THE PROPAGATION OF ORCHID THROUGH FORMATION OF PROTOCORM LIKE BODIES (PLB) FOR SUPPORTING THE RESQUE OF Phalaenopsis ORCHID IN INDONESIA

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Abstract

Indonesia is a tropical country that has large orchid germ plasm because it has tropical forests along the equatorial regions (islands of Sumatra, Kalimantan, Sulawesi and Papua) Recently, there has been changed become oil palm plantations and climate change, which has caused the extinction of orchid. The *Phalaenopsis* orchid is an orchid that has high exotic and commercial value. The purpose of this study was to maintain the *Phalaenopsis* through propagation of Protocorm Like Bodies (PLB). This study used the treatment of different kinds of basal medium Murashige & Skoog (MS), ½ Murashige & Skoog (1/2 MS) and Vacin& Went (VW) and 6-benzylaminopurine (BAP) and α-naphthylacetic acid (NAA) concentrations on growth and propagation of PLB. The results showed sequentially VW, ½ MS and MS media provided the best plb growth and media ½ MS provides regeneration plb into a good planlet in which shoots and roots formed. Treatment of plant growth regulator of BAP: NAA effect on diameter of plb colony and individual size of plb with 2.1 cm and 0.2 cm, respectively.

Keywords: Phalaenopsis sp, Protocorm Like Bodies (PLB), Benzyl Amino Purin (BAP) and Naphthyl Acetic Acid (NAA)

BACKGROUND

Indonesia is a tropical region with large germplasm of orchids because tropical forests lying along the line of the equator (the islands of Sumatra , Kalimantan , Sulawesi and Papua). Recently, there has been change by the presence of oil palm plantation and climate change, which has caused the extinction of orchids. There were kind of *Phalaenopsis* sp that preserved, and tissue culture technique was used to preserved it by propagation of protocorm like body. Medium was an important role in plant growth of tissue culture. The suitable medium for plant growth was influenced by composition and concentration of salt.

There were many mediums could used to grow of the orchid for example Vacin & Went (VW), a half concentration of Murashige & Skoog (½ MS) and Murashige & Skoog (MS) itself. The composition and concentration salt of it mediums were different, but have the same form of nitrogen that were ammonium and nitrate (George E.F.1987). Nitrogen as a nitrate and ammonium fix growth of plant in tissue culture (Gunawan, 1988).

Add of plant growth regulator into medium help the growth of culture to be speed up. Ratio concentration of cytokinine and auxine affected the direction of growth culture, where cytokinine tend to grow of shoots and leaves while auxine tend to grow of roots and callus. The added of 2 mgL^{-1} 6-benzylaminopurine and 0.5 mgL^{-1} α-naphthylacetic acid were the most suitable in multiplication of shoots *Phalaenopsis* orchid (Košir, *et al*. 2004). Other authors expressed different but have equal inclination in the direction growth of plants. This study aim to know of propagation and regeneration of plb phalaenopsis in medium of VW, ½ MS and MS with the difference ratio of concentration 6-benzylaminopurine and α-naphthylacetic acid.

METHODS

Preparation of medium

The medium used salt accordance to composition basal of VW and MS, ½ MS medium used half of MS concentration. Each medium was added coconut water 15 % concentrations as organic compound. Plant growth regulator of 6-benzylaminopurine (BAP) and α-naphthylacetic acid (NAA) were given in some ratio concentrations into each medium which it ratio were (1) BAP 1 mgL^{-1} : NAA 0.5 mgL^{-1} (2) BAP 0.75 mgL^{-1} : NAA 0.75 mgL^{-1} (3) BAP 0.5 mgL^{-1} : NAA 1 mgL^{-1}. The mediums with treatment of BAP and NAA were used to induced of plb from explan (medium of induction). And, the other mediums were made from salt accordance to composition of VW, MS and ½ MS without plant growth regulator, this mediums were used to induce shoot and root from plb as the regeneration of plb to planlet (medium of regeneration).

Planting of explant .

Plant material used plb that was been planted for 4-5 months, which has round shaped with the colony of plb. Plb colony was separated use scalpel order to obtain plb single. Plb single then disposed at the bottom and hilt and cut into pieces cuboid with 2-3 mm side. Pieces of plb cuboid were used as a explant, and then put into induction medium. Tissue culture of plb incubated in dark condition with temperature about 24-25^{o}C until the plb respond to grow callus, and then transferred to light condition. Before incubated, the plb was photographed to record the beginning condition. Photography of plb takes every week to acknowledge the changing of it. Every photography uses the same size an enlargement and plb position. Photography used a stereo microscope Leica EZ4 HD with enlargement 8x. Two months after planting obtained growth colonies of plb with green colour and globular shape, then plb was subculture to the regeneration medium to induce shoots and the root from plb. The end of growth in induction medium, number of plb formed was calculated and diameter of plb was measured. Really, measurement of diameter plb was measure of width of plb by microscope, because it will has difficult technique if measure diameter by calipers with sterile condition.

Plb which have grown and developing to green colour and globular shape then do subculture to the regeneration medium, in the regeneration medium plb grown and developed produce shoots and roots. Plb that growth in the induction medium grouped in accordance each treatment of concentration BAP : NAA and characterized for observation shoots and roots formed. Shoots and roots formed were calculated in 2 months later.

The variables of observation were 1. The number of plb/eksplan, 2. The number of shoot/eksplan, 3. The number of planlet/eksplan, 4.Growth plb (diameter colonies and individual). Analysis statistic of data used Randomize Complete Design with 2 factors and 3 replications.

RESULT AND DISCUTION

Protocorm like body formed.

The propagation of protocorm like body starting to become apparent on the 7th day, started growing a thin callus greenish yellow colour on the edge eksplan and thicken in the 16th day (Figure.1a, b). On day 23 , the edge eksplan overgrown with callus almost equally and eksplan respond to form callus in the midle part of characterized formed bubbles convex as will be plb (Figure 1c). Then, in 30th day bubble convex clearer in almost all the part of eksplan surface as plb young , and the 46thday in the center point bubble convex growing candidates shoots like the tip of leaves and in the edge of every bubble convex formed clear

the borderline, this stage phase of plb, on the 59 day plb to grow larger (Figure 1d, e,f). Then plb done subculture to the media regeneration .

Plb formed in each eksplan and propagation of plb most was 35.3 plb per eksplan in VW medium with treatment of BAP 0.75 mgL^{-1}: NAA 0.75 mgL^{-1} and the lowest 24 plb per eksplan in MS medium with treatment of BAP 0.75 mgL^{-1}: NAA 0.75 mgL^{-1} and MS medium with treatment of BAP 0.5 mgL^{-1}: NAA 1 mgL^{-1} (Table 1). The used of plant growth regulator cytokinin and auxine single or combination could produced callus candidates of plb (Zhao *et al*. 2008). Performed of each plb different typical, in the medium treatment of BAP 0.5 mgL^{-1}: NAA 1 mgL^{-1} plb shaped relatively long and large with a slight amount, treatment of BAP 0.75 mgL^{-1}: NAA 0.75 mgL^{-1} plb shaped shorter and small with many, and treatment of BAP 1 mgL^{-1}: NAA 0.5 mgL^{-1} plb shaped short quite large numbers (Figure 2). The form of plb relatively large and long influenced by auksin, where constituent cells a lot due to lengthening cells. Lengthening cells caused by salt dissolved absorb into the cell (Commoner and Mazia, 1942). Diameter of plb colony and individual largest were 2.1 cm and 0.3 cm plb individual respectively. It was possibility caused by auxine dominant than cytokinine.

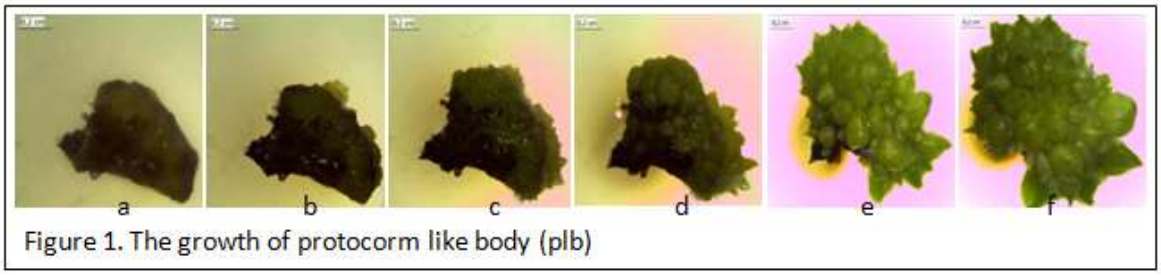

Figure 1. The growth of protocorm like body (plb)

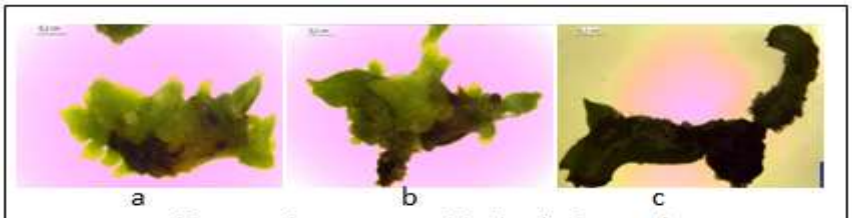

Figure 2. Different of protocorm like body formed.
Notes: a. BAP 1 mg L^{-1} : NAA 0.5 mg L^{-1}; b. BAP 0.75 mg L^{-1} : NAA 0.75 mg L^{-1}; c. BAP 0.5 mg L^{-1} : NAA 1 mg L^{-1}

Table 1. Plb formed & diameter in induction medium and shoot & planlet formed in regeneration medium

Induction medium	Plb formed (per explan)	Diameter of plb colony (cm)	Diameter of plb individual (cm)	Regeneration medium	Shoot (per explan)	Planlet (per explan)
½ MS B1:N1	26	1.0	0.2	½ MS	2	1
½ MS B2:N2	28	1.4	0.2	½ MS	1.7	0
½ MS B2:N2	29	1.3	0.22	½ MS	1.3	1.6
MS B1:N1	30.3	1.5	0.26	MS	2.7	0
MS B2:N2	24	1.4	0.2	MS	2.7	0
MS B3:N3	24	2.1	0.3	MS	0.7	0
VW B1:N1	31	1.5	0.17	VW	3	1
VW B2:N2	35,7	1.3	0.15	VW	2	0
VW B3:N3	33.3	1.5	0.2	VW	1.3	0

Notes: B1= BAP 1 mg L^{-1}; B2= BAP 0.75 mgL^{-1}; B3= 0.5 mg L^{-1}; N1= 0.5 mg L^{-1}; N2= 0.75 mg L^{-1}; N3= 1 mg L^{-1}

The shoot and planlet formed

The shoot was induced from plb that transferred to the regeneration medium, each plb could produced shoot but different in total number. Difference of shoot formation was affected by composition of induction medium before, where the BAP as cytokinin and NAA as auxine applied with different concentration. Cytokinin and auxine in medium influenced the concentration of endogen hormone, and the growth of plb depends on endogen compound. Formation of shoot most consisting of 3 shoots per eksplan in VW medium, with treatment of BAP 1 mg L^{-1}: NAA 0.5 mg L^{-1}in induction medium before, while shoot at least 0.7 shoots per eksplan in MS medium with treatment of BAP 0.5 mg L^{-1}: NAA 1 mg L^{-1} (table 1) in induction medium. The same used in concentration of BAP 1 mg L^{-1} produced higher in shoot multiplication of *Brassocattleya* (Cardoso & Ono, 2011), shoots of pineapple (Ibrahim *et al*. 2013). Cytokinin stimulate to grow of shoots, and combination of cytokinin higher than auksin had a role synergy in developing shoots. The combination treatment of BAP 0 – 6 mg L^{-1}: IAA 0.2 mg L^{-1} produced most of shoot in banana culture (Sipen & Davey, 2012). Performed of shoot varying in medium, in ½ MS medium shoot was dark-green colour with the number of numerous leaves, in VW medium shoot was green-pale colour with the number of leaves a little, and in MS medium shoot was dark-green colour, thicker and number of leaves a little. The different performed of shoots possibility of influenced by salt composition medium. An half concentration of MS and MS itself have content ammonium and nitrate higher than VW medium, so the leaf buds dark-green colour as an indication sufficiency nitrogen and leaf buds in the MS medium thicker and a slight amount as an indication nitrogen abundant.

A B C

Figure 3: Shoot and planlet formed from different basal medium a. ½ MS medium. b. VW medium. c. MS medium.

Each of eksplan does not induced planlet, except explan that plant in ½ MS and VW mediums, most 1.6 planlet per eksplan in the ½ MS medium with treatment of BAP 0.5 mg L^{-1}: NAA 1 mg L^{-1} (table 1) in induction medium. Similarly Zhao *et al* (2008) expressed that the use of NAA concentration 0 - 2.7 µM induce shoot and concentration 5.4 µM or higher induce root at regeneration orchids used the ½ MS medium. Also, Danso *et al* (2008) expressed that applied combination of NAA and IBA could increase the number of root induction from pineapple shoot. Eksplan in the MS medium only produce buds leaves, but not producing the root (planlet) (figure 3). Growth of planlet in the ½ MS medium relatively better from the VW medium, where planlet has more leaves with dark-green colour while planlet in the VW medium has few leaves with pale-green colour. Planlet formation was influenced by plant growth regulators, and the possibility of composition of salt in medium affected root formation. MS medium has complete composition and high concentration of salt did not produced roots so there was no planlet formed. Cardoso & Ono (2011) expressed the

percentage growth of roots most almost 40 % grow up with reduce the nitrogen until a quarter concentration left in MS medium with treatment of BAP 0.5 - 1 mg L^{-1}.

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