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The effect of coconut water and naphthalene acetic acid (NAA) application on the in vitro growth of *Paraphalaeonopsis serpentilingua* from West Kalimantan

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Abstract. *Mukarlina, Listiawati A, Mulyani S. 2010. The effect of coconut water and naphthalene acetic acid (NAA) application on the in vitro growth of* Paraphalaeonopsis serpentilingua *from West Kalimantan. Nusantara Bioscience 2: 62-66.* The 'Ekor tikus' orchid (*Paraphalaenopsis serpentilingua* J.J. Sm.) is an epidemic orchid in West Kalimantan. Now this orchid is facing a great conservation problem and threatened to be in extinction due to human exploitation. This research was conducted to find out the in vitro growth effect of *P. serpentilingua* by supplementation of NAA and coconut water in culture medium. The experiment was carried out using a Completely Randomized Design with two factors and five replicates. The result showed that supplemented NAA and coconut water on MS medium affected the emergence timing of buds, the average buds number, the average leaf number/buds, percentage of buds, the emergence timing of root, average root number and percentage of root. Medium that supplemented with 1.5 ppm NAA and 10% coconut water showed the fastest emergence timing of apical bud that is 13 days after planting. Medium supplemented with 0.5 ppm of NAA and 7.5% of coconut water shown the highest average number of bud was 11 buds.

Keywords: *Paraphalaenopsis serpentilingua*, NAA, coconut water.

Abstrak. *Mukarlina, Listiawati A, Mulyani S.* 2010. *Pengaruh aplikasi air kelapa dan asam naftalen asetat (*NAA) *pada pertumbuhan in vitro dari* Paraphalaeonopsis serpentilingua *dari Kalimantan Barat. Nusantara Bioscience 2: 62-66.* Anggrek 'ekor tikus' (*Paraphalaenopsis serpentilingua* J.J. Sm.) adalah anggrek endemik di Kalimantan Barat. Sekarang anggrek ini menghadapi masalah konservasi yang serius dan terancam punah akibat eksploitasi manusia. Penelitian ini dilakukan untuk mengetahui pengaruh pertumbuhan *P. serpentilingua* oleh suplementasi NAA dan air kelapa secara in in vitro dalam medium kultur. Percobaan dilakukan menggunakan Rancangan Acak Lengkap dengan dua faktor dan lima ulangan. Hasil penelitian menunjukkan bahwa penambahan NAA dan air kelapa pada medium MS berpengaruh terhadap waktu munculnya tunas, rata-rata jumlah tunas, rata-rata jumlah daun / tunas, persentase kuncup, waktu munculnya akar, rata-rata jumlah akar dan persentase akar. Medium yang mengandung 1,5 ppm NAA dan 10% air kelapa menunjukkan waktu tercepat munculnya tunas apikal adalah 13 hari setelah tanam. Medium dengan penambahan 0,5 ppm NAA dan 7,5% air kelapa menunjukkan rata-rata tertinggi jumlah tunas adalah 11 kuncup.

Kata kunci: *Paraphalaenopsis serpentilingua*, NAA, air kelapa.

INTRODUCTION

The 'ekor tikus' orchid (*Paraphalaenopsis serpentilingua* J.J. Sm) is an endemic orchid in West Kalimantan. This orchid has unique flowers with two branches lips (labellum) like snake's tongue so that to be called serpentilingua (serpentines are snake, lingua is tongue). This orchid is not only useful for ornament plant, but also for medicine plant. People use this leaf as medicine that neutralizes snake's poison (Chan et al. 1994; Siregar et al. 2005)

Population of this orchid has begun to decrease and classified as endangered species. One of the reasons is limited factor on the reproduction via seed. Production of seed on the August until December only and the seed does not have food reserve for embryo growth. The seed can be sprouting only if it symbiosis with mycorrhiza. Conservation problem due to human exploitation and forest burned too (Siregar et al. 2005).

Tissue culture technique constitutes an important component of biotechnology and has the potential not only to improve the existing cultivars, but also for the generation of plants in a comparatively short time compared to conventional breeding (Dixon and Gonzales 1994). The successful of tissue culture was influenced by modification of culture medium with addition of growth regulator substances and organic compounds. Growth regulator substance Naphthalene Acetic Acid (NAA) from auxin group used to increase in vitro root growth. Organic compounds like coconut water were added on the culture medium because it contains amino acid, vitamin, mineral and growth regulator substances like auxin and cytokinin that can be exhibit plant growth (George and Sherrington 1984; Hendaryono and Wijayani 1994).

The advantage of application synthetic grow regulator and organic compound like coconut water on the orchid culture medium was much be done (Bey et al. 2006; Untari and Puspaningtyas 2006; Widiastoety and Santi 1994).

This research aims to know effect of application combination concentration NAA and coconut water on the *in vitro* growth of the orchid *Paraphalaenopsis serpentilingua*.

MATERIALS AND METHODS

Materials used in the research are explants' stems that come from plantlets of *Paraphalaenopsis serpentilingua* ('ekor tikus' orchid) (Figure 1) from seed culture in Vacient and Went medium without supplement growth regulator substances, activated charcoal, agar, basal medium Murashige-Skoog (MS), NAA and coconut water.

The media were variously supplemented with NAA alone, coconut water alone or combination NAA (0.5 ppm, 1 ppm, 1.5 ppm) and coconut water (5%, 7.5%, 10%). The pH was adjusted to 5.8 before adding agar.

Cultures were incubated at 25 C at photoperiod of 16h/day with an illumination 0f 30 µmol m-2 sec-1 provided by 40 W cool white in fluorescent light. The cultures were regularly subcultured at four weeks intervals on new medium and twice to do with at least five cultures per treatment. (Listiawati et al. 2006) Observation has been done every day until three months after planting.

Parameter that observed were emergence timing of apical bud and emergence timing of axillary bud (day), number of bud (bud), number of leaves each bud (blade), emergence timing of root (day) and number of root (blade).

The experiment was carried out using a Completely Randomized Design with two factors. The first factor is NAA with four levels (0 ppm; 0.5 ppm; 1 ppm and 1.5 ppm), and the second factor is coconut water with four levels (0%; 5%; 7.5% and 10%) and five replicates. The result analyzed by ANOVA test and continued by using the Duncan Multiple Range Test (DMRT).

Figure 1. *Paraphalaenopsis serpentilingua* ('ekor tikus' orchid)

RESULTS AND DISCUSSION

The emergence timing of bud

The result showed that the apical bud has emergence timing faster than axillary bud. This reason caused that on the tip of stem found the meristem tissue that always to have meristematic characteristic. When the apical meristem was to divide, the axillary meristem to go through dormant so that at the beginning of bud growth especially to go on the apical bud growth. Apical shoot meristem will be synthesis auxin that necessary for apical bud growth. The growth of apical bud will inhibit growth of axillary bud (apical dominance) (Hidayat 1995; Salisbury and Ross 1995).

Treatment without supplemented (control) can be forming apical bud on the 24 days after planting (Table 1). This reason indicated that endogen growth regulator has capable of induction apical bud growth. The plant growth was influenced by internal factors, among them is endogen growth regulator (Hopkins 1995). Similarly, stem explants of *Paraphalaenopsis serpentilingua*. On the treatment with 0 ppm NAA + 0 ppm BAP can be forming apical bud at 37.33 days after planting (Maryam 2008).

Table 1. Effect of supplemented NAA and coconut water on emergence timing of bud

Treatment	Emergence timing of bud (day)	
	Apical	Axillary
0 ppm NAA + 0% coconut water	24 abc	47cd
0 ppm NAA + 5% coconut water	34 abcd	60 cd
0 ppm NAA + 7,5% coconut water	35 bcde	58 cd
0 ppm NAA + 10% coconut water	25 abc	65 de
0.5 ppm NAA + 0% coconut water	25 abc	104 e
0.5 ppm NAA + 5% coconut water	46 de	0 a
0.5 ppm NAA + 7.5% coconut water	23 abc	38 bcd
0.5 ppm NAA + 10% coconut water	28 abcd	49 cd
1 ppm NAA + 0% coconut water	53 e	0 a
1 ppm NAA + 5% coconut water	30 abc	54 cd
1 ppm NAA + 7.5% coconut water	43 cde	38 bcd
1 ppm NAA + 10% coconut water	37 bcde	31 bcd
1.5 ppm NAA + 0% coconut water	40 cde	18 ab
1.5 ppm NAA + 5% coconut water	13 a	21 ab
1.5 ppm NAA + 7.5% coconut water	26 abc	19 ab
1.5 ppm NAA + 10% coconut water	18 ab	13 a

Note: Figure in same column in each group followed by the same letter is not significantly different according to DMRT, P<0.05

Treatment with 1.5 ppm NAA + water coconut 5% is the most effective concentration for induction apical bud, this case could be shown by fastest of emergence timing of bud that is 13 days after planting (Table 1). Opinion that there is a proportion on interaction among 1.5 ppm NAA, growth regulators on coconut water and endogen growth regulators, so that they are optimum for induction bud. Optimum interaction among endogen growth regulators and exogenous regulators can be activated enzymes for growth increase (Wattimena 1992).

The fastest emergence timing of axillary bud is in treatment 1.5 ppm NAA + 10% coconut water that is 13 days after planting (Table 1). This reason indicates that interaction among 1.5 ppm NAA, 10% coconut water and endogen growth regulators are efficient to rule the apical dominance. Salisbury and Ross (1995) said that ratio cytokinin higher to auxin will stimulate growth of axillary bud, but ratio cytokinin lower to auxin will be excited apical dominance.

Number of buds

The combination concentration 0.5 ppm NAA + 7.5 % coconut water is the most efficient to give much axillary bud that is 11 buds (Table 2). Opinion that proportion among NAA, growth regulators primary cytokinin on coconut water and endogen growth regulators will be more activated enzymes that needed in bud multiplication. Responds a plant towards growth regulators were depend with species, part of the plant and interaction among growth regulators (Salisbury and Ross 1995; Hopkins 1995). Otherwise, coconut water consists of Nitrogen (N), Potassium (K), Calcium (Ca), vitamins, amino acids, nucleic acids, and gibberellic acid that function as stimulator of tissue proliferation, to carry on metabolism and respiration (Gunawan 1987; Hendaryono and Wijayani 1994).

All treatment conducted without addition of coconut water only to give 0-2 buds (Table 2). This reason can be caused by incapability endogen cytokinin to increase bud multiplication without exogenous cytokinin that comes from coconut water. George and Sherrington (1984) said that if the cytokinin on sub-optimum condition, therefore, required exogenous cytokinin to obtain proportion between endogen cytokinin and exogenous cytokinin to bud multiplication.

Application 1.5 ppm NAA in all level concentration of coconut water only give 0-1 axillary bud (Table 2). Axillary bud multiplication only requires effective concentration of cytokinin without auxin or with low auxin concentration (Wattimena 1992). Endogen auxin and 1.5 ppm NAA interaction can stimulate synthesis of ethylene. Ethylene on the plant cells can inhibit plant growth (George and Sherrington 1984; Hopkins 1995). Similarly, application 20 ppm NAA+ 150 g/L sweet potato on black orchid (*Coelogyne pandurata* Lindl) culture was given only 1.5 buds (Untari and Puspitaningtyas 2006).

The treatment of 0.5 ppm NAA + 5% coconut water was showed that explants cannot form axillary bud and growth slower than the other treatments. This case can be realized by lasting of emergence timing of apical bud that is 46 days after planting. Eventuality, interaction between endogenous cytokinin and cytokinin on 5% coconut water more effective to form the chlorophyll, whereas all leaves' plantlets on this treatment greener than leaves' plantlets on other treatment. Salisbury and Ross (1995) state that once of cytokinin function was increase synthesis of protein that chlorophyll attaches.

Table 2. Effect of supplemented NAA and coconut water on average number of buds and average number of leaves

Treatment	Average number of buds		Average number of leaves	
	Apical	Axillary	Apical	Axillary
0 ppm NAA + 0% coconut water	1	1.33 a	5.33 d	2.23 cde
0 ppm NAA + 5% coconut water	1	3 abc	3.33 ab	2.75 de
0 ppm NAA + 7.5% coconut water	1	6 bc	3 ab	2.87 e
0 ppm NAA + 10% coconut water	1	2 ab	4 abc	2.83 de
0.5 ppm NAA + 0% coconut water	1	2 ab	4.33 cd	2 bcde
0.5 ppm NAA + 5% coconut water	1	0 a	2.67 a	0 a
0.5 ppm NAA + 7.5% coconut water	1	11 d	4 abc	2.02 bcde
0.5 ppm NAA + 10% coconut water	1	7.3 cd	4 abc	2.27 cde
1 ppm NAA + 0% coconut water	1	0 a	2.33 a	0 a
1 ppm NAA + 5% coconut water	1	6 bc	3.67 ab	1.7 abcde
1 ppm NAA + 7.5% coconut water	1	1.67 ab	2.33 a	1.67 abcde
1 ppm NAA + 10% coconut water	1	1.33 a	2.67 a	1.17 abcd
1.5 ppm NAA + 0% coconut water	1	1 a	2.67 a	0.43 ab
1.5 ppm NAA + 5% coconut water	1	0.33 a	4 abc	0.33 ab
1.5 ppm NAA + 7.5% coconut water	1	0.67 a	3.67 ab	0.83 abc
1.5 ppm NAA + 10% coconut water	1	1 a	3.67 ab	0.77 abc

Note: Figure in same column in each group followed by the same letter is not significantly different According to DMRT, $P<0.05$

Number of leaves

Variations of average number of leaf are 2.23-5.33 leaves (Table 2). Formatting of leaf was related to the emergence timing of bud. The last emergence timing of apical bud is 30 days until 53 days after planting only produce 2.33 until 3.33 leaves, whereas the treatment that gives to emergence timing 13 days until 28 days after planting produce 3.67 until 5.33 leaves. Proportion among of endogen growth regulators, NAA and growth regulator on coconut water was used to growth apical bud before. Forming of leaves achieved after growth of apical bud. Exogen growth regulators can be reaches growth primordial of leaf (George and Sherrington 1994; Hidayat 1995).

The average number of axillaries bud's leaves that was achieved in coconut water alone treatments was 2.23 – 2.87 leaves (Table 2). This result showed that cytokinin on coconut water has been able to induction divided of leaf cells. Dixon and Gonzales (1994) state that application of cytokinin without auxin was completely optimum for divide and extent of leaf cells. Otherwise, coconut water contained some elements that are Ca and vitamins that used to stimulate addition number of leaf (Hendaryono and Wijayani 1994). Application of 15 ppm NAA + 250 mL/L coconut water can stimulate addition number of leaf of black orchid culture that is 3.3 leaves (Untari and Puspaningtyas 2006).

Number of roots

The result showed that forming root in the plantlets can be achieved only on four treatments that are 1 ppm NAA + 7.5 % coconut water; 1 ppm NAA + 10% coconut water; 1.5 ppm NAA + 5% coconut water and 1.5 ppm NAA + 7.5% coconut water. Eventuality, the treatments mentioned, have an effect of proportion among NAA, auxin on coconut water and auxin endogen that stimulate forming of root. Auxin is a phytohormone used to stimulate initiation primordia of root. When ratio auxin is higher than cytokinin initiation of root can be stimulated (George and Sherrington 1995; Wattimena 1992).

The fastest of emergence timing of root achieved on combination 1.5 ppm NAA + 5% coconut water that is 23 days after planting. Opinion that ratio NAA and growth regulator on coconut water was efficient to induction growth of root. Otherwise, this treatment has the fastest emergence timing of apical bud that is 13 days after planting. The apical bud will be synthesis auxin, auxin will be translocated polar basipetal to induction growth of root (Hopkins 1995; Salisbury and Ross 1995).

CONCLUSION

Based on the result of analysis, it showed that there was a significant effect of the NAA and coconut water application of emergence timing of bud, number of bud and number of leaf produced by the explants. The treatment of 1.5 ppm NAA and 10 % coconut water has a good effect on the emergence timing of axillary bud that is 13 days after planting. Combination of 0.5 ppm NAA and 7.5% coconut water have a good effect on number of bud multiplication that is 11 buds.

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