American Journal of Agriculture and Forestry
2022; 10(3): 111-117
http://www.sciencepublishinggroup.com/j/ajaf
doi: 10.11648/j.ajaf.20221003.15
ISSN: 2330-8583 (Print); ISSN: 2330-8591 (Online)

Industrial Propagation of *Chukrasia Tabularis* A. Fuss by Bioreactor Technique

Tran Van Minh

International University, Vietnam National University, Ho Chi Minh City, Vietnam

Email address:
drminh.ptntd@yahoo.com

To cite this article:
Tran Van Minh. Industrial Propagation of *Chukrasia Tabularis* A. Fuss by Bioreactor Technique. *American Journal of Agriculture and Forestry.* Vol. 10, No. 3, 2022, pp. 111-117. doi: 10.11648/j.ajaf.20221003.15

Received: May 23, 2022; **Accepted**: June 9, 2022; **Published**: June 20, 2022

Abstract: *Background: Chukrasia Tabularis* A. Fuss is a valuable woody plant and difficult in reproduction in nature. *Objectives:* There are a needs to conserve and develop for re-forest. *Method:* Manipulation of tissue culture via bioreactor techniques was the effective new way to resolve re-forest. Stems and leaves of in vitro plantlets were used as planting materials. Somatic embryo callus was initiated on medium MS + BA (2mg/l) + PVP (500 mg/l) supplemented with 2,4D (2mg/l). Somatic cell suspension was cultured for initiation and for proliferation. On medium MS + 2,4D (2mg/l) supplemented with BA (1mg/l). The volume of somatic cell suspension for bioreactor cultivation was 20%. Somatic embryo suspension was cultured in bioreactor for initiation and proliferation on the medium MS + 2,4D (2mg/l) supplemented with BA (1mg/l). Embryogenic suspension was stimulated on the medium MS + 2,4D (1mg/l) + CW (10%) + sucrose (45g/l) supplemented with BA (0.5mg/l) + NAA (0.1mg/l). In vitro shoots of *Chukrasia Tabularis* a. Fuss were regeneration on the medium MS + 2,4D (1mg/l) + CW (10%) + sucrose (45g/l) supplemented with TDZ (0,5mg/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 *Chukrasia Tabularis* a. Fuss by bioreactor technique was set up to produce 4,680 plantlets per one liter of somatic embryogenesis suspension *Conclusion:* a process of rapid industrial multiplication of woody plants by bioreactor technology has been built.

Keywords: *Chukrasia Tabularis* A. Fuss, Micropropagation, Bioreactor Technology

1. Introduction

Conventional micropropagation of woody plants currently leads to a problem that micropropagation laboratories which is that tissue cultures are often slow-growing, labor-intensive, and time-consuming to propagate produce seedlings in large quantities when marketed at high seedling costs [1]. The embryo cloning system [2] solves the above barrier with the following advantages: rapid multiplication in the form of cells, the cloned embryo is a differentiated organism with high regeneration coefficient and lower labor costs and costs [3]. In somatic embryo technology, liquid culture is the basic technique performed on shakers or bioreactors [4, 5] with the aim of increasing biomass, inducing homogenous somatic embryogenesis and leading to the ability to regenerate somatic embryos with high efficiency [6]. Bioreactor techniques have been studied and applied to micropropagation in order to reduce the cost of tissue culture products [3]. Culture materials in micropropagation by bioreactor technology such as embryogenic callus cells, clonal embryonic cells, protocorm, bud clusters [6]. There are 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 [3]. Each type of bioreactor has different features, depending on the physiological properties of the cultured plants, aiming to increase biomass rapidly and enhance growth [5]. Physical and chemical factors are important factors affecting cell proliferation and cell regeneration [7]. There has been much success in culturing somatic embryos on woody plants [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 C*hukrasia tabularis a. fuss* by bioreactor technology.

2. Material and Methods

2.1. Material

Breed: *Chukrasia tabularis* A. Fuss of Con Dao.
Culture sample: young leaf buds in vitro 20 days old.

2.2. Methods

The culture mineral nutrient medium was MS [9], WPM (Lloyd & McCown, 1980) with the addition of growth regulators: BA (6-benzylaminopurine), TDZ (thidiazuron), 2,4D (2.4-dichlorophenoxy acetic acid), PVP (polyvynylpyrrolidone), CW (10%), sucrose sugar (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.

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 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. 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

Young leaves, stems, and in vitro shoot-roots of 20 days old were used as culture materials. Culture medium for embryogenic callus generation: MS + BA (2mg/l) + PVP (500 mg/l) supplemented with 2,4D (0.5-1-2-4mg/l). Research results show that (Tables 1 and 2) after 15 days of culture: On the culture medium, callus embryos develop MS + BA (2mg/l) + 2,4D (2mg/l). Embryonic callus arose on the cuts of leaf and stem specimens and soon reached maximum mass at day 20. The root samples took 15 days for callus to begin to appear and proliferation was also very slow. This shows that the root sample is completely unsuitable for callus cell culture. Between treatments also showed that the time to start forming callus was also not different. In terms of color, embryogenic callus appeared in the treatments where the leaf samples were mainly milky white, slightly yellowish. This embryonic callus is structured by many clusters of cells with a clear structure, observed under an optical microscope, these are clusters of cells containing cell nuclei with many organelles, which are clusters of cells potential for somatic embryogenesis. Callus arising from the stem and roots is yellow brown, rather porous, with no clear structure. When cultured for a long time on medium, the callus hardens and turns black. Microscopic examination shows that the callus contains cells with small nuclei and mainly vacuoles. During prolonged in vitro culture, shoots secreted resin when cultured on callus cell-generating medium, causing browning and death of the specimen. The addition of PVP (500 mg/l) a phenol sorbent via the hydrogen ring, resulted in the prevention of browning of *Chukrasia tabularis a. fuss* explants.

3.1.2. Selection Culture of Fast-Growing Embryogenic Callus 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 was 16 cycles. The mass of cultured callus clusters was 100 mg/cluster. Selected medium MS + BA (2mg/l) + 2,4D (2mg/l) + CW (10%). Culture samples were placed under diffused light of 22.2 µmol/m^2/s. The results showed that (Table 3) the volume of proliferating cells increased with each cycle of selection. At cycle 4, there was a mass of 202 mg/cluster with a proliferation coefficient of 2.02. The highest was 452 mg/cluster with a proliferation coefficient of 4.52 in cycle 14 and not much increase in the following cycles. The cyclic 14 callus was used as raw materials for bioreactor studies.

Table 1. *Callus generation time in the cultures.*

Treatment	Sample	Time (days)								
		10	11	12	13	14	15	16	17	18
	Leaf	-	-	-	+	+	+	++	++	+++
1	Stem	-	-	-	-	-	+	+	+	++
	Root	-	-	-	-	-	-	-	-	+
	Leaf	-	-	-	+	+	++	++	++	+++
2	Stem	-	-	-	-	+	+	+	+	++
	Root	-	-	-	-	-	-	-	+	+
	Leaf	-	-	-	+	+	+	++	+++	+++
3	Stem	-	-	-	-	-	+	+	+	++
	Root	-	-	-	-	-	-	-	-	+
	Leaf	-	-	+	+	++	++	+++	+++	+++
4	Stem	-	-	-	-	+	+	+	++	++
	Root	-	-	-	-	-	-	+	+	+

(-): no somatic cells appear (+): somatic cells appear, (++): a lot of somatic cells appear, (+++): a lot of somatic cells appear.

Table 2. *Effect of explants and culture medium on the rate of callus formation of the explants.*

Medium	2,4D (mg/l)	Formation rate of callus (%)		
		Leaf	Stem	Root
MS + BA (2mg/l)	0.5	33.4c	13.4d	7.3b
	1	80.1a	73.5b	14.1ab
	2	46.7b	53.3c	12.8ab
	4	88.6a	86.6a	19.3a
CV%		6.62	9.3	17.76

Table 3. *Selection fast growing callus lines through culture cycles.*

Year 1 (2007) (mg/cluster)	10 Cycles 1	Cycle 2	Cycle 3	Cycle 4
	162	170	185	202
	20 Cycles 5	Cycles 6	Cycles 7	Cycles 8
	224	250	268	294
Year 2 (2008) (mg/cluster)	30 Cycles 9	Cycles 10	Cycles 11	Cycles 12
	320	355	384	404
	30 Cycles 13	Cycles 14	Cycles 15	Cycles 16
	428	452	466	472

3.2. Cultivation and Proliferation of Cell Suspensions in Bioreactor

3.2.1. Culturing the Embryonic Callus Suspension in Bioreactor

Selected callus was used as culture material. Callus 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 generated the MS embryonic callus suspension supplemented with BA (0.1-0.5-1-2mg/l) and 2,4D (0.1-0.5-1- 2mg/l). Research results show that (Table 4) after 30 days of culture, the suitable culture medium is MS + 2,4D (2mg/l) + BA (1mg/l). Cells proliferate slowly in the first week, and proliferate rapidly at 3-4 weeks, less clumps, forming cell suspension. The suspension has a beautiful ivory-white color, the cells were uniform in shape and size. A biomass growth factor was 7.3 times during the generation stage.

Table 4. *Effect of culture medium on the generation of embryonic callus suspension in bioreactor.*

Culture medium	BA (mg/l)	2,4D (mg/l)	Growth coefficient
MS	0.1	0.1	2.8
		0.5	3.2
		1.0	3.8
		2.0	4.6
	0.5	0.1	3.2
		0.5	3.8
		1.0	5.0
		2.0	5.8
	1.0	0.1	4.2
		0.5	5.8
		1.0	6.2
		2.0	7.3
	2.0	0.1	3.8
		0.5	5.2
		1.0	6.4
		2.0	7.5
CV%			11.8

3.2.2. Proliferation of Embryonic Callus Suspensions in Bioreactor

The callus suspension from the above experiment was used cultured in a bioreactor of 3 liters, with a culture volume of 1 liter with rate of 10%, and a stirring speed of 30 rpm. The culture medium in the bioreactor proliferates in MS embryonic callus suspension supplemented with BA (0.1-0.5-1-2mg/l) and 2,4D (0.1-0.5-1-2mg/l). Research results show that (Table 5) after 20 days of culture, the appropriate culture medium is MS + 2,4D (2mg/l) + BA (1mg/l). Cells proliferate slowly in the first week, and rapidly proliferate at 2-3 weeks with little clustering. The suspension was smooth, with the color of coffee, milk and biomass growth factor of 11.3 times.

Table 5. *Effect of culture medium on proliferation of embryonic callus cell suspension in bioreactor.*

Culture medium	BA (mg/l)	2,4D (mg/l)	Growth coefficient
MS	0.1	0.1	4.0
		0.5	5.4
		1.0	6.2
		2.0	6.4
	0.5	0.1	6.0
		0.5	5.8
		1.0	7.8
		2.0	8.2
	1.0	0.1	7.2
		0.5	3.6
		1.0	9.4
		2.0	11.3
	2.0	0.1	6.5
		0.5	7.0
		1.0	8.6
		2.0	10.0
CV%			10.4

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

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

There was culture volume 20% in bioreactor with temperature 26±2°C and agitator speed 30rpm. Culture medium for proliferation of embryonic callus cell suspension: MS + 2,4D (2mg/l) + BA (1mg/l). Research results show that

(Table 6) after 30 days of culture: The proliferation coefficient of embryonic callus increased by 6.6-7.3 times at both light intensities 11.1-33, 3 mol/m^2/s.

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

There was culture volume 20% in bioreactor with agitator speed 30rpm. Culture medium for proliferation of embryonic callus cell suspension: MS + 2,4D (2mg/l) + BA (1mg/l). Research results show that (Table 7) after 30 days of culture: The density of embryogenic callus cells increased by 6.2 times at 26±2°C compared to 5 times at 30±2°C. The appropriate culture temperature for embryonic callus cell proliferation is 26±2°C.

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

Embryonic callus suspension was used as culture material. There was culture volume 20% in bioreactor with illumination intensity 11.1 µmol/m^2/s and temperature 26±2°C. Culture medium for proliferation of embryonic callus cell suspension: MS + 2,4D (2mg/l) + BA (1mg/l). Research results show that (Table 8) after 30 days of culture: The density of embryonic callus cells increased by 7.3 times at the stirring speed of 30rpm compared to 5.2 times at 60rpm. The appropriate stirring speed for embryonic callus cell proliferation culture is 30rpm.

Table 6. *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 + 2,4D (2mg/l) + BA (1mg/l)	Cell suspension (after 30 days of culture)	6.6	7.3

Table 7. *Effect of temperature on cell suspension proliferation and PLB.*

Culture medium	Culture sample	26±2°C	30±2°C
MS + 2,4D (2mg/l) + BA (1mg/l)	Cell suspension (after 30 days of culture)	6.2	5.0

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

Culture medium	Culture sample	30rpm	60rpm
MS + 2,4D (2mg/l) + BA (1mg/l)	Cell suspension (after 30 days of culture)	7.3	5.2

3.4. Cell Suspension Regeneration in Bioreactor

3.4.1. Effect of TDZ on Induction of Somatic Embryogenesis

Embryonic callus suspension was used as culture material. There was 20% culture volume. Embryo induction culture medium MS + 2,4D (1mg/l) + CW (10%) + Sucrose (45g/l) supplemented with TDZ (0-0.05-0.1-0.3-0.5mg/l). After a 20-day culture period, spread the cells on agar with the same medium composition as the liquid culture. The cell suspension spread volume was 10 ml/60 ml of semi-solid medium. Research results show that (Table 9) after one week of drying the cell solution, follow-up in the next 4 weeks, the layer of smooth cells covered the surface of agar, appearing embryo-like structure. Observation under the stereo microscope, these forms can be seen with the morphology of somatic embryos with blue and milky white in spherical and elongated forms. The results of embryogenesis in the treatments were significantly different, with low TDZ concentrations (0.05-0.1mg/l) giving high embryogenesis results (19.2 and 14.6 embryos/10ml). For other concentrations of TDZ (0.3-0.5mg/l), although the embryogenesis results were low (12.8 and 11.9 embryos/10ml) but compared with the control treatment (without adding TDZ) can only generate 3.4 embryos/10ml of cell suspension, there was still a big difference. The appropriate concentration of TDZ was 0.05mg/l for somatic embryogenesis on agar medium.

3.4.2. Effect of Sucrose on Induction of Somatic Embryogenesis in Bioreactor

For different crops and at different stages of embryonic development, sugar and osmotic pressure requirements are different. Embryonic callus suspension was used as culture material with 20% culture volume. The culture medium was MS + TDZ (0.05mg/l) + 2,4D (1mg/l) supplemented with sucrose (45-55-65g/l). The research results showed that (Table 10) after 45 days of culture: Somatic embryo formation at the sugar concentrations of 65g/l compared with the concentrations of 45g/l and 55g/l had a very significant difference. With a high concentration of sucrose (65g/l) resulted in higher embryogenesis compared to two lower sugar concentrations (45-55g/l). On the same medium, blue somatic embryos form a lot, which was the type of embryo with high regeneration potential. Research on somatic embryogenesis obtained the best results (19.2 embryos/10ml) on MS + TDZ medium (0.05mg/l) + 2,4D (1mg/l) + sucrose (65g) /l) + CW (10%).

3.4.3. Somatic Embryo Regenerative Culture

(i). Regeneration of Embryos Directly from Suspension

The suspension before going through the regeneration phase on agar was inoculated on: MS + BA (2mg/l) + sucrose (45g/l) + CW (10%). The cell suspension spread volume was 10 ml/60 ml of semi-solid medium. Regenerating medium MS + CW (10%) + Sucrose (45g/l) supplemented with BA (0.1mg/l) and kinetin (0.1mg/l). The objective was to investigate the possibility of direct embryogenesis in liquid medium to proceed to direct regeneration from suspension. The results showed that (Table 11) in all treatments, after 3 weeks, regenerative shoots began to appear. However, during follow-up, regeneration appeared only at 3 weeks after culture, then no more

regenerative shoots appeared. The highest number of shoots regenerated was only 11.8 shoots/10ml of cell culture. Direct regeneration from suspension has low efficiency.

(ii). Regeneration of Activated Embryos Induces Embryogenesis

At this stage, the embryonic cells develop into morphologies that are clearly observed from spherical, elongated, cardiac, and cotyledon under the stereomicroscope. Regenerating medium MS + CW (10%) + Sucrose (45g/l) supplemented with BA (0.1mg/l) and kinetin (0.1mg/l). The results showed that (Table 12) the number of regenerative shoots on each cluster of somatic cells was significantly different in the treatments. With regenerative capacity in all treatments, flower slices proved to be a plant with a high ability to regenerate in vitro from somatic embryos. All treatments had regenerative shoots even though the control medium was not supplemented with growth stimulants. The regenerated shoots did not appear mutant or degenerate. With the addition of growth stimulant BA (0.1mg/l) gave the highest regeneration results (29.4 shoots/10ml of cell culture) and elongated shoots. Obtained 2,940 shoots per liter of cultured somatic embryonic cell suspension.

Table 9. *Effect of TDZ on somatic embryogenesis.*

Medium	TDZ (mg/l)	Number of embryos/10ml of embryonic cell suspension
MS + 2,4D (1mg/l) + CW (10%) + Sucrose (45 g/l)	0.0	3.4 d
	0.05	19.2a
	0.1	14.6b
	0.3	12.8 c
	0.5	11.9c
CV%		8.9

Table 10. *Effect of Sucrose on somatic embryogenesis.*

Medium	Sucrose (g/l)	Number of embryos/10ml of embryonic cell suspension
MS + TDZ (0.05mg/l) + 2,4D (1mg/l) + CW (10%)	45	19.6b
	55	18.4b
	65	19.2a
	45	19.6b
	55	18.4b
CV%		8.6

3.5. Shoot Growth in Temporary Immersion System (TIS) Bioreactor

3.5.1. New Shoots Regeneration

The culture sample was a newly regenerated shoot of 1-2cm. Culture medium to stimulate rooting was WPM + NAA (0.1mg/l) + Sucrose (20g/l). The control was cultured under the same media conditions on agar. Culture the TIS bioreactor in the mode of interrupting for 1-6 hours floating, submerged for 1-2 minutes. Research results show that (Table 13) after 30 days of culture. In the TIS bioreactor, 100% regeneration rate was not different from the control. The shoot height reached 8.2cm compared with 5.5cm of control and roots developed in both cultures. Shoots grow faster and stronger in the semi-submersible bioreactor with floating/sinking time 1-3 hours/1-2 minutes the tree dies due to waterlogging, and 5-6 hours/1-2 minutes the top of the tree was dry.

3.5.2. Cluster of Shoots

The culture sample was a cluster of shoots with size was 1-2cm high, there are 2-3 shoots/culture cluster. Culture medium for shoot multiplication was WPM + BA (0.5mg/l) + CW (10%) + Sucrose (20g/l). The control was cultured under the same media conditions on agar. Culture the TIS bioreactor in the mode of interrupting for 4 hours, submersion for 1 minute. Research results show that (Table 13) after 30 days of culture. In the TIS bioreactor, 100% shoot regeneration rate was not different from the control. The height of the shoot cluster reached 5.4cm compared with 3.2cm of the control. The number of shoots reached 7.8 shoots/cluster compared to 3.5 shoots/cluster. Floating time 1-3 hours and 5-6 hours and flooding time 1-2 minutes was not suitable, shoots were waterlogged and tops dry.

Table 11. *Effect of culture medium on regeneration of somatic embryos (not yet cultured to activate embryogenesis).*

Medium	BA (mg/l)	KIN (mg/l)	Number of embryos/10ml of embryonic cell suspension
MS + Sucrose (45g/l) + CW (10%).	-	-	8.5a
	0.1	-	4.2a
	-	0.1	11.8a
	0.1	0.1	6.4a
CV%			18.4

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

Medium	BA (mg/l)	KIN (mg/l)	Number of shoots/clusters	Number of embryos/10ml of embryonic cell suspension
MS + Sucrose (45g/l) + CW (10%)	-	-	0.32d	3.4
	0.1	-	5.89a	29.4
	-	0.1	2.87b	17.5
	0.1	0.1	1.53c	10.2
CV%			14.3	12.2

3.6. Rapid Multiplication of Woody Plants in Large Scale by Temporary Immersion System (TIS) Bioreactor Technology

Table 13. *Effect of culture rhythm on shoot development in TIS bioreactor.*

Rhythm		Cluster of shoots		New shoots reborn
Floating (hour)	Sinking (minutes)	Number of shoots	Shoot cluster height (cm)	Stem height (cm)
1	1	-	-	-
1	2	-	-	-
2	1	-	-	-
2	2	-	-	-
3	1	-	-	-
3	2	-	-	-
4	1	7.8	5.4	8.2
4	2	-	-	-
5	1	-	-	-
5	2	-	-	-
6	1	-	-	-
6	2	-	-	-
Control (agar)		3.5	3.2	5.5
Test f (0.05)		*	*	*

Previous study carried out on fruit crop, vegetables, medicinal plants, ornamental plants, eucalyptus by temporary immersion sytem. Gingseng was produced via embryonic cell in bioreactor for saponin obtained. Rarely, using bireactor techniques to propagate woody plant in re-forest. As a result of the research process, a process of rapid industrial multiplication of woody plants by bioreactor technology has been built.

4. Conclusion

On the culture medium generated callus embryos MS + BA (2mg/l) + 2,4D (2mg/l) after 20 days of culture. Embryonic callus appeared in the treatments where the leaf samples were mainly milky white, slightly yellowish. This embryonic callus is structured by many clusters of cells with a clear structure, observed under an optical microscope, these are clusters of cells containing cell nuclei with many organelles, which are clusters of cells potential for somatic embryogenesis.

Proliferative cell biomass increased with each cycle of selection. The highest was 452 mg/cluster with a proliferation coefficient of 4.52 in cycle 14 and not much increase in the following cycles. Cyclic callus cells were used as raw materials for bioreactor studies.

The suspension has a beautiful ivory-white color, the cells are uniform in shape and size. Has a biomass growth factor of 7.3 times. The suspension is smooth, with the color of coffee and milk. Has a biomass growth factor of 11.3 times.

Cell suspensions proliferated under conditions of light condition achieves cell proliferation coefficient of 6.6-6.2-7.3; Embryo induction with 19.2 embryos/10ml of culture medium;. Regeneration when the cell suspension was stimulated reached 29.4 shoots/10 ml of cell fluid.

In the TIS bioreactor after 30 days of culture: 100% regenerated shoots were not different from the control.

References

[1] Mamood, M. (1993). Application of plant in vitro technology. Proceeding, 16-18 Nov 1993, Univ. of Malaysia, Malaysia.

[2] Evans, D. A., Sharp, W. R. and Flick, C., E. (1981). Growth and behavior or cell cultures: embryogenesis and organogenesis. In: Thorpe TA (ed.): Plant Tissue Culture. Methods and Applications in Agriculture. Academic Press, 45-114.

[3] Aitken-Christie, J., Kozai, T. and Smith, M. A. L., (1994). Automation and environmental control in plant tissue culture. Kluwer.

[4] Shakti, M., Goel, M. K., Kukreja, A. K. and Mishra, B. N. (2007). Efficiency of liquid culture systems over conventional micropropagation: A progress towards commercialization. African J Biotechnology (13), 1484-1492.

[5] Son, S. H., Choi, S. M., Yun, S. R., Kwon, U. W., Lee, Y. H. and Paek, K., Y. (1999). Large scale culture of plant cell and tissue by bioreactor system. J Plant Biotech (1), 1-7.

[6] Paek, K. Y., Hahn, E. J. and Son, S. H. (2001). Application of bioreactors for large-scale micropropagation systems of plants. In vitro Cell. Dev. Biol – Plant (37), 149-157.

[7] Park, S. Y., Murthy, H. N. and Paek, K. Y. (2003). PLB induction and subsequent plant regeneration from root tip cultures of Doritaenopsis. Plant Sci (164), 919-923.

[8] Berthouly, M. and Michaux-Ferriere, N., M. (1996). High frequency somatic embryogenesis in offea canephora: Induction conditions and histological evolution. Plant Cell Tissue. Org. Cult. (44), 169-176.

[9] Murashige, T. and Skoog, R. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant (15), 431-497.

[10] Shri Mohan Jain, Jameel M. Al-Khayri and Dennis V. Johnson (2011). Date Palm Biotechnology, Springer.

[11] Anne Kathrine Hvoslef-Eide and Walter Preil (2005). Liquid culture system for in vitro plant propagation, Springer.

[12] Lian M. L., Chakrabarty D. and Paek K. Y. (2003). Bulblet Formation from Bulbscale Segments of *Lilium* Using Bioreactor System. Biologia plantarum (46), 199-203.

[13] Etienne H. and Berthouly M. (2002). Temporary immersion systems in plant micropropagation. Plant Cell, Tissue and Organ Culture (69), 215-231.

[14] Niemenak N., Saar-Surminski K., Rohsius C., Ndoumou D. O. and Lieberei R. (2008). Regeneration of somatic embryos in Theobroma cacao L. in temporary immersion bioreactor and analyses of free amino acids in different tissues. Plant Cell Report (27), 667-676.

[15] Youn Hee Kim, Gee Young Lee, Hye Hyeong Kim, Jae Hong Lee, Jae Hong Jung and Sang Deok Lee (2019). *In vitro* mass propagation and acclimatization of *Haworthia truncata.* J Plant Biotechnol 46: 127-135.