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

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**Abstract:** *Backgroud:* Dendrobium sp. are production by multishoot system and it is required high of labor, energy, cost, large area. *Objectives:* there are needs of a new production system by using of plant cell culture techniques. *Methods:* manipulation of bioreactor techniques was effective ways to resolve the chalenges. Protocorm like bodies were used as planting materials. Somatic embryo callus was initiated on medium MS supplemented with 2.4D (0,3mg/l) + CW (30%). Somatic cell suspension was cultured for initiation and for proliferation and on medium MS + 2.4D (0,3mg/l) + CW (30%) and MS + NAA (0,5mg/l) + 2.4D (0.1mg/l). The volume of somatic cell suspension for bioreactor cultivation was 20%. The volume for plating was 5ml/60ml semi-solid medium. Somatic embryo suspension was cultured in bioreactor for initiation and proliferation on the medium MS + NAA (0,5mg/l) + 2.4D (1mg/l). Embryogenic suspension was stimulated on the medium MS supplemented with BA (0.2mg/l) + NAA (0.2mg/l). In vitro shoots of dendrobium were regeneration on the medium MS supplemented with BA (0.2mg/l) + NAA (0.2mg/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. *Results:* Micropropagation of Dendrobium sp. by bioreactor technique was established to produce 6,200 plantlets per one liter of somatic embryogenesis suspension.

**Keywords:** Bioreactor, *Dendrobium* sp., Embryonic Callus, Protocorm Like Bodies, Somatic Embryogenesis Suspension

## 1. 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 advantages: rapid multiplication in the form of cells, cloned embryo is a differentiated organism with high regeneration coefficient, low labor cost and low cost. lower city [2]. In somatic embryo technology, liquid culture is the basic technique performed on shakers or bioreactors [3] with the aim of increasing biomass, inducing homogenous somatic embryogenesis and leading to reproducibility. somatic embryos have high yield [4]. Bioreactor techniques have been studied and applied to micropropagation in order to reduce the cost of tissue culture products [2]. 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 for micropropagation such as airlift bubble column-bioreactor, airlift bubble ballon-bioreactor, propeller tank-bioreactor, and semi-bioreactor contemporary bioreactor [2]. Each type of bioreactor has different features, depending on the physiological properties of the cultured plants, aiming to increase biomass rapidly and enhance growth [6]. Physical and chemical factors are important factors affecting cell proliferation and cell regeneration [6]. There have been many successes through somatic embryo culture on orchids [7], dendrobium [8], and micropropagation via bioreactor and temporary immersion system of date palm [10], orchid [11], lily [12], ruber [13], cocoa [14], Haworthia truncata [15]. This paper studies the rapid multiplication of Dendrobium sp. orchids by bioreactor technology.

# 2. Material and Methods

### *2.1. Material*

Varieties: orchids like Dendrobium iryasakult cv. Sonia imported from Singapore.

Culture samples: young leaf sheath, PLB.

### *2.2. Methods*

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The culture mineral nutrient medium was MS [9].

Added: BA (6-benzylaminopurine), TDZ (thidiazuron), NAA (α-naphthalene acetic acid), 2.4D (2,4-dichlorophenoxy acetic acid), adenine (10mg/l), peptone (1g/l), B1 (10mg/l), CW coconut water (10%), sucrose (30g/l).

Culture conditions: room temperature 26+2°C, RH = 65%, lighting time 10 hours/day, light intensity 11.1-33.3µmol/$m^2$/s, bioreactor speed 30- 60rpm.

# 3. Results and Discussion

### *3.1. Cultivation and Regeneration of PLB*

#### *3.1.1. Culturing PLB*

The culture sample is the growing peak corm: The culture medium was MS + Adenine (10mg/l) supplemented with 2iP (1.5mg/l) + IBA (0.1mg/l). Research results show that (Table 1): After 30 days of culture: The shoots arising on the culture samples are the growing apical corms, the young shoots arise in clusters of buds, (6-8 buds/sample).

Culture specimen is young leaf sheath: The culture sample was young shoot after 14 days of age (with 20mm bud height). Culture medium was MS + Adenine (10mg/l) supplemented with BA (1.5mg/l) + IBA (0.1mg/l). Research results show that (Table 1): After 30 days of culture, young shoots appeared on the young leaf sheath with the number of buds arising from 3-5 buds/leaf sheath.

Comment: The culture samples are the growing apical corms or the young leaf sheaths are capable of direct shoot regeneration and a small part of PLB embryogenesis: with 4.2-4.6 PLB/ corm sample growth and 3.2-3.8 PLB/sample of young leaf sheath.

#### *3.1.2. Fast Multiplication of PLB on Agar Medium*

PLBs obtained through thin-layer cell culture were used as raw materials for protocorm clones. PLBs were sliced thinly (1mm thick) and cultured on cloning medium MS + Adenine (10mg/l) + IBA (0.1mg/l) supplemented with BA (0.1-0.5-1-2mg/l). The results showed that (Table 2): after 30 days, PLB continued to arise and most of the PLBs converted into small shoot clusters, the number of shoots regenerated from protocorm was 10.8 shoots/cluster on MS + Adenine medium. (10mg/l) + BA (1mg/l) + IBA (0.1mg/l).

#### *3.1.3. Fast Multiplication of PLB on Liquid Medium*

PLBs obtained through thin-layer cell culture were used as raw materials for protocorm clones. PLBs were sliced thinly (1mm thick) and cultured on cloning medium MS + Adenine (10mg/l) + IBA (0.1mg/l) supplemented with BA (0.1-0.5-1-2mg). /l). Research results showed that (Table 3): after 30 days, PLB continued to arise (4.6 PLB/sample) and transformed into small shoot clusters, the number of shoots regenerated from protocorm decreased completely by 5.3 shoots/cluster on MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l) medium.

***Table 1.*** *Effect of samples on thin layer cell culture.*

| Culture medium | Culture sample | Formation of protocorm/sample | Formation of bud cluster (number of shoots/cluster) |
|---|---|---|---|
| MS + 2iP (1.5mg/l) + IBA (0.1mg/l) | Area of growth apical corm | 4.6 | 7.8a |
| | Young leaf sheath | 3.2 | 4.8c |
| MS + BA (1.5mg/l) + IBA (0.1mg/l) | Area of growth apical corm | 4.2 | 5.2b |
| | Young leaf sheath | 3.8 | 3.6d |
| CV (%) | | 9.4 | 10.8 |

***Table 2.*** *Effects of BA and IBA on shoot multiplication and protocorm.*

| BA (mg/l) + IBA (0.1mg/l) | Formation of protocorm | Formation of bud cluster (number of shoots/cluster) |
|---|---|---|
| 0.1 | 0.6b | 2.6c |
| 0.5 | 1.2b | 4.8b |
| 1.0 | 2.8a | 10.8a |
| 2.0 | 3.2a | 11.2a |
| CV (%) | 18.2 | 12.4 |

#### *3.1.4. Fast Multiplication of PLB on Bioreactor*

PLBs obtained through thin-layer cell culture were used as raw materials for protocorm clones. PLBs were sliced thinly (1mm thick) and cultured on cloning medium MS + Adenine (10mg/l) + IBA (0.1mg/l) supplemented with BA (0.1-0.5-1-2mg/l). The results showed that (Table 4): after 30 days, PLB continued to arise (5.4 PLB/sample) and transformed into small shoot clusters, the number of shoots regenerated from protocorm decreased by 4.2 buds/cluster. on MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l) medium.

***Table 3.*** *Effects of BA and IBA on shoot multiplication and protocorm.*

| BA (mg/l) + IBA (0.1mg/l) | Formation of protocorm | Formation of bud cluster (number of shoots/cluster) |
|---|---|---|
| 0.1 | 2.2b | 1.4c |
| 0.5 | 3.4b | 2.1b |
| 1.0 | 4.6a | 5.3a |
| 2.0 | 5.2a | 4.8a |
| CV (%) | 12.2 | 10.4 |

***Table 4.*** *Effects of BA and IBA on shoot multiplication and protocorm.*

| BA (mg/l) + IBA (0.1mg/l) | Formation of protocorm | Formation of bud cluster (number of shoots/cluster) |
|---|---|---|
| 0.1 | 3.6b | 0.2c |
| 0.5 | 4.2b | 1.4b |
| 1.0 | 5.4a | 4.2a |
| 2.0 | 6.1a | 3.5a |
| CV (%) | 10.8 | 11.4 |

***Table 5.*** *Effect of IBA on root formation (after 30 days).*

| IBA (mg/l) | Number of roots | Root Length (mm) |
|---|---|---|
| 0.0 | 1.8 | 25 |
| 0.1 | 2.2 | 38 |
| 0.5 | 3.0 | 42 |
| 1.0 | 4.2 | 56 |
| CV (%) | 12.4 | 18 |

#### *3.1.5. PLB Regeneration*

Regeneration: PLB through propagation was used as culture material. Root growth medium: MS + BA (0.1mg/l) supplemented with IBA (0-0.1-0.5-1mg/l) (Table 5). Research results show that (Table 5): In vitro shoots grow and thrive on culture medium. Roots emerged after 14 days of culture. After 30 days of culture: the number of roots generated 4-8 roots/bud, the length of the roots reached 30-50mm, the height of the shoots was 40-50mm. Dendrobium orchids were introduced to grow in natural conditions before being domesticated. After 30 days of domestication, the shoots had strong stems and leaves, dark green leaves, the height of the shoots reached 110mm, the roots developed strongly and the root diameter reached 0.3-0.8mm. The transplanted plants were then introduced to the nursery.

### *3.2. Cultivation of Starting Material*

#### *3.2.1 Embryonic Callus Culture*

The culture samples were PLBs that were cut into thin layer and cultured on MS + CW embryogenic callus growth medium (30%) supplemented with 2.4D (0-0.3-0.5mg/l). The results showed that (Table 6): After 4 weeks of culture, callus formed on the slice. Slices with callus were cultured in liquid medium, after 8 weeks of culture, the PLB slices were filtered and removed, and callus cell suspension was obtained. The suspension was cultured for scar tissue regeneration on MS + 2.4D medium (0.3mg/l).

***Table 6.*** *Effect of culture medium on callus formation.*

| Culture medium | | Rate of callus generation (%) | Creation a callus cell suspension (mg/50ml) |
|---|---|---|---|
| On agar (4 weeks) | In liquid (4 weeks) | | |
| 2.4D (0.3mg/l) | 2.4D (0.3mg/l) | 50 | 1.286 |
| 2.4D (0.5mg/l) | 2.4D (0.5mg/l) | 70 | 1.864 |
| 2.4D (0.0mg/l) | 2.4D (0.3mg/l) | 100 | 1.483 |
| CV% | | 12 | 10.8 |

#### *3.2.2. Proliferative Culture of Embryonic Callus Suspension in Bioreactor*

The callus suspension from the above experiment was used as the mother solution 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 embryonic callus cell suspension supplemented with NAA (0.1-0.5-1mg/l) and 2.4D (0.1-0.5-1-2mg/l). Research results show that (Table 7): After 20 days of culture, the appropriate culture medium is MS supplemented with NAA (0.5mg/l) + 2.4D (0.1mg/l). Cells proliferate slowly in the first week, and rapidly proliferate at 2-3 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 9.9 times.

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

| | Cycle1 | Cycle 2 | Cycle 3 | Cycle 4 |
|---|---|---|---|---|
| Year 1 (2007) | 114 | 122 | 134 | 146 |
| (mg/cluster) | Cycle 5 | Cycle 6 | Cycle 7 | Cycle 8 |
| | 174 | 206 | 241 | 282 |
| | Cycle 9 | Cycle 10 | Cycle 11 | Cycle 12 |
| Year 2 (2008) | 315 | 340 | 374 | 406 |
| (mg/cluster)) | Cycle 13 | Cycle 14 | Cycle 15 | Cycle 16 |
| | 424 | 457 | 462 | 479 |

### *3.3. Cultivation and Proliferation of Cell Suspensions in Bioreactor*

#### *3.3.1. Culturing 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 generated MS embryonic callus cell suspension supplemented with NAA (0.1-0.5-1mg/l) and 2.4D (0.1-0.5-1-2mg/l). Research results show that (Table 8): After 30 days of culture, the appropriate culture medium is MS supplemented with NAA (0.5mg/l) + 2.4D (0.1mg/l). Cells proliferate slowly in the first 1-2 weeks, and rapidly proliferate at 3-4 weeks, less clumps, forming cell suspensions. There was a part of embryonic callus cells that differentiate into somatic embryonic cells. There was a biomass growth factor of 6.2 times during the generation stage.

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

| Culture medium | NAA (mg/l) | 2,4D (mg/l) | Growth coefficient |
|---|---|---|---|
| MS | 0.1 | 0.1 | 4.8 |
| | | 0.5 | 6.4 |
| | | 1.0 | 8.0 |
| | | 2.0 | 8.4 |
| | 0.5 | 0.1 | 6.2 |
| | | 0.5 | 8.0 |
| | | 1.0 | 10.6 |
| | | 2.0 | 10.8 |
| | 1.0 | 0.1 | 6.6 |
| | | 0.5 | 8.2 |
| | | 1.0 | 10.4 |
| | | 2.0 | 9.2 |
| CV% | | | 11.6 |

### *3.3.2. Proliferative Culture of Embryonic Callus Suspension in Bioreactor*

The callus suspension from the above experiment was used as the mother solution 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 embryonic callus cell suspension supplemented with NAA (0.1-0.5-1mg/l) and 2.4D (0.1-0.5-1-2mg/l). Research results show that (Table 9): After 20 days of culture, the appropriate culture medium is MS supplemented with NAA (0.5mg/l) + 2.4D (0.1mg/l). Cells proliferate slowly in the first week, and rapidly proliferate at 2-3 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 9.9 times.

## *3.4. Effect of Physical Conditions on Cell Suspension Culture in Bioreactor*

### *3.4.1. 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 is the embryonic callus suspension: 20% culture volume in bioreactor. Culture temperature 26±2°C. Stirring speed 30rpm. MS + NAA (0.5mg/l) + 2.4D (0.1mg/l) culture medium. The results showed that (Table 10): After 30 days of culture: The efficiency of embryogenic cell proliferation was 7.8-8.2 times. The ability to increase embryonic cell biomass under two different light conditions.

Culture sample is PLB pseudo-embryotype: Culture temperature 26±2°C. Stirring speed 30rpm. PLB samples cut into thin slices were cultured on MS + Adenine (10 mg/l) + IBA (0.1 mg/l) + BA (1 mg/l) medium. Research results show that (Table 10): After 60 days of culture: PLB formed strongly, on average, 8.2-14.6 PLBs/culture piece. The ability to increase PLB biomass in high light was better.

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

| Culture medium | NAA (mg/l) | 2,4D (mg/l) | Growth coefficient |
|---|---|---|---|
| MS | 0.1 | 0.1 | 7.6 |
| | | 0.5 | 10.2 |
| | | 1.0 | 12.8 |
| | | 2.0 | 13.4 |
| | 0.5 | 0.1 | 9.9 |
| | | 0.5 | 12.8 |
| | | 1.0 | 16.2 |
| | | 2.0 | 17.2 |
| | 1.0 | 0.1 | 10.5 |
| | | 0.5 | 13.1 |
| | | 1.0 | 18.0 |
| | | 2.0 | 14.7 |
| CV% | | | 12.8 |

***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 + NAA (0.5mg/l) + 2,4D (0.1mg/l). | Cell suspension (after 30 days of culture) | 7.8 | 8.2 |
| MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l) | PLB (after 60 days of culture) | 8.2 | 14.6 |

### *3.4.2. Effect of Temperature (26-30±2°C) on Proliferation of Embryonic Cells and PLB in Stirrer Bioreactor Culture*

The culture sample is the embryonic callus suspension: 20% culture volume in bioreactor. Illumination intensity 11.1µmol/m$^2$/s. Stirring speed 30rpm. Culture medium MS + NAA (0.5mg/l) + 2,4D (0.1mg/l). The research results showed that (Table 11). After 30 days of culture: The efficiency of embryogenic cell proliferation was different at two temperatures: 7.8 times at 26±2°C compared to the increase in biomass 2, 6 times at 30±2°C. The temperature of 26±2°C was suitable for culturing and proliferating embryonic cells.

Culture sample was PLB pseudoembryonic: Light intensity 11.1µmol/m$^2$/s. Stirring speed 30rpm. PLB was cut into thin slices cultured on: MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l). Research results show that (Table 11): After 60 days of culture: PLB formed strongly, averaging 8.2 PLBs/culture piece at 26±2°C compared to 2.4 PLB/culture piece. At 30±2°C. PLB was suitable for rapid multiplication at the temperature of 26±2°C.

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

| Culture medium | Culture sample | 26±2°C | 30±2°C |
|---|---|---|---|
| MS + NAA (0.5mg/l) + 2,4D (0.1mg/l). | Cell suspension (after 30 days of culture) | 7.8 | 2.6 |
| MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l) | PLB (after 60 days of culture) | 8.2 | 2.4 |

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

The culture sample is the embryonic callus suspension: 20% culture volume in bioreactor. Illumination intensity 11.1µmol/$m^2$/s. Culture temperature 26±2°C. Culture medium MS + NAA (0.5mg/l) + 2,4D (0.1mg/l). Research results show that (Table 12): After 30 days of culture: embryonic cell mass is 8.2 times at stirring speed of 30rpm compared to the biomass growth rate of 2.2 times at 60rpm. The stirring speed of the impeller 30rpm is suitable for proliferation of embryonic cells.

Culture sample is PLB pseudoembryonic: Light intensity 11.1µmol/$m^2$/s. Culture temperature 26±2°C. The sample was cut into thin slices. Culture medium: MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l). The results showed that (Table 12): After 60 days of culture: PLB formed strongly, averaging 8.8 PLB/piece cultured at 30rpm compared to 2.3 PLB/piece cultured at 60rpm. The 30rpm impeller stirring speed suitable for PLB proliferation.

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

| Culture medium | Culture sample | 30rpm | 60rpm |
|---|---|---|---|
| MS + NAA (0.5mg/l) + 2,4D (0.1mg/l). | Cell suspension (after 30 days of culture) | 8.2 | 2.2 |
| MS + Adenine (10mg/l) + IBA (0.1mg/l) + BA (1mg/l) | PLB (after 60 days of culture) | 8.8 | 2.3 |

### *3.5. Regeneration of Cultured Cell Suspensions in Bioreactor*

#### *3.5.1. Induced Culture of Somatic Embryonic Cell Suspension in Bioreactor*

Embryonic callus cell suspension was used as the culture material in the bioreactor. 10% culture volume. Activated culture medium for MS embryogenesis was supplemented with NAA (0.1-0.2-0.5mg/l), TDZ (0.3-0.5-1mg/l), BA (0.1). -0.2-0.5mg/l). The results showed that (Table 13): After 45 days of culture: TDZ did not stimulate embryogenesis. The suitable medium for embryogenesis was the medium supplemented with the combination of NAA (0.2mg/l) + BA (0.2mg/l). Differentiated cells form embryos in culture. The proliferation rate decreased rapidly, whereas the embryo differentiation rate increased rapidly. The suspension completely differentiated the embryos after 45 days of culture.

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

| Culture medium | NAA (mg/l) | BA (mg/l) | Activation efficiency (%) |
|---|---|---|---|
| | 0.1 | 0.1 | 64 |
| | | 0.2 | 80 |
| | | 0.5 | 82 |
| | 0.2 | 0.1 | 74 |
| MS | | 0.2 | 86 |
| | | 0.5 | 88 |
| | 0.5 | 0.1 | 68 |
| | | 0.2 | 77 |
| | | 0.5 | 78 |
| CV% | | | 10 |

#### *3.5.2. Plating Culture and Regeneration of Somatic Embryonic Cell Suspensions on Agar*

The somatic cell suspension was used as the culture material. Spread volume 5ml/60ml semi-solid medium. Spread culture medium of MS embryo suspension supplemented with NAA (0.1-0.2-0.5mg/l), TDZ (0.3-0.5-1mg/l), BA (0, 2-0.5-1mg/l), and no growth regulator (0-PGR) added. Research results show that (Table 14): After 45 days of culture: TDZ inhibits the process of callus generation, cells are highly necrotic. The culture medium supplemented with the combination of NAA (0.2mg/l) + BA (0.2mg/l) achieved shoot regeneration efficiency, reaching the yield of 6,200 shoots per liter of embryonic cell suspension.

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

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

Research results (Table 15) show that the suitable mineral medium for orchid protocorm is MS (M.0244), orchimax (O.0257). On the culture medium of orchid protocorm and ground orchid, the number of protocorms increased (2.2 and 8.2 protocorm/cluster) and the number of regenerated shoots (2.6 and 2.4 shoots/cluster). In contrast, orchids and cycads tended to go into shoot regeneration (2.6 and 2.4 buds/cluster).

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

| Culture medium | NAA (mg/l) | BA (mg/l) | Number of shoots/5ml of embryo suspension |
|---|---|---|---|
| | 0.1 | 0.1 | 19 |
| | | 0.2 | 24 |
| | | 0.5 | 25 |
| | 0.2 | 0.1 | 22 |
| MS | | 0.2 | 31 |
| | | 0.5 | 31 |
| | 0.5 | 0.1 | 21 |
| | | 0.2 | 23 |
| | | 0.5 | 23 |
| CV% | | | 10 |

***Table 15.*** *Effect of mineral nutrient medium on rapid protocorm multiplication on agar.*

| Culture medium | Code | Dendrobium | |
|---|---|---|---|
| | | PLB | Shoot |
| MS | M.0244 | 2.2a | 5.6a |
| Vacine-Went | V.0226 | 1.2c | 2.4d |
| Orchimax | O.0257 | 1.6b | 4.8b |
| Knudson-C | K.0215 | 1.2c | 2.2d |
| Lindemann | L.0216 | 1.4b | 3.4c |
| Test f (0.05) | | * | * |

### *3.6.2. Effect of Culture Rhythm on Protocorm Nucleus and Orchid Shoot Development*

The results showed that (Table 16), the 4-hour floating and 1-minute immersion rhythms 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 (2.4 protocorm compared with 1.8 semi-submersible culture) and vice versa, the ability to regenerate shoots cultured in TIS bioreactor better than those grown on agar (6.6 shoots regenerated versus 4.6 on agar). Single shoots growing strongly in semi-submersible culture reached 45 mm leaf length compared to 40 mm cultured on agar.

Rapid multiplication of orchid industry by bioreactor technology.

***Table 16.*** *Effect of culture rhythm on protocorm nucleus and orchid shoot development.*

| Rhythm | | Protocorm | | Single shoot |
|---|---|---|---|---|
| Floating (hour) | Sinking (minutes) | PLB | Number of shoots | Leaf length (mm) |
| 1 | 1 | 1.0c | 4.2c | 22c |
| 1 | 2 | 0.8c | 4.0c | 20c |
| 2 | 1 | 1.2c | 4.6c | 24c |
| 2 | 2 | 1.0c | 4.4c | 22c |
| 3 | 1 | 1.6b | 5.8b | 36a |
| 3 | 2 | 1.4b | 5.6b | 34a |
| 4 | 1 | 1.8b | 6.6a | 45a |
| 4 | 2 | 1.4b | 6.2a | 42a |
| 5 | 1 | 1.4b | 5.2b | 38a |
| 5 | 2 | 1.2c | 4.8c | 36a |
| 6 | 1 | 0.8c | 4.0c | 28c |
| 6 | 2 | 0.6c | 3.6d | 24c |
| Control (agar) | | 2.4a | 4.6c | 40a |
| Test f (0.05) | | * | * | * |

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

***Table 17.*** *Tentatives of industrial propagation of Cymbidium sp. by bioreactor techniques.*

| Step | Culture target | Time (days) | Culture conditions |
|---|---|---|---|
| 1 | Select culture samples | 00 | Young leaf sheath, PLB |
| 2 | Generation of embryogenic callus on agar medium | 30 | MS + 2.4D (0,3mg/l) + CW (30%). |
| 3 | Create a suspension of callus embryos in liquid medium | 20 | MS + 2.4D (0,3mg/l) + CW (30%) |
| 4 | Proliferation of embryonic callus suspension in liquid medium | 20 | MS + NAA (0,5mg/l) + 2.4D (0.1mg/l) |
| 5 | Proliferation of suspension in bioreactor | 20 | MS + NAA (0,5mg/l) + 2.4D (1mg/l). |
| 6 | Embryogenesis induction in bioreactor | 45 | MS + BA (0.2mg/l) + NAA (0.2mg/l). |
| 7 | Embryo regeneration on agar medium | 45 | MS + BA (0.2mg/l) + NAA (0.2mg/l). |
| 8 | PLB and single shoot regeneration in TIS bioreactor | 30 | MS + IAA (0.1mg/l) |
| | Breeding Industry | | 6,200 orchid buds/litter of embryo cultured in bioreactor |

# 4. Conclusion

Cultivation and regeneration of PLB: PLBs were induced from cultivation of the apical corms and young leaf sheaths. Thinly sliced PLBs were cultured on semi-solid and liquid MS medium for growth, generation and performed clusters small buds. PLBs were regenerated on MS + BA (0.1mg/l) + IBA (1mg/l) medium.

Cultivation of starting material: with callus formed on the slices after 4 weeks of culture in liquid medium and after 8 weeks of culture, the PLB slices were filtered and removed, and callus cell suspension was obtained.

On the selected medium: the highest was 457 mg/cluster in cycle 14$^{th}$ and the following cycles did not increase much. Cyclic callus cells were used as raw materials for bioreactor studies.

Cultivation and proliferation of cell suspensions in bioreactor: cells proliferate rapidly proliferate at 3-4 weeks, less clumps, forming cell suspensions after 30 days of culture. There is a part of embryonic callus cells that differentiate into somatic embryonic cells. There was a growth factor of 6.2 times in the growth stage and there was a biomass growth factor of 9.9 times.

Effect of physical conditions on cell suspension in bioreactor: on MS + NAA (0.5mg/l) + 2.4D (0.1mg/l) culture medium after 30 days of culture, cell suspensions proliferated rapidly under culture conditions with light intensity. 11.1 mol/m$^2$/s, temperature 26±2°C and agitator speed 30rpm achieve cell suspension proliferation coefficient 7.8-7.8-8.2.

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. light of 11.1 μmol/$m^2$/s, temperature of 26±2°C and agitator speed of 30rpm achieved PLB proliferation coefficient 8.2-8.2-8.8.

Cell suspension regeneration in bioreactor: embryogenesis cells was induced in embryogenesis activation medium in culture medium MS + NAA (0.2mg/l) + BA (0.2mg/l). Embryo differentiation rate increased rapidly and the suspension completely differentiated to embryos after 45 days of culture and the yield was 6,200 shoots per liter of cell suspension in plating. TDZ inhibits the process of scar tissue generation, cells are highly necrotic.

Shoot growth and PLB in TIS bioreactor: The suitable mineral medium for orchid protocorm nucleus is MS (M.0244). The 4-hour float and 1-minute immersion rhythms are suitable for protocorm culture, shoot regeneration, and shoot growth. PPM at a concentration of 2‰ was suitable for liquid culture to limit the infection rate leading to shoot matter.

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