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# Industrial Propagation of *Phalaenopsis* sp. by Bioreactor Technique

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**Abstract:** Protocorm like bodies (PLB) were used as planting materials. Somatic embryo callus were initiated on medium supplemented with NAA (1mg/l) or 2.4D (1mg/l). Somatic cell suspension were cultured for initiation and for proliferation. on medium MS supplemented with NAA (1mg/l) and NAA (0.5mg/l). The volume of somatic cell suspension for bioreactor cultivation was 20%. Somatic embryo suspension were cultured in bioreactor for initiation and proliferation on the medium MS supplemented with NAA (0.5mg/l). Embryogenic suspension was stimulated on the medium MS supplemented with BA (0.5mg/l) + NAA (0.1mg/l). In vitro shoots of phalaenopsis were regeneration on the medium MS supplemented with BA (0.5mg/l) + NAA (0.1mg/l). Plantlets were enhanced growth and development in immersion-bioreactor cultivation by sinking/rising floated 1min/4hrs. Temperature, light intensity and stirring in stirring-bioreactor cultivation were favored at 26±2°C, 11,1-22,2µmol/$m^2$/s, and 30rpm. Micropropagation of *phalaenopsis* sp. by bioreactor technique was set up to produce 7,580 plantlets per one liter of somatic embryogenesis.

**Keyworks:** *Phalaenopsis* sp., somatic embryogenesis, micropropagation, protocorm like bodies, bioreactor technique**.**

## I. Introduction

Traditional breeding micro [1] on orchids leads to a problem that the common micro -breeding laboratories were that the tissue transplantation often grows slowly, costs a lot of labor costs, lot of time to produce seedlings in large quantities when marketed at a high price of seedlings. Asexual breeding system [2] solves the above barrier with the advantages: fast multiplying in the form of cells, asexual embryo is a different differentiation of regeneration coefficient, low labor costs and low cost [3]. In soma embryo technology, liquid culture was a basic technique made on a shake or Bioreactor [4,5] aims to increase biomass, induction that generates a homogeneous and conductor to the ability to regenerate soma with high performance [6], orchids [7]. Bioreactor technique has been researched and applied to the breeding microwave to reduce the cost of tissue transplanting products [3]. Cultivation materials in micro propagation with Bioreactor technology such as embryonic callus, cloned embryo cells, protocorms, shoots [7]. There are also many types of bioreactors used for micro-multiplication such as Bioreactor of cylindrical gas (Airlift Bubble Column-Bioreactor, Bioreactor Bubble Balloon-Balloon-Bioreactor, Bioreactor Footer Tank-Bioreactor) Contemporary Bioreactor [6]. Each type of Bioreactor has different features, depending on the physiological properties of farming plants, aiming to increase biomass quickly and enhance growth. Physical and chemical factors are factors that greatly affect cell proliferation and affect cell regeneration later [8]. Successfully taken through soma embryo culture on orchids [9] and micropropagation via bioreactor and temporary immersion system of date palm [11], orchid [12], lily [13], ruber [14], cocoa [15]. This paper studies the rapid multiplication of coffee plants using bioreactor technology.

This article studied propagation of orchids with Bioreactor technology on large scale.

## II. Materials and Methods

### Materials

Varieties: Phalaenopsis orchids imported from Singapore (Phalaenopsis sp).

Explants: Young leaf sheath, PLB

### Methods

The culture mineral nutrient medium is MS [10]. Added: BA (6-benzylaminopurine), TDZ (thidiazuron), IBA (β-indol butyric acid), NAA (α-naphthalene acetic acid), adenine, kinetin (6-furfurylaminopurine), B1 (10mg/l), peptone (1g/l), coconut water (10%), sucrose (30g/l), activated carbon (1g/l).

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Culture conditions: room temperature 26±2°C, RH = 65%, lighting time 10 hours/day, light intensity 11.1-33.3µmol/m2/s, shaker speed 90rpm, speed impeller bioreactor 30-60rpm.

Experimental design: randomized complete block design, 3 replicates, 3 conical flasks each time (containing 60ml of semi-solid medium or 50ml of liquid medium). The volume of callus cells put into culture 10g/100ml of liquid medium generates suspension. The volume of cell fluid was cultured 20% in liquid with shake and bioreactor 3-5 liters. The cell spread volume was 5ml/60ml of semi-solid medium. Data were analyzed using MSTATC software (t=0.05).

## III. Results and Discussion

### Cultivation and Regeneration of PLB

### Culturing PLB

The cultures were (i) thinly sliced apical corms (ii) thinly sliced PLB (iii) and young leaf sheaths (0.5cm2) used as inoculum on growth culture protocorm medium: MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l). The results showed that (Table 1): After 3 months of culture: PLB arose on all three types of cultured samples. The number of PLBs arising was different in the cultures in the order of thinly sliced apical corms (11.8 PLB/acre), young leaf sheaths (7.4 PLB/sample) and thin layer PLBs. (6.2 PLB/sample). However, the PLB arising from the apical corms is suitable for further studies and the PLB is a suitable culture for the study of somatic embryogenesis.

**TABLE 1**: Effect of explants on protocorm formation.

| Culture medium | Explants | Protocorm formation |
|---|---|---|
| MS + BA (1mg/l) + NAA (0.1mg/l) | Corm apical bud | 11.8a |
| | Thinly sliced PLB | 6.2c |
| | Young leaf sheath | 7.4b |
| CV(%) | | 8.2 |

### Fast Multiplication of PLB on Agar

The cultures were PLBs formed through thin-layer cell cultures, thinly sliced (1mm thick), and cultured on PLB phylogenetic media: MS + peptone (1g/l) + sucrose (20g/l) l) supplemented with BA (0.1-1-5mg/l) and NAA (0.1-0.5-1mg/l). Research results showed that (Table 2): After 2 months of culture: PLB appeared more on MS + peptone (1g/l) + sucrose (20g/l) + BA (1-5mg/l) culture medium. + NAA (0.1mg/l), generated 11.4 PLB/culture sample. The green PLB is used in micropropagation or somatic cell culture.

**TABLE 2**: Effect of BA and NAA on protocorm formation

| BA (mg/l) | NAA (mg/l) | Protocorm formation |
|---|---|---|
| 0.1 | 0.1 | 2.4d |
| | 0.5 | 1.4e |
| | 1.0 | 0.8e |
| 1.0 | 0.1 | 11.4a |
| | 0.5 | 4.6c |
| | 1.0 | 3.2d |
| 5.0 | 0.1 | 6.8b |
| | 0.5 | 2.8c |
| | 1.0 | 2.1d |
| CV(%) | | 16.4 |

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**Rapid Multiplication of PLB with Suspension**

The cultures were PLBs formed through thin-layer cell cultures, thinly sliced (1mm thick), and cultured on PLB phylogenetic media: MS + peptone (1g/l) + sucrose (20g/l) l) supplemented with BA (0.1-1-5mg/l) and NAA (0.1-0.5-1mg/l). The results showed that (Table 3): After 2 months of culture: PLB appeared more on the culture medium MS + peptone (1g/l) + sucrose (20g/l) + BA (1mg/l) + NAA (0.1mg/l), generated 13.1 PLB/culture. The green PLB is used in micropropagation or somatic cell culture.

**TABLE 3**: Effect of BA and NAA on protocorm formation

| BA (mg/l) | NAA (mg/l) | Protocorm formation |
|---|---|---|
| 0.1 | 0.1 | 4.2d |
| | 0.5 | 3.8e |
| | 1.0 | 2.2e |
| 1.0 | 0.1 | 13.1a |
| | 0.5 | 6.4c |
| | 1.0 | 5.6d |
| 5.0 | 0.1 | 8.7b |
| | 0.5 | 4.2c |
| | 1.0 | 4.0d |
| | CV(%) | 14.6 |

**Fast Multiplication of PLB on Bioreactor**

The cultures were PLBs formed through thin-layer cell cultures, thinly sliced (1mm thick), and cultured on PLB phylogenetic media: MS + peptone (1g/l) + sucrose (20g/l) l) supplemented with BA (0.1-1-5mg/l) and NAA (0.1-0.5-1mg/l). Research results showed that (Table 4): After 2 months of culture: PLB appeared more on MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l) culture medium. There were generated 13.1 PLB/culture.

**TABLE 3:** Effect of BA and NAA on protocorm formation

| BA (mg/l) | NAA (mg/l) | Protocorm formation |
|---|---|---|
| 0.1 | 0.1 | 4.2d |
| | 0.5 | 3.8e |
| | 1.0 | 2.2e |
| 1.0 | 0.1 | 13.1a |
| | 0.5 | 6.4c |
| | 1.0 | 5.6d |
| 5.0 | 0.1 | 8.7b |
| | 0.5 | 4.2c |
| | 1.0 | 4.0d |
| | CV(%) | 14.6 |

**PLB Regeneration**

PLBs were used as cultures on regenerating media: MS + peptone (1g/l) supplemented with NAA (0.1-0.5-1mg/l), BA (0.1-0, 3-0.5mg/l). The results showed that (Table 5): After 3 months of culture: PLB on regeneration medium MS + peptone (1g/l) + BA

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(0.1mg/l) + NAA (1mg/l) gave the ratio regenerated into highly complete shoots. The regeneration efficiency was 100%. The number of shoots generated per PLB is a single shoot.

**TABLE 4**: Effect of BA and NAA on protocorm formation

| BA (mg/l) | NAA (mg/l) | Regeneration rate (%) | Number of buds/PLB | Shoot height (mm) |
|---|---|---|---|---|
| 0.1 | 0.1 | 68c | 1 | 36b |
| | 0.5 | 85b | 1 | 32b |
| | 1.0 | 100a | 1 | 40a |
| 0.3 | 0.1 | 48d | 1 | 18d |
| | 0.5 | 56d | 1 | 24c |
| | 1.0 | 72c | 1 | 28c |
| 0.5 | 0.1 | 32e | 1 | 16d |
| | 0.5 | 47e | 1 | 26c |
| | 1.0 | 65d | 1 | 32b |
| | CV(%) | 14.6 | | 18.2 |

**Cultivation of Starting Material**

**Embryonic Callus Culture**

The PLB embryonic form has a slow in vitro multiplication rate due to the increasing size of the PLB, limiting the micropropagation process. PLB was used as a raw material for embryogenic callus cells. PLB was sliced thinly, placed on culture medium for embryogenic callus cells: MS + BA (0.1mg/l) supplemented with NAA (0.1-0.5-1-3mg/l), 2.4D (0.1-0.5-1-2mg/l). Research results show that (Table 6): After 45 days of culture. The culture medium MS + BA (0.1mg/l) + NAA (1mg/l) or 2.4D (1mg/l) both stimulated embryogenic callus formation (Table 2). In the culture process, there was light, the embryonic callus was blue; on the contrary, leave in diffused light ivory white. Embryonic callus cells, ivory white, suitable for biomass multiplication in a shake suspension with diffused light. Embryonic callus cells with green color were cultured for increased biomass on contemporary bioreactor with light.

**TABLE 5**: Effect of 2,4D and NAA on callus formation

| MS + BA (0.1 mg/l) culture medium supplemented with | | The rate of friable callus induction (%) | Area of callus (mm) |
|---|---|---|---|
| 2.4D (mg/l) | NAA (mg/l) | | |
| 0.1 | | 38 | 2.4 |
| 0.5 | | 52 | 3.8 |
| 1.0 | | 98 | 7.4 |
| 2.0 | | 82 | 6.4 |
| 3.0 | | 76 | 5.2 |
| | 0.1 | 36 | 2.2 |
| | 0.5 | 54 | 3.6 |
| | 1.0 | 96 | 6.2 |
| | 2.0 | 78 | 5.8 |
| | 3.0 | 62 | 4.6 |
| | CV% | 12 | 14.8 |

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**Selective Culture of Rapidly Growing Embryogenic Callus Cell Lines**

Callus obtained from the above experiment was cultured with selected cell lines on agar. The criterion was to select a cluster of fast-growing callus. The line selection cycle was 45 days, and then repeats. The sampling rate was 20% for each selection. Each cluster of callus was selected, inoculated on agar for subsequent selection with clone numbering. The number of line selections in 2 years is 16 cycles. The mass of cultured callus clusters was 100 mg/cluster. Selected medium MS + BA (0.1mg/l) + NAA (1mg/l) + CW (10%). Culture samples were placed under diffused light of 22.2 μmol/$m^2$/s. The results showed that (Table 7): The volume of proliferating cells increased with each cycle of selection. At cycle 6, the weight was 214 mg/cluster with a proliferation factor of 2.14. The highest was 371 mg/cluster and the proliferation coefficient was 3.71 in cycle 14 and the following cycles did not increase much. Callus cells at cycle 14 were used as raw materials for bioreactor studies.

**TABLE 6**: Callus growth through culture cycles

| | **Cycle 1** | **Cycle 2** | **Cycle 3** | **Cycle 4** |
|---|---|---|---|---|
| **Year 1 (2007)** | 127 | 142 | 156 | 166 |
| **(mg/ cluster)** | **Cycle 5** | **Cycle 6** | **Cycle 7** | **Cycle 8** |
| | 188 | 214 | 223 | 242 |
| | **Cycle 9** | **Cycle 10** | **Cycle 11** | **Cycle 12** |
| **Year 2 (2008)** | 257 | 284 | 306 | 322 |
| **(mg/ cluster)** | **Cycle 13** | **Cycle 14** | **Cycle 15** | **Cycle 16** |
| | 348 | 371 | 387 | 408 |

**Cultivation and Proliferation of Cell Suspensions in Bioreactor**

**Cultivation of Embryonic Callus Suspension in Bioreactor**

Post-selective callus was used as the raw material. A shaker was used for the suspension, with a shaking speed of 100 rpm. The volume of cells put into culture was 10g/100ml of the environment. The culture generates medium in Bioreactor: MS + BA (0.1 mg/l) admenment of MS + BA (0.1-0.5-1-3 mg/l). Research results show that (Table 8): After 30 days of culture, the appropriate culture medium is MS + BA (0.1mg/l) + NaA (0.5mg/l). Slow proliferating cells for the first 1-2 weeks, and rapid proliferation in the week 3-4, less clusters, forming cell suspension. The suspension was ivory white, cells have uniform shape and size. There was a part of the tissue cells that differentiate into soma embryo cells. There was a coefficient of biomass increase in the period of 2.8 times.

**TABLE 8**: Effect of culture medium on the generation of embryonic callus cell suspension in bioreactor.

| **Culture medium** | **NAA (mg/l)** | **Coefficient of proliferation** |
|---|---|---|
| MS + BA (0.1mg/l) | 0.1 | 2.6 |
| | 0.5 | 2.8 |
| | 1 | 2.4 |
| | 3 | 2.0 |
| CV% | | 11.8 |

**Proliferative Culture of Embryonic Callus Suspension in Bioreactor**

The suspension from the above experiment was used as the stock solution and put into culture in 3-liter Bioreactor, 1 liter culture volume, with 10% stock solution, 30rpm stirring speed. The culture medium in Bioreactor is MS + BA (0.1 mg/l) has additional NAA supplementation (0.1-0.5-1-3 mg/l). Research results show that (Table 9): After 20 days of culture, the appropriate culture environment is MS + BA (0.1mg/l) + NAA (0.5mg/l). The first week, and giving proliferation on 2-3 weeks, less clusters. There

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was evened suspension and milk color. There was a part of the tissue cells that differentiate into soma embryo cells. There was a coefficient of biomass increasing 15.9 times.

**TABLE 9**: Effect of culture medium on proliferation of embryonic callus cell suspension in bioreactor

| Culture medium | NAA (mg/l) | Coefficient of proliferation |
|---|---|---|
| MS + BA (0.1mg/l) | 0.1 | 14.1 |
| | 0.5 | 15.9 |
| | 1 | 13.2 |
| | 3 | 8.8 |
| CV% | | 14.2 |

**Effect of Physical Conditions on Cell Suspension Culture in Bioreactor**

Effect of Light (11.1-33.3 µmol/$m^2$/s) on Proliferation of Embryonic Cells and PLB in Stirrer Bioreactor

The culture sample was an embryonic callus suspension: The initial culture biomass was 20% in the bioreactor. The incubation temperature was 26±2ºC and stirring speed 30rpm. Somatic cell suspension biomass growth medium: MS + BA (0.1mg/l) + NAA (0.5mg/l). Research results show that (Table 10): After 30 days of culture. The efficiency of embryogenic callus proliferation was 16.8 times at the light intensity of 11.1 µmol/$m^2$/s compared to 8.3 times at 33.3 µmol/$m^2$/s. The suspension has a beautiful ivory-white color, and the cells were uniform in shape and size. Part of the somatic cell suspension differentiates into somatic embryos. The appropriate light intensity for proliferation of embryonic callus was 11.1 µmol/$m^2$/s.

Culture specimen was pseudo embryonic PLB that were culture temperature 26+2ºC and stirring speed 30rpm. PLB was cut into thin slices and cultured on PLB nuclear bioreactor liquid medium: MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l). Research results show (Table 10): After 60 days of culture. The rate of PLB embryo formation was high (80-90%), with 8.9-10.2 PLB embryos/culture at both light intensities. The results of PLB generation were not different at two light intensities.

**TABLE 10**: Effect of light on cell suspension proliferation and PLB

| Medium | Culture sample | 11.1 µmol/$m^2$/s | 33.3 µmol/$m^2$/s |
|---|---|---|---|
| MS + BA (0.1mg/l) + NAA (0.5mg/l) | Cell suspension (after 30 days of culture) | 16.8 | 8.3 |
| MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l) | Cell suspension (after 60 days of culture) | 8.9 | 10.2 |

**Effect of Temperature (26-30±2ºc) on Proliferation of Embryonic Cells and PLB in Stirrer Bioreactor**

The culture sample was an embryonic callus suspension: The initial culture biomass was 20% in the bioreactor. Illumination intensity was 11.1 µmol/m2/s and stirring speed 30rpm. Somatic cell suspension biomass growth medium: MS + BA (0.1mg/l) + NAA (0.5mg/l). Research results show that (Table 11): After 30 days of culture. The efficiency of embryogenic callus cell proliferation was 16.2 times at 26±2ºC compared to 7.4 times at 30+2ºC. The suspension has a beautiful ivory-white color, and the cells were uniform in shape and size. Part of the somatic cell suspension differentiates into somatic embryos. The appropriate culture temperature for proliferation of embryonic callus cells was 26+2ºC.

The culture sample was the pseudo embryonic PLB that were cut into thin slices and cultured on liquid medium bioreactor PLB: MS + peptone (1g/l) + BA (1mg/l) + NAA (0) ,1mg/l). Research results show that (Table 11): After 60 days of culture. The embryo formation rate was high (80-90%), with 10.2 PLB embryos/culture at 26±2°C compared to 5.8 PLB/cultivation at 30±2°C. The suitable temperature for PLB rapid multiplication was 26±2ºC.

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**TABLE 11**: Effect of temperature on cell suspension proliferation and PLB

| Medium | Culture sample | 26±2ºC | 30±2ºC |
|---|---|---|---|
| MS + BA (0.1mg/l) + NAA (0.5mg/l) | Cell suspension (after 30 days of culture) | 16.2 | 7.4 |
| MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l) | Cell suspension (after 60 days of culture) | 10.2 | 5.8 |

**Effect of Stirring Speed of Propeller (30-60rpm) on Proliferation of Embryonic Cells and PLB in Stirrer Bioreactor**

The culture sample was an embryonic callus suspension: The initial culture biomass was 20% in the bioreactor. Illumination intensity was 11.1 μmol/m2/s and incubation temperature were 26±2ºC. Somatic cell suspension biomass growth medium: MS + BA (0.1mg/l) + NAA (0.5mg/l). Research results show that (Table 12): After 30 days of culture. The proliferation efficiency of embryonic callus cells was 18.4 times at a stirring speed of 30rpm compared to 9.6 times at 60rpm. The suspension has a beautiful ivory-white color, and the cells are uniform in shape and size. Part of the somatic cell suspension differentiates into somatic embryos. The appropriate stirring speed for embryogenic callus cell proliferation culture is 30rpm.

Culture specimen was pseudo embryonic PLB: The light intensity was 11.1 μmol/$m^2$/s and incubation temperature were 26±2ºC. PLB was cut into thin slices (1mm thick) and cultured on PLB liquid medium bioreactor: MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l). Research results show that (Table 12): After 60 days of culture. The embryo formation rate was high (80-90%), with 9.2 PLB embryos/culture at 30rpm compared to 7.3 PLB embryos/culture at 60rpm.

**TABLE 12**: Effect of stirrer speed on cell suspension proliferation and PLB

| Medium | Culture sample | 30rpm | 60rpm |
|---|---|---|---|
| MS + BA (0.1mg/l) + NAA (0.5mg/l) | Cell suspension (after 30 days of culture) | 18.4 | 9.6 |
| MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l) | Cell suspension (after 60 days of culture) | 9.2 | 7.3 |

**Cell Suspension Regeneration Culture in Bioreactor**

**Induced Culture of Somatic Embryo Suspension in Bioreactor**

The somatic cell suspension was used as the culture material for the suspension. The initial culture biomass was 10% in the bioreactor. Somatic embryonic cell suspension culture: MS supplemented with NAA (0.1-0.5mg/l) and BA (0.1-0.5-1mg/l). Research results show that (Table 13): After 45 days of culture. MS + NAA (0.1mg/l) + BA (0.5mg/l) culture medium stimulates the induction of somatic embryogenesis. Embryo differentiation time increased gradually with culture time from 15-45 days after culture. The cells were pale yellowish white, larger in size.

**TABLE 13**: Effect of culture medium on somatic embryo suspension induction activation in bioreactor

| Culture medium | NAA (mg/l) | BA (mg/l) | Activation efficiency |
|---|---|---|---|
| MS | 0.1 | 0.1 | 42 |
| | | 0.5 | 72 |
| | | 1.0 | 58 |
| | 0.5 | 0.1 | 30 |
| | | 0.5 | 52 |
| | | 1.0 | 44 |
| CV% | | | 12 |

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**Spread Culture and Regeneration of Somatic Embryo Suspension in Bioreactor**

Somatic embryonic cell suspension was used as culture material. The initial culture biomass was 10%. The cell spread volume was 5ml/60ml of semi-solid medium. Somatic cell suspension spread culture medium: MS supplemented with NAA (0.1-0.5-1-3mg/l) and BA (0.1-0.5-1-3mg/l). Research results show that (Table 14): After 30 days of culture. The culture medium spread the suspension of embryonic callus cells MS + NAA (0.1 mg/l) + BA (1 mg/l) was suitable for homogenous differentiation of somatic embryos, forming a single layer of embryonic cells. soma, which is blue. The somatic embryos fully differentiated and regenerated after 45 days of culture. High regeneration efficiency 100% of samples put into culture. The number of arising shoots was 3-5 shoots/cluster. The buds per liter of embryo cultured in bioreactor was efficiency of 7,580 buds.

**TABLE 14**: Effect of culture medium on regeneration of somatic embryo suspension

| Culture medium | NAA (mg/l) | BA (mg/l) | Number of shoots/5ml of embryonic cell suspension |
|---|---|---|---|
| MS | 0.1 | 0.1 | 26.4 |
| | | 0.5 | 32.8 |
| | | 1.0 | 37.9 |
| | | 3.0 | 18.2 |
| | 0.5 | 0.1 | 16.8 |
| | | 0.5 | 22.4 |
| | | 1.0 | 24.6 |
| | | 3.0 | 12.2 |
| CV% | | | |

**Shoot Growth AND PLB in Semi-Submersible Bioreactor**

**Effect of Mineral Nutrient Medium in Protocorm Rapid Multiplication on Agar**

Research results (Table 15) show that the suitable mineral environment for phalaenopsis protocorm was Vacine-Went (V.0226), and cymbidium was Lindemann (L.0216). In culture medium, phalaenopsis protocorm increased the number of protocorms (6.8 protocorms/cluster) and the number of regenerated shoots (2.6 shoots/cluster). In contrast, jade had an increased number of protocorms (2.2 protocorms/cluster) and the number of regenerative shoots (1.8 shoots/cluster).

**TABLE 15**: Effect of mineral nutrient medium on rapid protocorm multiplication on agar

| Base medium | Code | Phalaenopsis | | Cymbidium | |
|---|---|---|---|---|---|
| | | PLB | Shoots | PLB | Shoots |
| MS | M.0244 | 5.6b | 2.2b | 1.8b | 2.2a |
| Vacine-Went | V.0226 | 6.8a | 2.6a | 2.2a | 1.8b |
| Orchimax | O.0257 | 4.8b | 2.6a | 2.0a | 2.1b |
| Knudson-C | K.0215 | 3.2c | 1.8b | 1.8b | 2.0b |
| Lindemann | L.0216 | 6.2a | 2.6a | 2.4a | 2.4a |
| Test f (0.05) | | * | * | * | * |

**Effect of Culture Rhythm on Protocorm Proliferation and Phalaenopsis Shoot Development.**

The results showed that (Table 16), a 4-hour floating rhythm and 1-minute immersion were suitable for protocorm culture and shoot regeneration. Compared with the control, the ability to generate protocorm on agar medium was better than semi-submersible culture (5.8 protocorm compared with 5.6 sub-submersible culture) and vice versa, the ability to regenerate shoots cultured in semi-

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submersible better than those grown on agar (2.8 shoots regenerated versus 2.2 on agar). Single shoots that thrived in semi-submerged culture reached 38mm leaf length compared to 32mm grown on agar.

**TABLE 16**: Effect of culture rhythm on protocorm proliferation and Phalaenopsis shoot development

| Rhythm | | Protocorm | | Single bud |
|---|---|---|---|---|
| **Floating (hour)** | **Sinking (minutes)** | **PLB** | **Number of buds** | **Leaf length (mm)** |
| 1 | 1 | 4.0b | 1.6b | 20c |
| 1 | 2 | 3.8c | 1.0c | 18c |
| 2 | 1 | 4.6b | 1.8b | 24b |
| 2 | 2 | 4.2b | 1.4b | 20b |
| 3 | 1 | 5.2a | 2.4a | 30a |
| 3 | 2 | 4.8b | 2.0b | 26b |
| 4 | 1 | 5.6a | 2.8a | 38a |
| 4 | 2 | 5.2a | 2.4a | 32a |
| 5 | 1 | 5.4a | 2.6a | 32a |
| 5 | 2 | 4.6b | 2.2a | 28b |
| 6 | 1 | 4.2b | 1.8b | 26b |
| 6 | 2 | 2.8d | 1.4b | 22b |
| Control (agar) | | 5.8a | 2.2a | 32a |
| Test f (0.05) | | * | * | * |

**Rapid multiplication of phalaenopsis orchids by bioreactor technology**

As a result of the research process, a process of rapid industrial multiplication of phalaenopsis orchids by bioreactor technology has been established.

| Step | Culture target | Time (days) | Culture conditions |
|---|---|---|---|
| 1 | Select culture samples | 00 | Sheath young leaves, PLB, hybrid seed |
| 2 | Generation of embryogenic callus on medium agar | 45 | MS + 2.4D (1mg/l) / NAA (1mg/l) |
| 3 | Creation of embryonic callus suspension in liquid medium | 30 | MS + NAA (1mg/l) |
| 4 | Proliferation of embryonic callus suspension in liquid medium | 20 | MS + NAA (0.5mg/l |
| 5 | Proliferation of suspension in bioreactor | 20 | MS + NAA (0.5mg/l) |
| 6 | Embryogenesis induction in bioreactor | 45 | MS + BA (0.5mg/l) + NAA (0.1mg/l) |
| 7 | Embryo regeneration on medium agar | 45 | MS + NAA (0.1mg/l) + BA (1mg/l) |

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|---|---|---|---|
| 8 | Single PLB regeneration in semi-submersible bioreactor | 60 | MS + BA (0.3mg/l) + NAA (0.1mg/l) |
| 9 | Breeding industry | | 7,580 Phalaenopsis buds/liter of embryo cultured in bioreactor |

## VI. Conclusion

PLBs were cultured arising from apical corms, thinly sliced or sheathed PLBs on MS + peptone (1g/l) + BA (1mg/l) + NAA (0.1mg/l) cultures. PLBs were thinly sliced and rapidly multiplied on MS agar + peptone (1g/l) + sucrose (20g/l) + BA (1-5mg/l) + NAA (0.1mg/l) medium, on liquid medium MS + peptone (1g/l) + sucrose (20g/l) + BA (1mg/l) + NAA (0.1mg/l), on bioreactor MS + peptone (1g/l) + sucrose (20g/l) medium ) + BA (1-5mg/l) + NAA (0.1mg/l) for PLB generation/culture was 11.4-13.1-14.5. PLBs were regenerated on MS medium + peptone (1g/l) + BA (0.1mg/l) + NAA (1mg/l).

On culture medium MS + BA (0.1mg/l) + NAA (1mg/l) or 2.4D (1mg/l) both stimulated embryogenic callus formation After 45 days of culture. The culture process has light, the embryonic callus was green.

On the medium select the line MS + BA (0.1mg/l) + NAA (1mg/l) + CW (10%). The volume of proliferating cells increased with each cycle of selection. The highest was 371 mg/cluster and the proliferation coefficient was 3.71 in cycle 14 and the following cycles did not increase much.

On suitable culture medium is MS + BA (0.1mg/l) + NAA (0.5mg/l) cells proliferate rapidly at 3-4 weeks, less clustering, formed cell suspension after 30 days of culture. The suspension has a beautiful ivory-white color, and the cells are uniform in shape and size.

On suitable culture medium was MS + BA (0.1mg/l) + NAA (0.5mg/l) cells that proliferate slowly in the early stages, and proliferate rapidly at 2-3 weeks, with little clustering. There has a biomass growth factor of 15.9 times.

On MS + BA (0.1mg/l) + NAA (0.5mg/l) culture medium after 30 days of culture, cell suspensions proliferated rapidly under culture conditions with light intensity 11.1 µmol/$m^2$/s, temperature 26$\pm$2$^o$C and agitator speed 30rpm achieved cell suspension proliferation coefficient 16.8-16.2-18.4.

On MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l) cultures after 60 days of culture, thinly sliced PLBs proliferated under intense culture conditions with light of 11.1 µmol/$m^2$/s, temperature of 26$\pm$2$^o$C and agitator speed of 30rpm achieved PLB proliferation coefficient 8.9-10.2-9.2.

On culture medium MS + NAA (0.1mg/l) + BA (0.5mg/l) stimulated the induction of somatic embryogenesis after 45 days of culture.

The culture medium spread with the embryonic callus cell suspension MS + NAA (0.1 mg/l) + BA (1 mg/l) was suitable for homogenous somatic embryo differentiation, forming a single layer of somatic embryonic cells, which turns green after 30 days of culture. The somatic embryos fully differentiated and regenerated after 45 days of culture. The high regeneration efficiency 100% of samples put into culture. The buds per liter of embryo cultured in bioreactor was efficiency of 7,580 buds.

The suitable mineral medium for phalaenopsis protocorm nucleus was Vacine-Went (V.0226), The 4-hour float and 1-minute immersion rhythms are suitable for protocorm culture, shoot regeneration, and shoot growth. PPM at a concentration of 0.2% is suitable for liquid culture to limit the rate of infection resulting in shoot matter.

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