

Low-Cost Media for in vitro Multiplication and Development of Protocorm Like Bodies (PLBs) of *Eulophia graminea* Orchid

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Abstract— *Eulophia graminea* is a saprophytic orchid easily found in a high salinity environment along the coastline. A study aimed to explore low-cost media compositions enriched with organic matters for in vitro multiplication and development of protocorm-like bodies (PLBs) of *E. graminea*. This study consisted of two stages. The first stage was to determine the best basic media for in vitro PLBs multiplication by testing NPK 20-20-20, Murashige and Skoog (MS), Knudson C (KC), and Vacin and Went (VW). The second stage was to compare the development of PLBs of *E. graminea* using NPK 20-20-20 as the basic media, supplemented with three types of organic matters (Cavendish banana, potato and coconut water) and four organic matter concentrations (50,100, 150, 200 ml L⁻¹), arranged under Factorial Completely Randomized Design with ten replications. The results showed that orchid PLB of *E. graminea* could be planted on NPK medium with similar success in growth and development, in term of PLB number, PLB diameter, leaf number in plantlet, plantlet height, root number and root length, to those planted on MS medium. Among complex organic growth supplements, including coconut water, potato, and banana, coconut water at a concentration of 57.17 – 98.67 ml L⁻¹ resulted in the best multiplication of PLB, in term of the greatest number, PLB diameter, and fresh weight. Coconut water promotes the greatest conversion of PLB into plantlets of *E. graminea* at an optimum concentration of 91.63 – 102.89 ml L⁻¹. The price of using a complete fertilizer medium NPK is much lower than those of using MS medium, Knudson C medium, or Vacin and Went medium; this media was the most cost-effective media for mass micropropagation of *E. graminea*.

Keywords— orchid; *Eulophia graminea*; in vitro; organic matter; multiplication

I. INTRODUCTION

Eulophia graminea is a saprophytic orchid which poses a ± 6 cm-diameter of the pseudobulb. Leaflets are arranged intermittently with leaf size of 9-20 cm length and ± 5 cm width. Inflorescences, with the length of 26-70 cm, emerge at leaf axil. Flowers consist of dorsal sepal, lateral sepal, and labellum. Corolla color is greenly red-brown, with white flower lips and pale red in the middle of flower lips. Fruits are capsule shape 5-7 cm length [1]. In their natural habitat, this orchid is endangered as seeds of *E. graminea* have no endosperm which hinders their germination without the assistance of mycorrhiza [2] and [3].

Tissue culture techniques have been used to conserve germplasm of many kinds of orchid. These techniques pose several advantages including having high multiplication rate, propagules relatively being a similar size, and relatively faster. In addition, the resulted plantlets have the same genetic material with their parents (true to type) and being pathogen free [4]. When orchid seeds are cultured, they may develop protocorm-like bodies (PLB), a round structure that further develop to become plantlet [5].

Naturally, the orchid plant often grows profusely under

various media which may not contain enough nutrients, attaching on a tree (epiphyte), a rock (lithophyta), organic matter (saprophyte) or soil (terrestrial) [6]. Under in vitro system, MS (Murashige and Skoog), Vacin and Went (VW), and Knudson C (KC) are media commonly used, as they are made of macro and micro minerals, vitamins and sucrose from chemicals which are expensive. There were some studies on the most suitable culture media for sowing orchids, such as MS medium for *Cymbidium sp.* [6], ½ MS for *Dendrobium sp.* [7] and [8], XER medium for *Phalaenopsis sp.* [9] and [10], KC medium for Teixeira, *Cymbidium sp.* and *Dactylorhiza fuchsia* [11] and [12], VW medium for *Vanda sp.* and *Mokara sp.* [13], [14].

Complete fertilizer formulations have been used to overcome high price of chemicals for composing tissue culture media. NPK (20-20-20) is a complete fertilizer formula containing a total of 20% Nitrogen in form of 3.9% Ammonium-Nitrogen, 5.7% Nitrate-Nitrogen, and 10.6% Urea-Nitrogen, 20% P₂O₅ 20 %, 20% K₂O, 0.05% Ca, 0.10% Mg, 0.20% S, 0.02% B, 0.05% Cu, 0.10% Fe, 0.05% Mn, 0.0005% Mo, 0.05% Zn and 39% inert ingredient [15]. This complete fertilizer at a concentration of 1 g L⁻¹, enriched with 50 g L⁻¹ tomato paste, has been used

successfully for multiplication of *Dendrobium*, resulting 74 shoots per jar [16]. Complete fertilizer had also been used for micropropagation of *Spathoglottis plicata* orchid and its mutant [4].

Several complex organic matters have been used to enrich media for increasing multiplication rate of orchids. Addition of coconut water at 250 ml L⁻¹ speed up seed germination of Lion orchid (*Grammatohyllum scriptum*) twice as fast as those under control medium [17]. When coconut water was added at 150 ml L⁻¹ on VW medium, it increased PLB formation of orchid [18]. The addition of potato at 150 g L⁻¹ increased plantlet height and leaf number of *Phalaenopsis amabilis* [19].

This paper reports experiments conducted with objectives (1) to determine the best basic media for in vitro PLBs multiplication by testing NPK 20-20-20, Murashige and Skoog (MS), Knudson C (KC), and Vacin and Went (VW) and (2) to identify type (Cavendish banana, potato and coconut water) and concentration (50,100, 150, 200 ml L⁻¹), of complex organics matter, both for highest multiplication and development of PLB of *E. graminea* orchid in vitro.

II. MATERIAL AND METHOD

A. Plant Material and Culture Condition

This experiment used planting materials of *E. graminea* PLBs that were 8 weeks after planting (WAP) (Figure 1a). These materials previously were tissue cultured from PLBs that naturally grow from shoot tip meristem/corms of the plant that grows in their habitat along the long coastline of Bengkulu (Figure 1b), through the following protocol. Before planting, sterilization was done gradually as follows: (1) all leaves covering plant corms were removed, (2) the corms were then washed with 20% detergent solution and rinsed with running water until clean, (3) clean corms were sterilized for 60 min using pellets consisting of a mixture of 2 g L⁻¹ Benlate and 2 g L⁻¹ Agrimycin, subsequently rinsed 3 times with distilled water, (4) the corms were further sterilized by soaking them in 10% sodium hypochlorite solution for 30 minutes then rinsed with sterile distilled water 3 times, (5) shoot tip meristem of the sterile corms were taken with a size of 0.5 cm from the tip most and then planted on jars containing ½ MS medium enriched with 150 ml L⁻¹ young coconut water.

Jars containing planting material of *E. graminea* were incubated in culture rooms with a temperature of 24 ± 2°C under the light of 100 lux supplied by 20-watt LED lamps with 16 hours on and 8 hours off for a period of 8 weeks.

B. Preparation of Organic Extracts and Media

Coconut water was obtained from young green coconuts that were picked from an 8-m palm tree in farmer's garden located in Bengkulu City. Mature Cavendish Banana and Potato cv. Granola was obtained from a supermarket in Bengkulu City and prepared in modified method from Obsuwan and Thepsithar [13]. In brief, the banana was sliced into 1 cm³ sized cubes of each freshly-diced material were blended at a concentration of 50,100, 150, 200 g L⁻¹ with 200 ml of liquid ½ MS medium using kitchen blender (Philips) for one minute. The potato was peeled and then cut

into cubes of 1 cm³. The fresh-diced material was boiled in 200 ml of distilled water in 10 minutes. The potato extracts were strained and immediately added to ½ MS medium at a concentration of 50,100, 150, or 200 g L⁻¹ and blended [13].

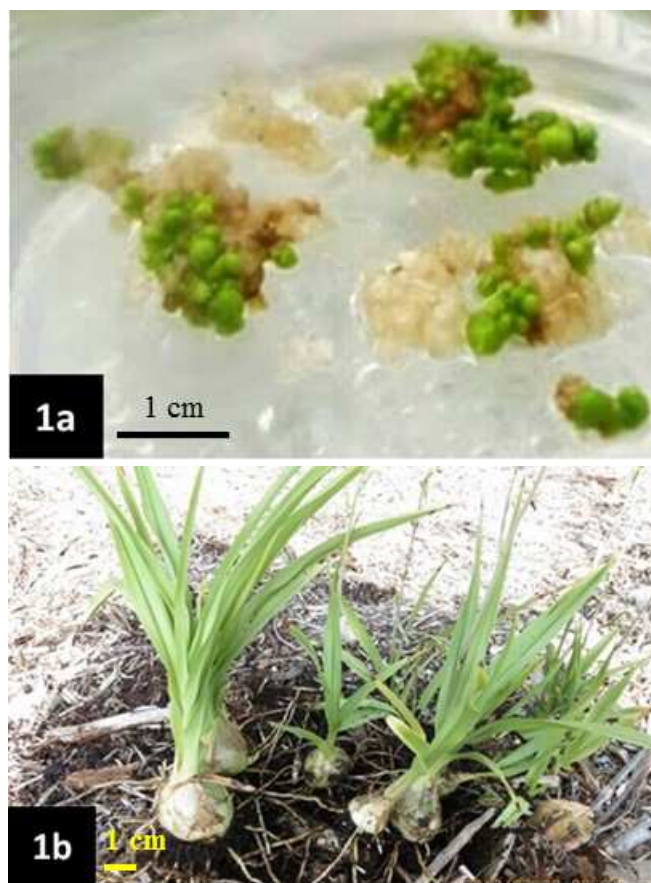


Fig. 1 *Eulophia graminea* plant material: (1a) PLBs aged 8 weeks under tissue culture media of ½ MS + 150 ml L⁻¹ young coconut water. (1b) Orchids of *E. Graminea*, growing under their natural habitat, were the origin of materials developed under cultured tissue media to obtain PLBs for experiments

Media were adjusted to pH 6.0, being cooked until boiling, and being poured into culture jar at 25 ml per jar. The media were subsequently autoclaved at 121°C with 15 psi for 20 minutes and incubated at ambient temperature for 7 days.

Sterilized media were planted with three PLBs of *E. graminea* orchid per jar. The jars were incubated in a culture room at 24 ± 2°C, under 16 hours light and 8 hours dark for 12 weeks.

C. Experimental Design and Data Analysis

Experiments were conducted in two phases. The first phase was a selection of in vitro medium for PLB of *E. graminea* orchid; whereas the second phase was a selection of type and concentration of organic complex to be added for the selected medium in the first phase. The first experiment used a Completely Randomized Design with ten replications. Selection of in vitro medium for PLBs of *E. graminea* orchid involved four in vitro basic media, including NPK (2 g L⁻¹ compound fertilizer NPK 20-20-20), MS (Murashige dan Skoog Medium) [6], KC (Knudson C Medium) [11] and VW (Vacin and Went Medium) [20]. Each medium was added with 30 g L⁻¹ sucrose and 7 g L⁻¹ agar powder. The first

experiment was conducted to evaluate the development and multiplication of PLBs. The growth of PLBs was measured with several variables including PLB number, PLB diameter, plantlet height, leaf number, root number and root length. These variables were observed at 12 weeks after plating. Variability of growth data was analyzed by using F test at 5% level by using X-Costat. Treatments of significant data were further separated by using Duncan's Multiple Range Test (DMRT) at 5% level.

The second experiment aimed to evaluate the role of organic matter on the conversion of PLBs to become plantlets. The experiment included the addition of three types of organic matters (Cavendish banana, potato, and coconut water) and four organic matter concentrations (50,100, 150, 200 ml L⁻¹). Type and concentration of organic matters were tested in a factorial design resulted in 15 treatment combinations, arranged under Factorial Completely Randomized Design with ten replications. We used 2 g L⁻¹ compound fertilizer NPK 20:20:20 (selected from the first experiment) added with 30 g L⁻¹ sucrose, 7 g L⁻¹ agar powder, and organic growth supplement for all treatments.

The conversion of PLBs to become plantlets were measured through growth variables, including plantlet height, leaf number, root number and root length. Data variant were analyzed by using F-test at 5% level with X-Costat program. The difference among the treatments was separated further by using Duncan's Multiple Range Test (DMRT) at the level of 5 percent.

III. RESULTS AND DISCUSSION

A. Selection of in vitro Basic Media for PLB Multiplication of *E. graminea* Orchid

Variant analysis at 5% demonstrated that basic culture media affected PLB multiplication in terms of PLB number, PLB diameter, and fresh weight of PLB at 12 weeks after planting (WAP – Table 1).

TABLE I
PLB MULTIPLICATION OF *E. GRAMINEA* AS AFFECTED BY MEDIA FORMULATION AT 12 WEEKS AFTER PLATING

Kind of Media	PLB Number (per jar)	PLB Diameter (mm)	PLB Fresh Weight (g)
NPK	29.2 a	2.3 a	1.04 a
MS	25.2 a	2.2 a	0.91 a
KC	5.8 b	1.2 b	0.31 b
VW	4.6 b	0.6 c	0.16 b

Note: Values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 5%, MS= Murashige and Skoog Medium, KC = Knudson C Medium and VW = Vacin and Went Medium

The best respond of PLB multiplication and development of PLB *E. graminea* to become plantlet was found on NPK 20-20-20 medium, being as good as those plated on MS medium. Multiplication and development PLB on both media were better than those on KC or VW media (Table 1). The greatest number of PLB (29.2 PLBs per jar) resulted on NPK medium, but similar to those on MS medium (25.2 PLBs per jar); whereas those on KC and VW, media were

4.6 and 5.8 PLBs per jar, respectively. Similarly, the biggest PLB diameters (2.3 mm and 2.2 mm) were also found on NPK and MS media, respectively. These were about twice bigger than those planted on KC medium, or about four-times bigger than those on VW medium. The high number and big diameter of PLBs resulted in the greatest fresh weight of PLBs on NPK. The heaviest fresh weight of PLB (1.04 g) was found on MS medium, similar to those resulted on NPK medium (0.91 g); both being higher than those PLBs on KC and VW media (Table 1).

The result of analysis of variant at 5% demonstrated that type of basic medium affects the development of PLBs to become plantlet, in term plantlet height, leaf number, root number, and root length at 12 WAP. Subsequent DMRT test at 5% results are presented in Table 2. In general, developmental responses of PLBs to become plantlet on NPK medium were similar to those on MS but differed than those on KC and VW medium. The highest plantlet (5.76 cm) was found on NPK medium, followed but were not different from those on MS medium (3.34 cm). Those plantlets were higher than those grown on VW and KC media (0.82 cm). The greatest leaf numbers were obtained on NPK and MS media (6.6 and 5.2, respectively), followed by those on KC and VW media (3.8 and 1.2, respectively). Root numbers per jar were greatest when PLB was grown on MS and NPK media (12.8 and 10.8, respectively), being greater than those grown on KC and VW media (1.4 and 0.6, respectively). In addition, the longest roots of plantlet were found when PLB was plated on MS and NPK media (3.68 and 2.96, respectively), being longer than those on KC and VW media (0.92 and 0.28, respectively).

High multiplication rate of PLBs and good development of PLB to become plantlet of *E. graminea* on NPK medium suggests that concentration of 2 g L⁻¹ with the complete and balanced composition of NPK and enriched with complete macro and micronutrients make NPK formulation suitable for PLB growth. As this experiment demonstrate that NPK is as good as MS medium, this means that NPK is very potent for use as an alternative to MS medium which is commonly used for in vitro culture of orchids.

The results of this experiment additionally demonstrated that the requirement of the particular composition of nutrients is highly specific for each species of orchids. In our experiment, optimal growth of *E. Graminea* under medium NPK 20-20-20 might be because the medium contains macro- and micro-elements similar with those in MS medium, but more complete as compared to those compositions in VW or in KC media. This result indicated that PLBs of *E. Graminea* orchid needs complete nutrients for their propagation and their development to become plantlets (Table 2). On the other sides, media MS, KC, and VW have been successfully used for multiplication and development of several orchids in vitro [13], [14]. VW medium added with coconut water was the best medium for PLBs proliferation of Vanda Kasem's Delight (VKD), VW medium supplemented with potato extract being the best medium for growth of Mokara orchid, and VW medium enriched with Namwa banana being the best medium for growth of Vanda orchid [13]. In addition, Knudson C medium has been demonstrated to be the most suitable for PLB's growth and development of *Vanda helvola* [31].

TABLE II
EFFECT OF MEDIA TYPE ON DEVELOPMENT OF PLB TO BECOME PLANTLET
OF *EULOPHIA GRAMINEA* ORCHID AT 12 WEEK AFTER PLATING

Types of Media	Plantlet Height (cm)	Leaf Number	Root Number	Root Length (cm)
NPK	5.76 a	6.6 a	10.8 a	2.96 a
MS	3.34 a	5.2 a	12.8 a	3.68 a
KC	1.60 b	3.8 b	1.4 b	0.92 b
VW	0.82 b	1.2 c	0.6 b	0.28 b

Note: Values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 5%. MS= Murashige and Skoog Medium. KC = Knudson C Medium and VW = Vacin and Went Medium.

A further advantage is that NPK formulation is also cheap, easily available and easy to prepare for culture medium. The price for making one-liter NPK medium is only Rp 23.500,-, being much cheaper when compared with those of MS, KC and VW media (Rp 572.500,-, Rp 375.500,- and Rp 315.700,-, respectively). On the other words, NPK medium price was only 4.1% of MS medium, 6.3% of KC medium or 7.4% of VW medium. Therefore, NPK medium can be a very cheap alternative medium for mass propagation of *E. graminea* orchid.

The proportion of nitrogen and phosphorus on a medium has been stated to have a pivotal role in determining the formation of leaf and root [15]. In term of NPK 20-20-20, according to [21] this medium at 1 g L⁻¹ + vitamin was the best medium for propagating *Paraphalaeonopsis serpentina* in vitro, as shown on the greatest number of leaf (4.95), of leaf length (16.75 mm), of plantlet height (8.28 mm), of leaf wide (4.88 mm) and of root length (44.98 mm).

B. Selection of Type and Concentration of Organic Growth Supplements

Results of variant analysis demonstrated that there were interaction effects between type and concentration of complex organic matter supplements on PLB multiplication variables, including PLB number, PLB diameter, and addition fresh weight. Interaction responses of these variables are presented in Fig. 2A, 2B, and 2C.

The polynomial orthogonal test revealed that most concentration responses from 50 ml L⁻¹ to 200 ml L⁻¹ of three types of organic matters (Cavendish banana, potato, and coconut water) on three variables of PLB multiplication were quadratic, except those of potato on PLB diameter having a linear response.

In case of coconut water supplementation, optimum responses of three multiplication variables (PLB number, PLB diameter, and PLB fresh weight) were achieved at different concentrations (Fig. 2). For PLB number, the response curve to coconut water concentration was $y_{B_3} = 1.9x^2 + 14.58x + 20.6$, $R = 0.997$, with maximum PLB number (18.38 PLBs per jar) being achieved at 57.17 ml L⁻¹ (Fig. 2A). For PLB diameter, the response curve to coconut water was $y_{B_3} = -0.04x^2 + 0.134x + 0.08$, $R^2 = 0.939$, with greatest diameter (0.214 cm) being achieved on coconut water at concentration of 98.67 ml L⁻¹ (Fig. 2B). On the other hand, for fresh weight gain, the response curve of coconut water was $y_{B_3} = 0.1525x^2 - 0.8695x + 1.4025$, $R^2 =$

0.926, with the highest fresh weight gain being 1.204 g achieved at 92.16 ml L⁻¹ (Fig. 2C).

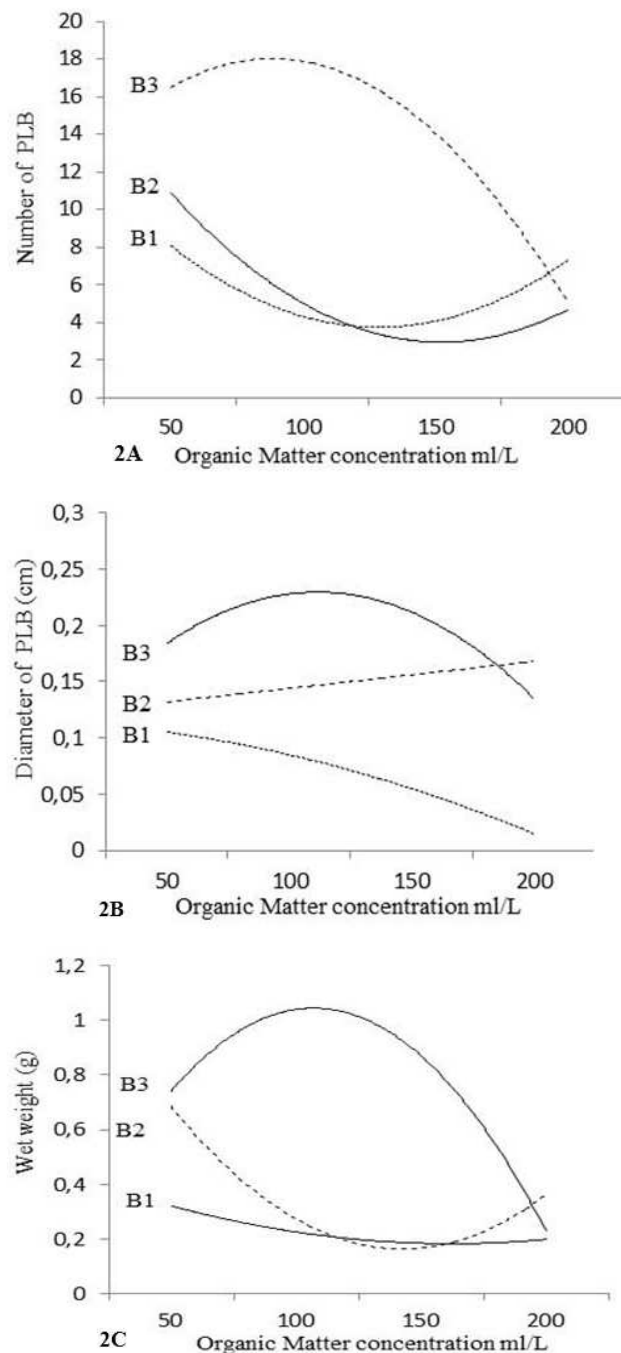


Fig. 2 Interaction effect between type and concentration of complex organic matter on PLB number (2A), PLB diameter (2B), and fresh weight (2C) of *Eulophia graminea* at 12 weeks after planting, B1 = banana, B2 = potato, B3 = coconut water

The best multiplication, based on criteria of the highest PLB number (18.38 PLB), the greatest PLB diameter (2.14 mm) and the heaviest fresh weight gain (1.204 g), was achieved on addition with coconut water at a concentration between 57.2 – 98.7 ml L⁻¹. Similarly, at [22] and [23] reported that coconut water contained urea, diphenyl urea, auxin (indole acetic acid), tryptophan, amino acid as IAA precursor and reduced nitrogen source, and zeatin and dihydrozeatin, which were needed by PLB of *E. graminea* orchid for cell division, for chlorophyll formation, and for

further development to form secondary, tertiary and so on of PLBs.

Variation analysis results demonstrated that interaction between type and concentration of complex organic matters significantly affected PLBs development to become plantlet. as shown in plantlet height, leaf number, root number, and root length, at 12 WAP. The type and concentration of complex organic matter supplementations interaction were further tested by using polynomial orthogonal test. This produced interaction response curves of the above growth variables (Fig. 3).

PLBs development of *E. graminea* responded to coconut water concentration from 50 ml L⁻¹ to 200 ml L⁻¹ in form of quadratic curve. Response of plantlet height to coconut water concentration was quadratic with regression equation of $yB_3 = -2.4x^2 + 12.48x - 9.7$, $R^2 = 0.8155$, with optimal height (7.68 cm obtained at 102.89 ml L⁻¹ coconut water (Fig. 3A). Response of plantlet leaf number also demonstrated quadratic response of $yB_3 = -x^2 + 4.48x + 0.6$, $R^2 = 0.9766$, with the greatest leaf number (5.98) obtained at coconut water concentration of 91.63 ml L⁻¹ (Fig. 3B). In addition, root number was also quadratic, $yB_3 = -5.1x^2 + 23.34x - 9.3$, $R^2 = 0.8305$, with the greatest root number of 18.41 root per jar obtained at coconut water concentration of 98.23 ml L⁻¹ (Fig. 3C). Finally, root length response was also quadratic. $yB_3 = -0.985x^2 + 4.667x - 2.035$, $R^2 = 0.831$, with the longest root (3.89 cm) was achieved at concentration of 98.44 ml L⁻¹ (Fig. 3D).

In general, our experiment demonstrated that coconut water was an organic matter which revealed better and dominant responses on all PLBs development are becoming plantlets variables of *E. graminea*, including plantlet height, leaf number, root number and root length than those of banana or potato. The optimal concentration range of coconut water for optimal plantlet growth was 91.63 – 102.89 ml L⁻¹, or about 100 ml L⁻¹ of coconut water.

Addition of coconut water on NPK medium for PLB development demonstrated positive effects indicating its potential for use in mass micropropagation of *E. graminea*. The positive effect of coconut water might be related to its content of organic compounds. such as vitamins, minerals, amino acids, nucleic acids, phosphorus and plant hormones auxin and gibberellic acids. These compounds have been demonstrated to promote tissue proliferation, facilitate metabolism and respiration in *Dendrobium anosmum* [22].

Coconut water addition also enriches nutrition of the media as every 100 g of coconut water contain 2.61 g sucrose, zeatin, dihydrozeatin, urea and diphenyl urea and vitamins (vitamin B, nicotinic acid, and biotin) which needed very importantly by orchid PLB to stimulate its growth [23].

In addition, coconut water also Nitrogen contain (in forms of 29 amino acids and their derivate), 20 mg Phosphorus, 31 mg Calcium, 25 mg Magnesium (Mg), 0.142 mg Mangan (Mn), and 0.29 mg Ferrum (Fe) [24]. Nitrogen is a constituent of chlorophyll [25], whereas Magnesium and Mangan are part of the chlorophyll [26]. According to [27] addition of coconut water into culture media induce cell division in *Phalaenopsis*.

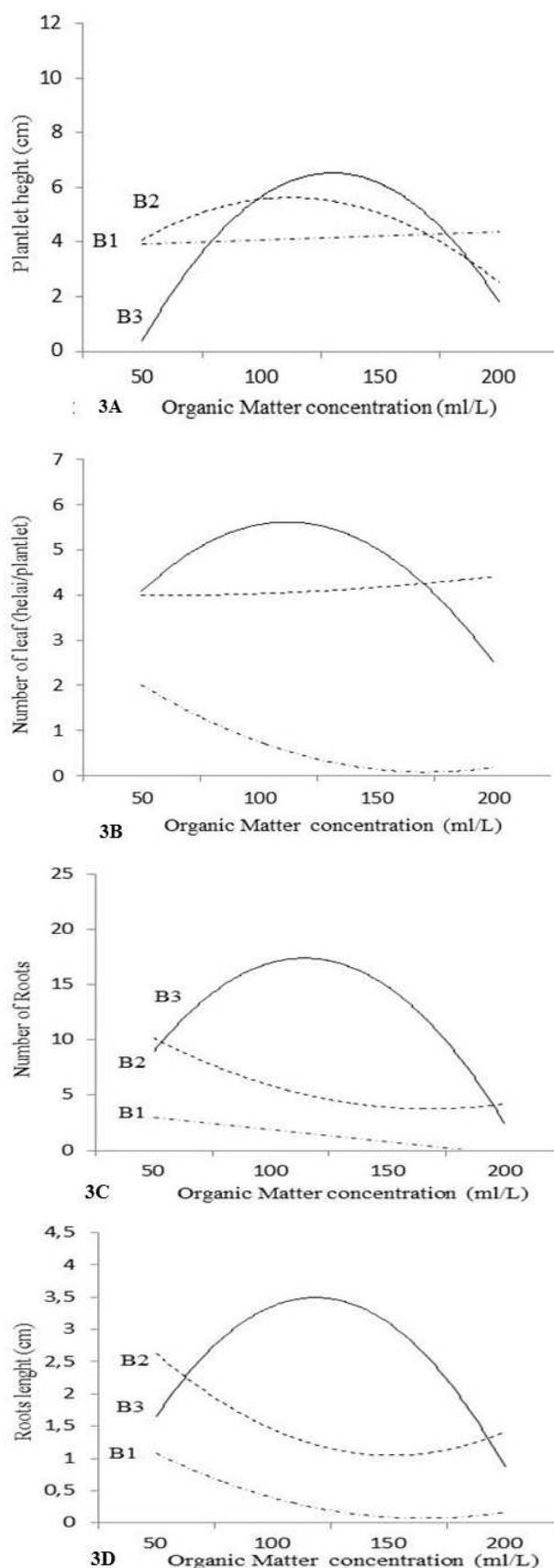


Fig. 3 The interaction effect between type and concentration of complex organic matter on the development of PLB to plantlet: plantlet height (2A), leaf number per plantlet (2B), root number per plantlet (2C), and average root length (2D) of *Eulophia graminea* at 12 WAP. B1 = banana. B2 = potato. B3 = coconut water.

Coconut water contains plant hormones which might play an important role in regulating PLB growth. Auxin in coconut water might function as a regulator in several phases of plant development, including embryogenesis, organogenesis and tissue formation. Cytokinin from coconut water might promote cell division that results in faster cell growth [28] and [22]. Young coconut water contains various organic substances that are crucial for growth and development of PLBs and plantlets of orchid. These substances includes 150.6 nM indole-3-acetic acid, 0.26 nM N6-isopentenyladenine, 0.14 nM dihydrozeatin, 0.09 nM trans-zeatin, 0.31 nM kinetin, 3.29 nM ortho-topolin, 46.6 nM dihydrozeatin O-glucoside, 48.7 nM trans-zeatin O-glucoside, 76.2 nM trans-zeatin riboside, 0.33 nM kinetin riboside, 10.2 nM trans-zeatin riboside-5'-monophosphate, 1.16 nM gibberellin, 37.8 nM gibberellin 3, and 65.5 nM Abscisic acid [23].

Addition of coconut water into the culture medium for micropropagation of several orchids, especially those using PLB as explant has been reported by [29], that the highest PLB number of hybrid *Phalaenopsis* was obtained in modified ½ MS medium supplemented with peptone and coconut water. The treatments with organic growth supplements resulted in better and early plantlets of *Cymbidium pendulum* (Roxb.) Sw, as compared to control without organic additions [30].

Based on the above results and other references, it is interesting that supplementation of coconut water into multiplication medium of orchid has many potential advantages. Coconut water can promote the development of PLBs to form plantlet, with significantly more efficient and cheaper than those of synthetic plant growth regulator. Coconut water is easily available at any place and anytime, especially in tropical regions. It is easy to prepare without using complicated calculation; hence, it can be used by ordinary people who want to establish an orchid nursery at household scale.

Other organic substance supplementation might be more suitable for other species of orchids as some reports have been published. For example, Vanda orchid produced a maximum number of roots (3.80 roots per shoot) when cultured on VW medium supplemented with 'Namwa' banana [13]. Mokara orchid showed maximum fresh weight (0.27 g) and root number (5.20 roots/shoot), both being greater than those cultured on other organic supplementation [13]. In another report, plantlet height and leaf number of *Phalaenopsis amabilis* were the greatest when cultured in VW with the addition of potato extract at 150 g L⁻¹ [18], and *Phalaenopsis amabilis* were the greatest when cultured in VW with the addition of coconut water at 150 ml L⁻¹ concentration [19].

IV. CONCLUSIONS

Orchid PLB of *E. graminea* can be plated on NPK medium with similar success in growth and development, in term of PLB number, PLB diameter, leaf number in plantlet, plantlet height, root number and root length, to those planted on MS medium. The price of using a complete fertilizer medium NPK is much lower than those of using MS medium, Knudson C medium, or Vacin and Went medium.

Among complex organic growth supplements, including young coconut water, potato extract and banana juice, coconut water at a concentration of 57.17 – 98.67 ml L⁻¹ resulted in the best multiplication of PLBs, in term of the greatest number, PLB diameter, and fresh weight. Coconut water promotes the greatest conversion of PLBs into plantlets of *E. graminea* at an optimum concentration of 91.63 – 102.89 ml L⁻¹.

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