Pertanian.

Ramdhini, R.N., Manalu, A.I., Ruwaida, I.P., Isrianto, P.L., Panggabean, N.H., Wilujeng, S., 2021. *Anatomi tumbuhan*. Yayasan Kita Menulis: Medan

Roostika, I., Darwati, I., Mariska, I., 2005. Micropropagation of purwoceng (*Pimpinella alpina* KDS) through organogenesis and somatic embryogenesis. *Seminar on Asean Science and Technology Week*: Jakarta

Satriawan, D., Nurliana, S., Pujiyanti, T., 2021. Effectiveness of BAP (6-*Benzyl Amino Purine*) for buds induction of nutmeg (*Myristica fragrans* Houtt.). *Proceedings of the 3rd KOBI Congress, International and National Conferences (KOBICINC 2020)*. 14(Kobicinc 2020), 12–15.

Setyowati, A., 2022. *Pengaruh penambahan beberapa konsentrasi air kelapa terhadap pertumbuhan eksplan kultur vanili (Vanila planifolia Andrews.).* Politeknik Negeri Jember.

Sharma, R., Bora, S., 2017. Influence of explants type and plant growth regulators on in vitro multiple shoots regeneration of *Vanilla planifolia*. *International Journal of Agricultural Science and Research*, *7*(2): 189–196.

Souza, R., Aparecida, V., Francyane, T.B., Tesfahun, A.S., Joao, V.N., Patricia, A.H., Virginia, H.A., Geraldo, M.A.C., 2013. Effect of coconut water on growth of olive embryos cultured in-vitro. *Ciencia Rural*. 43(2): 290-296.

Tan, B.C., Chin, C.F., Alderson, P., 2011. Optimization of plantlet regeneration from leaf and nodal derived callus of *Vanilla planifolia* Andrews. *Plant Cell, Tissue and Organ Culture*, *105*(3): 457–463.

Widyastuti, N., Deviyanti, J., 2018. *Kultur jaringan: Teori dan praktik perbanyakan tanaman secara in-vitro*. Yogyakarta: ANDI Yogyakarta.

Yunita, R., 2011. Pengaruh pemberian urine sapi, air kelapa, dan rootone f terhadap pertumbuhan stek tanaman markisa (*Passiflora edulis* var. flavicarpa). *Sciences, Health*, *4*(1): 1–23