# Industrial Propagation of Cymbidium Sp. by Bioreactor Technique

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***Abstract:*** **Micropropagation of orchid plant for conservation and development is needing. Traditional propagation of Cymbidium sp. requires energy cost, many labor, large area for growing, slow growth and development, and high cost input. It is a need to find new effective ways for in vitro propagation, and plant cell technology via bioreactor techniques effort the demands. Protocorm like bodies were used as planting materials. Somatic embryo callus were initiated on medium MS + BA (0.1 mg/l) supplemented with NAA (1 mg/l) or 2,4D (1 mg/l). Somatic cell suspension were cultured for initiation and for proliferation. on medium MS + BA (0.1mg/l) supplemented with 2.4D (1 mg/l) and NAA (1 mg/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 + BA (0.1mg/l) supplemented with 2.4D (1 mg/l) or NAA (1 mg/l). Embryogenic suspension was stimulated on the medium MS + BA (0.5 mg/l) + NAA (0.1 mg/l). In vitro shoots of Cymbidium sp. were regeneration on the medium MS + BA (0.1 mg/l). Plantlets were enhanced growth and development in immersion-bioreactor cultivation by sinking/rising floated 1min/6hrs. Temperature, light intensity and stirring in stirring-bioreactor cultivation were favoured at 26±2oC, 11,1-22,2 µmol/m2/s, and 30 rpm. Micropropagation of Cymbidium sp. by bioreactor technique was set up to produce 5,600 platlets per one liter of somatic embryogenesis suspension.**

***Keyword:*** **bioreactor, Cymbidium sp., homogenous cell, industrial propagation, somatic embryogenesis, temporary immersion system (TIS) bioractor,**

## I. INTRODUCTION

Traditional micropropagation [1] on orchids currently leads to a problem that micropropagation laboratories often face, which is that tissue culture plants often grow slowly, are very labor-intensive, and costly. It takes a long time to produce seedlings in large quantities when marketed at a high cost of seedlings. The embryo cloning system solves the above barrier with the advantages: rapid multiplication in the form of cells, cloned embryo is a differentiated organism with high regeneration coefficient, low labor and low energy cost [2]. In somatic embryo technology, liquid culture is the basic technique performed on shakers or bioreactors [3,4] with the aim of increasing biomass, inducing homogenous somatic embryogenesis and leading on the ability to regenerate somatic embryos with high efficiency [5]. Culture materials in micropropagation by bioreactor technology such as embryogenic callus cells, clonal embryonic cells, protocorm, bud clusters [5]. And there are also many types of bioreactors used for micropropagation such as airlift bubble column-bioreactor, airlift bubble balloon-bioreactor, propeller tank-bioreactor, and semi-bioreactor. contemporary bioreactor [6]. Each type of bioreactor has different features, depending on the physiological properties of the cultured plants, aiming to increase biomass rapidly and enhance growth [4]. Physical and chemical factors are important factors affecting cell proliferation and cell regeneration [7]. There has been success in culturing somatic embryos on cymbidium [8]. This paper studies the rapid multiplication of Cybidium sp. orchids by bioreactor technology.

## II. MATERIALS AND METHODS

*Materials*

Varieties: Cymbidium sp. imported from Australia.

Culture specimen: young leaf sheath, PLB

*Methods*

The culture mineral nutrient medium is MS [9]. Added with: BA (6-benzylaminopurine), TDZ (thidiazuron), NAA (α-naphthalene acetic acid), 2.4D (2.4-dichlorophenoxy acetic acid), peptone (1 g/l), B1 (10 mg/l), coconut water CW (10%), sucrose (30 g/l), activated carbon AC (1 g/l).

Culture conditions: room temperature 26±2°C, RH = 65%, lighting time 10 hours/day, light intensity 33.3 µmol/$m^2$/s, speed of bioreactor 30-60 rpm

Experimental design: randomized complete block design, 3 replicates, 3 conical flasks each time (containing 60ml of semi-solid medium or 50 ml 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 shaking and bioreactor 3-5 liters. Plating volume of cells 5ml/60ml of semi-solid medium. Data were analyzed by MSTATC software (t=0.05).

## III. RESULTS

Table 1: Effect of auxin on cell culture of protocorm

| Auxin (mg/l) | The culture specimen is the corm meristem region | The culture sample is the young leaf sheath |
|---|---|---|
| IAA (0.5 mg/l) | 2.1c | 1.9c |
| IBA (0.5 mg/l) | 2.6b | 2.4b |
| NAA (0.5 mg/l) | 5.9a | 3.8a |
| CV(%) | 8.6 | 10.2 |

Table 2. Effect of BA and NAA on protocorm . formation

| BA (mg/l) | NAA (mg/l) | Formation of protocorms |
|---|---|---|
| 0.1 | 0.1 | 0.8d |
| | 0.5 | 2.6c |
| | 1.0 | 1.2d |
| 1.0 | 0.1 | 2.4c |
| | 0.5 | 6.8a |
| | 1.0 | 2.2c |
| 2.0 | 0.1 | 2.0c |
| | 0.5 | 3.2b |
| | 1.0 | 1.6d |
| CV(%) | | 16.4 |

Table 3. Effect of BA and NAA on protocorm formation

| BA (mg/l) | NAA (mg/l) | Formation of protocorms |
|---|---|---|
| 0.1 | 0.1 | 2.4d |
| | 0.5 | 4.1c |
| | 1.0 | 3.6d |
| 1.0 | 0.1 | 4.0c |
| | 0.5 | 8.1a |
| | 1.0 | 4.4c |
| 2.0 | 0.1 | 4.0c |
| | 0.5 | 5.6b |
| | 1.0 | 3.8d |
| CV(%) | | 12.4 |

Table 4: Effect of BA and NAA on protocorm formation

| BA (mg/l) | NAA (mg/l) | Formation of protocorm |
|---|---|---|
| 0.1 | 0.1 | 3.2d |
| | 0.5 | 5.5c |
| | 1.0 | 4.1d |
| 1.0 | 0.1 | 5.6c |
| | 0.5 | 9.2a |
| | 1.0 | 5.2c |
| 2.0 | 0.1 | 5.1c |
| | 0.5 | 6.7b |
| | 1.0 | 4.1d |
| CV(%) | | 10.2 |

Table 5. Effect of BA, NAA and rhizogen on shoot rooting

| BA (mg/l) | rhizogen (mg/l) | NAA (mg/l) | Rooting rate (%) | Number of roots | Root Length (mm) |
|---|---|---|---|---|---|
| 0.1 | 5 | | 95a | 4.2a | 42a |
| | 10 | | 76b | 3.8b | 38b |
| 0.5 | 5 | | 82b | 2.4c | 36b |
| | 10 | | 64c | 2.2c | 34b |
| 0.1 | | 0.1 | 26e | 1.8d | 32b |
| | | 0.5 | 42d | 2.4c | 22c |
| 0.5 | | 0.1 | 22e | 1.6d | 34b |
| | | 0.5 | 34d | 1.8d | 24c |
| CV(%) | | | 15 | 18.4 | 12 |

Table 6. Effect of 2,4D and NAA on callus formation

| Culture medium: MS + BA culture medium (0.1 mg/l) supplemented with | | Callus formation rate (%) | Callus diameter (mm) |
|---|---|---|---|
| 2.4D (mg/l) | NAA (mg/l) | | |
| 0.1 | | 2 | 0.9 |
| 0.5 | | 2 | 0.9 |
| 1.0 | | 28 | 2.6 |
| 2.0 | | 24 | 1.4 |
| 3.0 | | 6 | 1.0 |
| | 0.1 | 1 | 0.8 |
| | 0.5 | 1 | 0.8 |
| | 1.0 | 22 | 2.2 |
| | 2.0 | 16 | 1.8 |
| | 3.0 | 4 | 1.2 |
| CV% | | 14.2 | 9.0 |

Table 7. Selection of fast growing callus lines through culture cycles

| | | | | |
|---|---|---|---|---|
| Year 1 (2007) (mg/cluster) | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 |
| | 108 | 122 | 136 | 142 |
| | Cycle 5 | Cycle 6 | Cycle 7 | Cycle 8 |
| | 158 | 174 | 185 | 206 |
| Year 2 (2008) (mg/cluster) | Cycle 9 | Cycle 10 | Cycle 11 | Cycle 12 |
| | 224 | 240 | 262 | 288 |
| | Cycle 13 | Cycle 14 | Cycle 15 | Cycle 16 |
| | 322 | 352 | 382 | 394 |

Table 8. Effect of culture medium on callus suspension production in bioreactor

| Culture medium | NAA (mg/l) | Growth coefficient |
|---|---|---|
| MS + BA (0.1 mg/l) | 0.1 | 2.2 |
| | 0.5 | 2.6 |
| | 1.0 | 3.2 |
| | 2.0 | 3.4 |
| CV% | | 10.6 |

Table 9. Effect of culture medium on proliferation of callus suspension in bioreactor

| Culture medium | NAA (mg/l) | Growth coefficient |
|---|---|---|
| MS + BA (0.1 mg/l) | 0.1 | 8.3 |
| | 0.5 | 12.4 |
| | 1.0 | 16.2 |
| | 2.0 | 18.4 |
| CV% | | 12.4 |

Table 10. Effect of light on cell suspension proliferation and PLB

| Culture medium | Culture sample | 11.1 µmol/m$^2$/s | 33.3 µmol/m$^2$/s |
|---|---|---|---|
| MS + BA (0.1 mg/l) + NAA (1 mg/l) | Cell suspension (after 30 days of culture) | 7.4 | 8.6 |
| MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) | PLB (after 60 days of culture) | 14.8 | 16.2 |

Table 11. Effect of temperature on cell suspension proliferation and PLB

| Culture sample | Culture medium | 26±2ºC | 30±2ºC |
|---|---|---|---|
| MS + BA (0.1 mg/l) + NAA (1 mg/l) | Cell suspension (after 30 days of culture) | 7.2 | 2.0 |
| MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) | PLB (after 60 days of culture) | 14.9 | 6.3 |

Table 12. Effect of stirrer speed on cell suspension proliferation and PLB

| Culture medium | Sample medium | 30rpm | 60rpm |
|---|---|---|---|
| MS + BA (0.1 mg/l) + NAA (1 mg/l) | Cell suspension (after 30 days of culture) | 8.5 | 3.2 |
| MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) | PLB (after 60 days of culture) | 15.8 | 6.9 |

Table 13. Effect of culture medium on induction activation of embryo suspension in bioreactor

| **Culture medium** | **NAA (mg/l)** | **BA (mg/l)** | **Activation efficiency (%)** |
|---|---|---|---|
| MS + BA (0.1 mg/l) | 0.1 | 0.1 | 62 |
| | | 0.5 | 88 |
| | | 1.0 | 80 |
| | 0.5 | 0.1 | 48 |
| | | 0.5 | 66 |
| | | 1.0 | 54 |
| CV% | | | 12 |

Table 14. Effect of culture medium on regeneration of somatic embryo suspension in bioreactor

| Culture medium | NAA (mg/l) | BA (mg/l) | Number of shoots/5ml of embryo suspension |
|---|---|---|---|
| MS + BA (0.1 mg/l) | 0.1 | 0.1 | 12 |
| | | 0.5 | 8 |
| | | 1.0 | 28 |
| | | 2.0 | 29 |
| | 0.5 | 0.1 | 16 |
| | | 0.5 | 12 |
| | | 1.0 | 10 |
| | | 2.0 | 12 |
| CV% | | | 10 |

Table 15. Effect of culture rhythm on protocorm nucleus and shoot development Cymbidium

| Rhythm | | Protocorm | | Single shoot |
|---|---|---|---|---|
| Floating (hours) | Sinking (minutes) | PLB | Number of shoots | Leaf length (mm) |
| 1 | 1 | 3.2d | 1.8b | 32b |
| 1 | 2 | 2.8e | 1.2c | 28c |
| 2 | 1 | 4.8c | 2.2b | 36b |
| 2 | 2 | 4.2c | 1.8c | 32b |
| 3 | 1 | 5.2b | 2.8a | 40a |
| 3 | 2 | 4.6c | 2.6a | 36b |
| 4 | 1 | 6.8a | 3.2a | 48a |
| 4 | 2 | 6.2a | 2.8a | 42a |
| 5 | 1 | 5.6b | 2.6a | 42a |
| 5 | 2 | 5.0b | 2.2b | 38b |
| 6 | 1 | 4.4c | 2.2b | 38b |
| 6 | 2 | 3.8d | 2.0b | 34b |
| Control (agar) | | 7.2a | 2.6a | 42a |
| Test f (0.05) | | * | * | * |

## IV. DISCUSSION

*Cultivation and Regeneration of PLB in Bioreactor*

*Cultivation to Produce PLB*

The culture sample was the growing apical corms: 45-day-old in vitro cymbidium shoots were used as culture material. Thinly sliced root-side corm was used as culture material. PLB culture medium: MS + BA (1 mg/l) + peptone (1 g/l) with separate addition of IAA, IBA, NAA with concentration of 0.5 mg/l. Research results show that (Table 1): After 45 days of culture: PLB bar formation was much on the culture media. Particularly, the medium supplemented with NAA (0.5 mg/l) had the formation of PLB bars with a frequency of 4-6 PLBs/culture. PLB was light green and dark green, indeterminate. The size of PLBs arising on the apical corms was larger than those arising on the white leaf sheath. Culture samples from the apical corms giving rise to PLB were suitable for research on micropropagation or somatic embryos.

The culture sample was young leaf sheath: The culture sample was the young leaf sheath (0.5 cm$^2$) used as the culture material. Young leaf sheaths were cultured on MS + peptone (1 g/l) + BA (1 mg/l) medium supplemented with IAA, IBA, and NAA separately at the concentration of 0.5 mg/l. Research results show that (Table 1): After 45 days of culture: PLB formation was not much on the culture media.

Particularly, the medium supplemented with NAA (0.5 mg/l) had the formation of PLB bars with a frequency of 3.8 PLB/culture. PLB was light green and dark green, indeterminate. PLB sizes arise on small white leaf sheaths. Culture samples from the apical corms giving rise to PLB are suitable for research on micropropagation or somatic embryos.

*Fast Multiplication of PLB on Agar*

PLBs obtained through thin-layer cell culture were used as culture material. PLBs were cut into thin layers (1 mm thick) and cultured on MS + peptone (1 g/l) medium supplemented with BA (0.1-1-2 mg/l) + NAA (0.1-0.5- 1 mg/l). The results of incubation showed that (Table 2): After 45 days, on culture medium MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) produced PLB 6.8/pattern of cultured slices.

*Fast Multiplication of PLB on Liquid Medium*

PLBs obtained through thin-layer cell culture were used as culture material. PLBs were cut into thin layers (1mm thick) and cultured on MS + peptone (1 g/l) medium supplemented with BA (0.1-1-2 mg/l) + NAA (0.1-0.5- 1 mg/l). The results of incubation showed that (Table 3): After 45 days, on culture medium MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) produced PLB 8.1 PLB/culture thin slice.

*Fast multiplication of PLB on bioreactor*

PLBs obtained through thin-layer cell culture were used as culture material. PLBs were cut into thin layers (1 mm thick) and cultured on MS + peptone (1 g/l) medium supplemented with BA (0.1-1-2 mg/l) + NAA (0.1-0.5-1 mg/l). The culture results showed that (Table 4): After 45 days, on the culture medium MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) produced the amount of 9.2 PLBs/culture thin slice.

*PLB Regeneration*

PLB was used as a regenerative culture material on the following media: MS + peptone (1 g/l) supplemented with rhizogen (5-10 mg/l), NAA (0.1-0.5-1 mg/l) and BA (0.1-0.5 mg/l). Research results showed that (Table 5): After 45 days of culture, the highest regeneration ability was 100% on MS + peptone (1 g/l) + BA (0.1 mg/l) + rhizogen culture medium (5 mg/l). Cymbidium shoots are vigorous in vitro and have developed roots. When combining NAA + BA, the rate of shoot regeneration was low, reaching 40-50% and PLB continued to arise. When BA alone was used, shoots were rod-shaped but did not form roots.

*Cultivation of Starting Material*

*Embryonic Callus Culture*

PLB has a slow in vitro multiplication rate. PLB has a large size, limiting the micropropagation process. PLB was used as a raw material for embryogenic callus. PLBs were thinly sliced and placed on embryogenic callus culture. Culture medium for embryogenic callus: MS + BA (0.1 mg/l) + peptone (1 g/l) supplemented with NAA (0.1-0.5-1-2-3 mg/l), 2,4D (0.1-0.5-1-2-3 mg/l). Research results show (Table 6): After 45 days of culture. The culture medium for embryogenic callus MS + BA (0.1 mg/l) + NAA (1 mg/l) or 2.4D (1 mg/l) both stimulate embryogenic callus and have a high rate of callus formation. low, the diameter of the callus generated was small. The culture process was left to the outside, the callus was green; On the contrary, leave it in the dark to be ivory white. Embryonic callus cells have an ivory white color suitable for mass multiplication in liquid shake with diffused light.

*Selective Culture of Fast-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 is 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 (0.1 mg/l) + NAA (1 mg/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 8 there was a weight of 206 mg/cluster with a proliferation coefficient of 2.06. The highest was 382 mg/cluster and the proliferation coefficient was 3.82 in cycle 15 and the following cycles did not increase much. The 15 cycle callus cells were used as raw materials for bioreactor studies.

*Cultivation and Proliferation of Cell Suspensions in Bioreactor*

*Culturing the Embryonic Callus Suspension in Bioreactor*

Selected callus was used as culture material. Callus cell suspension cultures were performed on a shaker with a shaking speed of 100 rpm. The mass of cells put into culture was 10g/100ml of medium. The culture medium in the bioreactor proliferates the MS + BA embryonic callus cell suspension (0.1 mg/l) supplemented with NAA (0.1-0.5-1-2 mg/l). Research results show that (Table 8): After 30 days of culture, the suitable culture medium is MS + BA (0.1 mg/l) + NAA (0.5 mg/l). Cells proliferate slowly in the first 1-2 weeks, and rapidly proliferate at 3-4 weeks, with little clustering. There is a part of embryonic callus cells that differentiate into somatic embryonic cells. There was a biomass growth factor of 3.2 times.

*Proliferative Culture of Embryonic Callus Suspension in Bioreactor*

The callus suspension from the above experiment was used as the mother fluid and cultured in a bioreactor of 2 liters, with a culture volume of 1 liter, with a parenting rate of 10%, and a stirring speed of 30 rpm. The culture medium in the bioreactor proliferates the MS + BA embryonic callus cell suspension (0.1 mg/l) supplemented with NAA (0.1-0.5-1-2 mg/l).

Research results show that (Table 9): After 20 days of culture, the appropriate culture medium is MS + BA (0.1 mg/l) + NAA (1 mg/l). Cells proliferate slowly in the first week, and rapidly proliferate at 2-3 weeks, with little clustering. There was a part of embryonic callus cells that differentiate into somatic embryonic cells. There was a biomass growth factor of 16.2 times.

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

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

The culture sample was the embryonic callus suspension: 20% culture volume in bioreactor. Culture temperature 26±2ºC. Stirring speed 30rpm. The culture medium proliferates the embryonic callus cell suspension MS + BA (0.1 mg/l) + NAA (1 mg/l). Research results show that (Table 10): After 30 days of culture: The efficiency of increasing embryonic cell mass is 7.4-8.6 times at both light intensities. The ability to increase embryonic cell biomass under two different light conditions

Culture sample is PLB pseudo-embryotype: Culture temperature is 26±2oC. Stirring speed 30rpm. PLBs were cut into thin slices (1mm thick) and cultured on MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) medium. Research results show that (Table 10): After 60 days of culture, PLB strongly formed 14.8-16.2 PLB/culture sample. The ability to increase PLB biomass in two different light conditions.

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

The culture sample was the embryonic callus suspension: 20% culture volume in bioreactor. Illumination intensity was 11.1 µmol/m2/s and Stirring speed 30rpm. The culture medium proliferates the embryonic callus cell suspension MS + BA (0.1 mg/l) + NAA (1 mg/l). The results showed that (Table 11). After 30 days of culture: the efficiency of embryogenic cell proliferation was 7.2 times at the temperature of 26±2oC compared to 2 times at the temperature of 30±2ºC. The suitable temperature for culturing the embryonic cell suspension was 26±2ºC.

Culture sample was PLB pseudo-embryotype: Light intensity 11.1 µmol/m$^2$/s and stirring speed 30 rpm. PLBs were cut into slices (1mm thick) and cultured on MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) medium. Research results show that (Table 11), after 60 days of culture, PLB strongly formed 10-20 PLBs/culture sample at 26±2ºC compared to 6.3 PLBs/culture sample at 30±2ºC. PLB was suitable for rapid multiplication at the temperature of 26±2ºC.

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

The culture sample was the embryonic callus suspension: 20% culture volume in bioreactor. Culture temperature was 26±2ºC. The culture medium proliferates the embryonic callus suspension MS + BA (0.1 mg/l) + NAA (1 mg/l). Research results show that (Table 12) after 30 days of culture: the efficiency of embryogenic cell proliferation was 8.5 times at a stirring speed of 30 rpm compared to 3.2 times at 60 rpm. The stirring speed of the impeller 30 rpm was suitable for proliferation of embryonic cells.

Culture sample was PLB pseudo-embryotype: PLB wass cut into small pieces and cultured on MS + peptone (1 g/l) + BA (1 mg/l) + NAA (0.5 mg/l) medium. Research results show that (Table 12) after 60 days of culture, PLB strongly formed 15.8 PLBs/cultivation at a stirring speed of 30 rpm compared with 6.9 PLBs/cultivation at 60 rpm. The 30 rpm impeller stirring speed suitable for PLB proliferation.

*Cell Suspension Regeneration in Bioreactor*

*Embryogenesis-induced Culture in Bioreactor*

Embryonic callus cell suspension was used as culture material. The 10% culture volume was used in bioreactor. The embryo induction culture medium MS + BA (0.1 mg/l) supplemented with NAA (0.1-0.5 mg/l) and BA (0.1-0.5-1 mg/l). Research results show that (Table 13) after 45 days of culture. The culture medium supplemented with NAA (0.1 mg/l) + BA (0.5 mg/l) stimulated the induction of somatic embryogenesis. Embryo differentiation time increased gradually with culture time from 15-45 days after culture. Cells are pale yellow-white, larger in size.

*Plating and Regenerate the Somatic Embryonic Cell Suspension on Agar*

Embryonic cell suspension was used as culture material. Culture medium spread with MS + BA somatic embryo suspension (0.1 mg/l) supplemented with NAA (0.1-0.5 mg/l) and BA (0.1-0.5-1-2 mg/l). Research results show that (Table 14) after 30 days of culture. Somatic cell spreading culture medium supplemented with NAA (0.1 mg/l) + BA (1 mg/l) was suitable for homogenous differentiation of somatic embryos, forming a layer of somatic embryonic cells with blue color. PLB regenerated into complete plants after 60 days of culture with high regenerative capacity. Shoots grow fast, uniform and strong. There was achieved yield of 5,600 orchid buds per liter of embryo cultured in bioreactor.

*Shoot Growth and PLB in Temporary Immersion System (TIS) Bioreactor*

*Effect of Culture Rhythm on Protocorm Nucleus and Shoot Growth of Cymbidium*

The results showed that (Table 15), 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 in semi-submersible culture (7.2 protocorm compared with 6.8 in semi-submersible culture) and vice versa, the ability to regenerate shoots cultured in semi-submersible better than those grown on agar (3.2 shoots regenerated versus 2.6 grown on agar). Single shoots growing strongly in semi-submerged

culture reached 48 mm leaf length compared to 42 mm cultured on agar.

*Rapid multiplication of the orchid industry by bioreactor technology*

As a result of the research, a process of rapid multiplication of the Catleya sp. orchid industry by bioreactor technology has been built.

| Step | Culture Objective | Time (days) | Conditions |
|---|---|---|---|
| 1 | Selection of culture samples | 00 | Young leaf sheath, PLB |
| 2 | Generation of embryogenic callus on agar medium | 45 | MS + BA (0.1 mg/l) + NAA (1 mg/l) or 2.4D (1 mg/l) |
| 3 | Create a suspension of callus embryos in liquid medium | 30 | MS + BA (0.1 mg/l) + NAA (1 mg/l) or 2.4D (1 mg/l) |
| 4 | Proliferation of embryonic callus suspension in liquid medium | 20 | MS + BA (0.1 mg/l) + NAA (1 mg/l) or 2.4D (1 mg/l) |
| 5 | Proliferation of suspension in bioreactor | 20 | MS + BA (0.1 mg/l) + NAA (1 mg/l) or 2.4D (1 mg/l) |
| 6 | Embryogenesis induction in bioreactor | 45 | MS + BA (0.5 mg/l) + NAA (0.1 mg/l) |
| 7 | Embryo regeneration on agar medium | 20 | MS + BA (0.1 mg/l) |
| 8 | PLB and single shoot regeneration in semi-submersible bioreactor | 60 | PLB: MS + BA (0.5 mg/l) Shoot: MS + BA (1 mg/l) + NAA (0.5 mg/l). |
| | Breeding Industry | | 5,600 orchid buds/liter of embryo suspension cultured in bioreactor |

## V. CONCLUSION

Cultivation and regeneration of PLB: PLBs were induced from the apical corm and young leaf sheath. PLBs were thinly sliced and cultured grow well in liquid medium and bioreactor. PLBs were developed and regenerated on MS medium + peptone (1 g/l) + BA (0.1 mg/l) + rhizogen (5 mg/l).

Cultivation of starting material: On the embryogenic callus culture medium supplemented with NAA (1 mg/l) or 2.4D (1 mg/l) both materials stimulated embryogenic callus formation after 45 days of culture. Embryonic callus cells have an ivory white color suitable for mass multiplication in liquid shake with diffused light.

On the medium select the line MS + BA (0.1 mg/l) + NAA (1 mg/l) + CW (10%). The volume of proliferating cells increased with each cycle of selection. The highest was 382 mg/cluster and the proliferation coefficient was 3.82 in cycle 15. The cycle 15 callus cells were used as raw materials for bioreactor studies

Cultivation and proliferation of cell suspensions in bioreactor: the appropriate suspension was MS + BA (0.1 mg/l) + NAA (1 mg/l). Cells proliferate rapidly at 3-4 weeks, less clumps, forming cell suspensions after 30 days of culture. There was a part of embryonic callus that differentiate into somatic embryonic cells and growth coefficient of biomass in the generation stage 16.2 times.

Effect of physical conditions on cell suspension in bioreactor: under culture conditions with light intensity of 11.1 µmol/$m^2$/s, temperature of 26±2°C and agitator speed of 30 rpm. Cell suspensions proliferated rapidly on MS + BA (0.1 mg/l) + NAA (1 mg/l) culture medium achieved cell suspension proliferation coefficient 7.4-7.2-8.5. Thinly sliced PLBs proliferated on MS + Adenine (10 mg/l) + IBA (0.1 mg/l) + BA (1 mg/l) cultures, achieve PLB proliferation coefficient 14.8-14.9-15.8.

Cell suspension regeneration in bioreactor: cell were stimulated the induction of somatic embryogenesis on the culture medium MS + BA (0.1 mg/l) + NAA (0.1 mg/l) + BA (0.5 mg/l) after 45 days of culture. Cells were pale yellow-white, larger in size.

Callus plating culture medium MS + BA (0.1 mg/l) + NAA (0.1 mg/l) + BA (1 mg/l) suitable for homogenous differentiation of somatic embryos, forming a layer of somatic embryonic cells, which were blue after 30 days of culture. PLB regenerated into complete plants after 60 days of culture with high regenerative capacity. Shoots grow fast, uniform and strong. There was achieved yield of 5,600 orchid buds per liter of embryo cultured in bioreactor.

The 4-hour float and 1-minute immersion rhythms are suitable for protocorm culture, shoot regeneration, and shoot growth. The PPM at a concentration of 2o/oo was suitable for liquid culture to limit the infection rate leading to shoot matter

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