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Industrial Propagation of *Coffea* SP. by Bioreactor Technique

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Abstract: Study is carried out at National Key Lab of Plant Cell Biotechnology, Ho Chi Minh City, Vietnam. Vietnam has planning to re-culture of coffea plant in area of over 40.000ha in South of Vietnam. Good quality of seedlings have a big demand. Using bioreactor technique is the first method to resolve the lack of seedlings. Stem, leaves of in vitro plantlets were used as planting materials. Somatic embryo callus was initiated on medium supplemented with MS+ BA (0.1mg/l) + 2iP (2mg/l) + IBA (2mg/l). Somatic cell suspension was created and proliferated on medium MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2.4D (1mg/l). Embryogenic suspension was stimulated on the medium MS + kinetin (1mg/l) + BA (4mg/l). In vitro shoots of coffee were regeneration on the medium MS + kinetin (1mg/l) + BA (4mg/l) + Adenin sulfate (40mg/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±2°C, 11.1-22.2µmol/m^2/s, and 30rpm. Micropropagation of *coffea* sp. by bioreactor technique was set up to produce 25,260 plantlets per liter of somatic embryogenesis suspension.

Keywords: *Coffea* sp, Micropropagation, Somatic Embryogenesis Suspension, Immersion-Bioreactor, Bioreactor Technique

1. Introduction

Conventional micropropagation on woody plants currently leads to a problem that micropropagation laboratories often face, which is that tissue transplants are often slow-growing, labor-intensive, and time-consuming to reproduce. produce seedlings in large quantities when marketed at high seedling costs [5]. The system of cloning embryo propagation [3] solves the above barrier with the advantages: rapid multiplication in the form of cells, the cloned embryo is a differentiated organism with high regeneration coefficient and low cost. labor costs and lower costs [1]. In somatic embryo technology, liquid culture is the basic technique performed on a shaker or bioreactor [9, 10] with the aim of increasing biomass, inducing homogenous somatic embryogenesis, and leading on the ability to regenerate somatic embryos with high efficiency [7]. Bioreactor techniques have been studied and applied in micropropagation in order to reduce the cost of tissue culture products [1]. Culture materials in micropropagation by bioreactor technology such as embryonic callus cells, clonal embryonic cells, protocorm, bud clusters [7]. And there are also many types of bioreactors used for micropropagation such as airlift bubble column-bioreactor, airlift bubble balloon-bioreactor, propeller tank-bioreactor, semi-circular bioreactor. contemporary bioreactor [1]. Each type of bioreactor has different features, depending on the physiological properties of the cultured plants, aiming to increase biomass rapidly and enhance growth [10]. Physical and chemical factors are important factors affecting cell proliferation and cell regeneration [8]. There has been success through somatic embryo culture on woody plants such as coffee trees [2] 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.

2. Materials and Methods

2.1. Materials

Varieties: K84 selected lines of Robusta coffea was used in experiments. Culture samples: (i) 20 days old in vitro young

leaves (ii) 2 years old vegetative leaves.

2.2. Methods

The culture mineral nutrient medium is MS [6] and WPM [4].

The addition of growth regulators: BA (6-benzylaminopurine), TDZ (thidiazuron), 2iP (2-isopentyl adenine), IBA (β-indol butyric acid), kinetin (6-furfurylaminopurine), casein hydrolysate, malt, adenine (20mg/l), B1 (10mg/l), peptone (1g/l), coconut water (10%), activated charcoal (1g/l).

Culture conditions: room temperature 26±2°C, RH = 65%, lighting time 10 hours/day, light intensity 11.1-33.3µmol/m^2/s, shaking speed 100rpm, speed 30rpm bioreactor stirrer.

Experimental design: randomized complete block design, 3 replicates, 3 conical flasks each time (containing 60 ml 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. Spread volume 5ml/60ml semi-solid medium. Data were analyzed using MSTATC software (t=0.05).

3. Results and Discussion

3.1. Cultivation of Starting Material

3.1.1. Phylogenetic Culture Creates Embryonic Callus

Tissue-transplanted coffee tree saplings and 2-year-old vegetative plants in the field were cultured on WPM/MS embryogenic callus growth medium + malt (400mg/l) + casein hydrolysate (100mg/l) supplemented with 2.4D (2mg/l), IBA (1mg/l), 2iP (2mg/l), BA (0.1-1mg/l), under complete darkness. The research results showed that: On the culture medium for embryogenic callus, embryogenic callus appeared on WPM/MS base mineral medium + malt (400mg/l) + casein hydrolysate (100mg/l) + 2.4 D (2mg/l) + 2iP (2mg/l) (Table 1). Callus has 3 different shapes and colors: white with a lot of water (not for culture), brownish white with a lot of water, both types of callus are not used in culture and spongy embryonic callus has a yellow-lemon color was used in the experiments after 6 weeks of culture (Table 2) and growth of embryonic callus with yellow-lemon was shown to be different in the treatments after 12 weeks of culture (Table 3). The basic mineral medium was suitable for culturing the lemon yellow embryo callus.

3.1.2. Embryonic Callus Proliferation Culture

Embryonic spongy callus (with lemon yellow color) was studied for proliferation on MS + malt (800mg/l) + casein hydrolysate (200mg/l) medium supplemented with 2.4D (1-2mg/l)), NAA (2mg/l), 2iP (4mg/l), BA (0.1-4mg/l), adenine (60mg/l), under dark conditions. Embryonic callus samples were put into culture with a mass of 500mg/sample. Research results showed that (table): MS + malt (800mg/l) + casein hydrolysate (200mg/l) basic culture medium supplemented with 2,4D growth regulators (1mg/l)) + 2iP (4mg/l) + adenine (60mg/l) is suitable for callus proliferation culture (Table 4) and embryogenic spongy callus generated from young leaves in vitro has higher growth power than yellow callus spongy is produced from vegetative young leaves after 6 weeks of culture.

Table 1. *Effect of basic mineral environment and growth regulators on callus formation.*

Basic mineral medium	Growth regulator (mg/l)	Proportion of callus cultured samples (%)	
		Young leaves in vitro	Young leaves sample
WPM	Control	00	00
	2,4D(2) + BA(0.1)	66	36
	2,4D(2) + 2iP(2)	86	73
	2,4D(2) + BA(0.1) + IBA(1)	73	46
	2,4D(2) + 2iP(2) + IBA(1)	83	56
	2,4D(2) + 2iP(2) + BA(0.1) + IBA(1)	83	46
MS	Control	00	00
	2,4D(2) + BA(0.1)	93	66
	2,4D(2) + 2iP(2)	100	83
	2,4D(2) + BA(0.1) + IBA(1)	96	63
	2,4D(2) + 2iP(2) + IBA(1)	100	66
	2,4D(2) + 2iP(2) + BA(0.1) + IBA(1)	100	76
CV%		12	14

3.1.3. 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 was 16 cycles. The mass of cultured callus clusters was 100 mg/cluster. Selected medium MS + malt (800mg/l) + casein hydrolysate (200mg/l) + 2.4D (1mg/l) + 2iP (4mg/l) + adenine (60mg/l). Culture samples were placed under diffused light of 22.2 µmol/m^2/s. The results showed that (Table 5): The volume of proliferating cells increased with each cycle of selection. At cycle 7 there was a mass of 202 mg/cluster with a proliferation coefficient of 2.02. The highest was 412 mg/cluster with a proliferation factor of 4.12 in cycle 15 and not much increase in the following cycles. Cycle 14 callus cells were used as raw materials for bioreactor studies.

Table 2. *Effect of basic mineral medium and growth regulators on the ability to create callus callus of lemon yellow spongy embryo.*

Basic mineral medium	Growth regulator (mg/l)	Percentage of cultured samples creating callus of lemon yellow spongy embryos (%)	
		Young leaves in vitro	Young leaves sample
WPM	Control	00	00
	2,4D(2) + BA(0.1)	16	13
	2,4D(2) + 2iP(2)	43	33
	2,4D(2) + BA(0.1) + IBA(1)	16	6
	2,4D(2) + 2iP(2) + IBA(1)	33	26
	2,4D(2) + 2iP(2) + BA(0.1) + IBA(1)	23	26
MS	Control	00	00
	2,4D(2) + BA(0.1)	26	16
	2,4D(2) + 2iP(2)	53	33
	2,4D(2) + BA(0.1) + IBA(1)	33	26
	2,4D(2) + 2iP(2) + IBA(1)	53	33
	2,4D(2) + 2iP(2) + BA(0.1) + IBA(1)	46	23
CV%		12	10

Table 3. *Effect of basic mineral medium and growth regulators on the growth of yellow spongy embryo callus.*

Basic mineral medium	Growth regulator (ml/l)	Growth (diameter of callus - cm)	
		Young leaves in vitro	Young leaves sample
WPM	2,4D(2) + BA(0.1)	1.4	0.9
	2,4D(2) + 2iP(2)	3.1	2.8
	2,4D(2) + BA(0.1) + IBA(1)	1.5	1.2
	2,4D(2) + 2iP(2) + IBA(1)	2.6	2.2
	2,4D(2) + 2iP(2) + BA(0.1) + IBA(1)	1.4	2.0
MS	2,4D(2) + BA(0.1)	1.9	1.6
	2,4D(2) + 2iP(2)	3.4	2.6
	2,4D(2) + BA(0.1) + IBA(1)	1.6	1.2
	2,4D(2) + 2iP(2) + IBA(1)	2.8	2.4
	2,4D(2) + 2iP(2) + BA(0.1) + IBA(1)	2.7	2.2
CV%		8.2	9.0

3.2. Cultivation and Proliferation of Cell Suspensions in Bioreactor

3.2.1. Phylogenetic Culture Produces Embryonic Callus Suspension in Bioreactor

Selected callus was used as culture material. Culturing the callus cell suspension was 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 generates the embryonic callus cell suspension MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) supplemented with 2,4D (1-2-3mg/l). Research results show that (Table 6): After 30 days of culture, the suitable culture medium is MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2,4-D (1mg/l). Cells proliferate slowly in the first 2 weeks, and proliferate rapidly at 3-4 weeks, less clumps, forming cell suspensions. The suspension has a beautiful ivory-white color, and the cells are uniform in shape and size. There has a biomass growth factor of 2.2 times.

3.2.2. Growth 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 3 liters, with a culture volume of 1 liter, with a mother infusion rate of 10%, and a stirring speed of 30 rpm. The culture medium in the bioreactor proliferates the embryonic callus cell suspension MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) supplemented with 2,4D (1-2-3mg/l). Research results show that (Table 7): After 20 days of culture, the suitable culture medium is MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2,4-D (2mg/l) + BA (1mg/l). Cells proliferate slowly in the first week, and proliferate rapidly at 2-3 weeks, with little clustering. The suspension was smooth, with the color of coffee and milk. There has a biomass growth factor of 12.8 times.

Table 4. *Effects of growth regulators on the growth ability of lemon yellow spongy embryo callus.*

Culture medium (mg/l)	proliferation of fresh weight callus embryos (mg)			
	Young leaves in vitro		Young leaves sample	
	Fresh weight (mg)	Growth Index	Fresh weight (mg)	Growth Index
Control	873	0.74	707	0.41
2.4D(1) + BA(4)	1.767	2.53	1.877	2.75
NAA(2) + BA(4)	2.057	4.01	1.773	2.54
2.4D(2) + BA(0.1)	2.147	3.29	2.013	3.02
NAA(2) + 2iP(4)	3.360	5.72	3.070	5.14
2.4D(2) + 2iP(4)	3.407	5.81	3.393	5.78
2.4D(1) + BA(4) + Ade(60)	5.210	9.42	3.810	6.62

Culture medium (mg/l)	proliferation of fresh weight callus embryos (mg)			
	Young leaves in vitro		Young leaves sample	
	Fresh weight (mg)	Growth Index	Fresh weight (mg)	Growth Index
2.4D(1) + 2iP(4) + Ade(60)	6.393	11.78	5.420	9.84
CV%	12.2	10.4	11.8	9.6

3.3. Effect of Physical Conditions on Cell Suspension Culture in Bioreactor

3.3.1. Effect of Light (11.1-33.3 µmol/m²/s) on Proliferation of Embryonic Cells and PLB in Stirrer Bioreactor Culture

Embryonic callus cell suspension was used as culture material. The volume of cells was cultured 30% in the bioreactor. Temperature 26±2°C. Rotary speed 30rpm. Culture medium for proliferation of embryonic callus cell suspension: MS + 2iP (2mg/l) + BA (0.1mg/l) + 2,4D (1mg/l) + Adenine + kinetin (1mg/l) + peptone (1g/l). Research results show that (Table 8): After 45 days of culture: Callus cell suspensions proliferate rapidly on bioreactor liquid medium. The proliferation rate of embryonic callus cells was 16.2-17.8 times at two light intensities of 11.1-33.3 mol/m^2/s.

Table 5. *Selection of fast-growing callus lines through culture cycles.*

	10 Cycle 1	Cycle 2	Cycle 3	Cycle 4
Year 1 (2007)	110	122	136	149
(mg/cluster)	20 Cycle 5	Cycle 6	Cycle 7	Cycle 8
	172	192	202	226
	30 Cycle 9	Cycle 10	Cycle 11	Cycle 12
Year 2 (2008)	252	288	302	338
(mg/cluster)	30 Cycle 13	Cycle 14	Cycle 15	Cycle 16
	362	384	412	426

Table 6. *Effect of culture medium on the generation of embryonic callus cell suspension in bioreactor.*

Culture medium	2,4D (mg/l)	Growth coefficient
	1	2.2
MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l)	2	1.8
	3	1.4
CV%		10.2

Table 7. *Effect of culture medium on proliferation of embryonic callus cell suspension in bioreactor.*

Culture medium	2,4D (mg/l)	Growth coefficient
	1	12.8
MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l)	2	10.4
	3	8.8
CV%		12.0

Table 8. *Effect of light on cell suspension proliferation and PLB.*

Culture medium	Culture sample	11.1 µmol/m²/s	33.3 µmol/m²/s
MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l)	Cell suspension (after 45 days of culture)	14.2	17.8

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

The cell volume was cultured 20% in the bioreactor. Illumination intensity 11.1 µmol/m^2/s. Rotary speed 30rpm. Culture medium for proliferation of embryonic callus cell suspension: MS + 2iP (2mg/l) + BA (0.1mg/l) + 2,4D (1mg/l) + Adenine (20mg/l) + kinetin (1mg/l) + peptone (1g/l). The results showed that (Table 9): After 45 days of culture: Callus cell suspension grew rapidly on bioreactor liquid medium. The proliferation rate of embryonic callus cells was 14.8 at 26±2°C compared to 7.4 times at 30±2°C. The suitable temperature for culturing embryonic callus cells was 26±2°C.

Table 9. *Effect of temperature on cell suspension proliferation and PLB.*

Culture medium	Culture sample	26±2°C	30±2°C
MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l)	Cell suspension (after 30 days of culture)	14.8	7.4

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

The volume of cells was cultured 30% in the bioreactor. Illumination intensity 11.1 μmol/m^2/s. The incubation temperature was 26±2°C. Culture medium for proliferation of embryonic callus cell suspension: MS + 2iP (2mg/l) + BA (0.1mg/l) + 2.4D (1mg/l) + Adenine (20mg/l) + kinetin (1mg/l) + peptone (1g/l). Research results show that (Table 10): After 45 days of culture: Callus cell suspensions proliferate rapidly on bioreactor liquid medium. The embryonic callus cell proliferation rate was 16.2 times at the stirring speed temperature of 30rpm compared to 9.4 times at 60rpm. The suitable stirring speed was 30rpm.

Table 10. *Effect of stirrer speed on cell suspension proliferation and PLB.*

Culture medium	Culture sample	30rpm	60rpm
MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l)	Cell suspension (after 30 days of culture)	16.2	9.4

3.4. Cell Suspension Regeneration in Bioreactor

3.4.1. Activated Culture Induces the Generation of Somatic Embryo Suspension in the Bioreactor

Callus cell suspension was used as culture material. Embryo induction culture medium supplemented with kinetin (1-2mg/l) + BA (1-2-4mg/l) + 2iP (1-2mg/l). Research results showed that (Table 11): On MS medium supplemented with kinetin (1mg/l) + BA (4mg/l) callus cells differentiated into pre-embryonic and somatic embryo cells after 2 weeks culture. Somatic embryo cells were large in size and appear germ cells after 4 weeks, with an activation coefficient of 75%. Embryonic cells are filtered on a filter, separating somatic pre-embryonic cells. Somatic pre-embryonic cells were re-cultured on semi-solid medium to induce embryogenesis.

Table 11. *Effect of culture medium on induction activation of somatic embryo suspension in bioreactor.*

Kinetin (mg/l)	BA (mg/l)	2iP (mg/l)	Activation efficiency (%)
1	1	-	48
1	2	-	65
1	4	-	75
1	1	1	50
1	2	1	62
1	4	1	68
1	1	2	36
1	2	2	48
1	4	2	62
2	1	-	52
2	2	-	60
2	4	-	72
2	1	1	44
2	2	1	56
2	4	1	62
2	1	2	26
2	2	2	38
2	4	2	52
CV%			14

3.4.2. Spread Culture and Regeneration of Somatic Embryonic Cell Suspension on Agar

Embryonic cells were used as culture material. MS culture medium supplemented with kinetin (1-2mg/l) + BA (1-2-4mg/l) + 2iP (1-2mg/l). Research results showed that (Table 12): On the medium supplemented with kinetin (1mg/l) + BA (4mg/l), callus cells differentiated into pre-embryonic and somatic cells after 2 weeks of culture transplant. Somatic embryo cells were large in size and germ cells appear after 4 weeks of culture. Regeneration rate was over 78.6% after 6 weeks of culture. A high regeneration rate for woody plants. Yield of 25,260 coffee buds per liter of embryo cultured in bioreactor.

3.5. Shoot Growth in Semi-Submersible Bioreactor

3.5.1. Newly Regenerated Shoots

Newly regenerated shoots from embryos have two scalloped leaves. Culture medium MS + BA (0.1mg/l) + Adenine (40mg/l) + CW (10%) + Suc (20g/l). The control was cultured under the same environmental conditions on agar. Cultivate semi-submersible bioreactors in the intermittent mode for 1-6 hours floating, submerged for 1-2 minutes. Research results show that (Table 13): After 60 days of culture. In the semi-submerged bioreactor: shoot regeneration 100% did not differ from the control; shoot height reached 5.2cm compared with 3.2cm of control; roots and stems develop vigorously. Floating time of 1-3 hours has a submerged time of 1-2 minutes and 5-6 hours has a submerged time of 2 minutes, indicating that it is not suitable because the shoots are waterlogged.

3.5.2. Axillary Bud

The explants were 30-day-old shoots, with the apical bud removed. Culture medium: MS + BA (0.1mg/l) + Adenine (40mg/l) + CW (10%) + Suc (20g/l). The control was cultured under the same environmental conditions on agar. Cultivate semi-submersible bioreactor in the mode of interrupting for 1-6 hours floating, submerged for 1-2 minutes. Research results show that (Table 13): After 60 days of culture. In the semi-submersible bioreactor: the axillary bud regeneration rate reached 100%, not different from the control; shoot height reached 6.2cm compared to 3.8cm. Floating time of 1-3 hours has a submerged time of 1-2 minutes and 5-6 hours has a submerged time of 2 minutes, indicating that it is not suitable because the shoots are waterlogged.

Table 12. *Effect of culture medium on regeneration of somatic embryo suspension in bioreactor.*

Kinetin (mg/l)	BA (mg/l)	2iP (mg/l)	Regeneration rate (%)	Number of shoots/5ml of cell suspension
1	1	-	50.2	80
1	2	-	68.0	108
1	4	-	78.6	126
1	1	1	52.3	83
1	2	1	64.8	102
1	4	1	71.1	113
1	1	2	37.6	59
1	2	2	50.3	80
1	4	2	64.8	102
2	1	-	54.4	86
2	2	-	62.8	99
2	4	-	75.4	120
2	1	1	46.1	73
2	2	1	58.6	92
2	4	1	64.4	102
2	1	2	27.2	43
2	2	2	39.8	62
2	4	2	54.4	86
CV%			12.8	10

Table 13. *Effect of culture rhythm on protocorm nucleus and orchid shoot development.*

Rhythm		Axillary bud		New shoot reborn
Floating (hour)	Sinking (minutes)	Regeneration rate (%)	Height of shoots (cm)	Height of shoots (cm)
1	1	-	-	-
1	2	-	-	-
2	1	-	-	-
2	2	-	-	-
3	1	100	5.2	4.8
3	2	-	-	
4	1	100	6.2	5.2
4	2	-	-	-
5	1	100	4.5	4.2
5	2	-	-	-
6	1	100	3.8	3.2
6	2	-	-	-
Control (agar)		65	3.8	3.2
Test f (0.05)				

3.6. Industrial Multiplication of Coffee Plants by Bioreactor Techniques

As a result of the research process, a process of rapid industrial multiplication of coffee plants by bioreactor technology has been built (Table 14).

Table 14. *A flush out system for coffee propagation.*

Step	Culture Objective	Time (days)	Culture conditions
1	Select culture samples	0	Young leaves shoot in vitro (or 2-year-old leaves sample)
2	Generation of embryogenic callus on agar medium	30	MS + BA (0.1mg/l) + 2iP (2mg/l) + IBA (2mg/l)
3	Create a suspension of callus embryos in liquid medium	20	MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2.4D (1mg/l)
4	Proliferation of embryonic callus suspension in liquid medium	20	MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2.4D (1mg/l)
5	Proliferation of suspension in bioreactor	20	MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2.4D (1mg/l)
6	Embryogenesis induction in bioreactor	30	MS + BA (4mg/l) + kinetin (1mg/l)
7	Embryo regeneration on agar medium	60	MS + BA (0.1mg/l) + kinetin (1mg/l)
8	Single shoot regeneration and stem rising in semi-submersible bioreactor	60	MS + BA (0.1mg/l) + adenin sulfate (40mg/l): single shoot
9	Breeding industry		25.260 coffee plants/liter of embryo cultured in bioreactor

4. Conclusion

Cultivation of starting material: on the culture medium for embryogenic callus, embryogenic callus appeared on WPM/MS base mineral medium + malt (400mg/l) + casein hydrolysate (100mg/l) + 2.4D (2mg/l) + 2iP (2mg/l).

On basic culture medium MS + malt (800mg/l) + casein hydrolysate (200mg/l) + 2,4D (1mg/l) + 2iP (4mg/l) + adenine (60mg/l) suitable for callus proliferation and embryogenic

callus produced from young leaves in vitro had higher growth than spongy yellow callus generated from young leaves after 6 weeks of culture.

On selected medium MS + malt (800mg/l) + casein hydrolysate (200mg/l) + 2.4D (1mg/l) + 2iP (4mg/l) + adenine (60mg/l). Cycle 14 callus cells were used as raw materials for bioreactor studies.

Cultivation and proliferation of cell suspensions in bioreactor: on suitable culture medium is MS + kinetin (1mg/l) + BA (0.1mg/l) + 2iP (2mg/l) + 2,4-D (1mg/l) slow proliferative cells in the first 2 weeks, and rapid proliferation at 3-4 weeks, less clustering, cell suspension formation after 30 days of culture. The suspension is smooth, with the color of coffee and milk. Has a biomass growth factor of 12.8 times.

Effect of physical conditions on cell suspension in bioreactor: on culture medium MS + 2iP (2mg/l) + BA (0.1mg/l) + 2,4D (1mg/l) + Adenine (20mg/l) + kinetin (1mg/l) + peptone (1g/l), the cell suspension proliferated under the conditions of light intensity of 11.1 μmol/m^2/s, temperature of 26±2°C, stirring speed of 30rpm to achieve cell proliferation coefficient of 14.2-14.8 -16.2.

Regeneration of cell suspension in bioreactor: on MS + kinetin (1mg/l) + BA (4mg/l) medium, callus cells differentiated into pre-embryonic and somatic embryonic cells after 2 weeks of culture. Regeneration rate was over 78.6% after 6 weeks of culture. A high regeneration rate for woody plants. The yield of 25,260 coffee buds per liter of embryo cultured in bioreactor.

In the immersion-bioreactor: after 60 days of culture: shoot regeneration 100% did not differ from the control; shoot height reached 5.2cm compared with 3.2cm of control; roots and stems develop vigorously.

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