MICROPROPAGATION OF *CHUKRASIA TABULARIS* A. FUSS BY SOMATIC EMBRYOGENESIS TECHNIQUE

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Abstract: *Chukrasia tabularis* belongs to the Meliaceae family, which is native to many Asian countries, including Vietnam. It is a precious wood species with beautiful wood grain, light-loving tree with evergreen leaves, widely grown in many places Cameroon, Costa Rica, Nigeria, Puerto Rico, South Africa, United States, China ... for many different purposes: planting economic forests, alternative forests, protective forest belts and urban greenery. In Vietnam, *Chukrasia tabularis* are usually distributed naturally in the northern, central highland and southern provinces. From Nghe Tinh onwards, there are many varieties such as Dong Nai *Chukrasia tabularis* (*Chukrasia tabularis* var. *Dongnaiensis*), Con Dao *Chukrasia tabularis* (*C. tabularis* sp.), small fruit *Chukrasia tabularis* (*C. tabularis* var. *Microcarpa*), and hairy *Chukrasia tabularis* (*C. tabularis* var. *Velutina*). *Chukrasia tabularis* often grow on well-drained soil, at an altitude of 300-800 m.asl, the annual average temperature is from 2-43°C, rainfall is 1,800-3,800 mm. Singing and development occurs in high limestone mountains, meaty soil, mixed meat, nutrient poor sandy soil at an altitude of less than 100 m. The tree is regenerated by seed but grows slowly and with high genetic diversity. Techniques of somatic embryo culture are applied to preserve and develop *Chukrasia tabularis* plants with urgent needs.

UDC Classification: 57.01, **DOI:** 10.12955/cbup.v7.1485

Keywords: *Chukrasia tabularis* a. fuss, callus, cell suspension, micropropagation

Introduction

Traditional micropropagation of woody plants has a problem where the tissue culture plants often grow slowly and the costs of labor, to produce the seedlings and the seedlings cost themselves, are high (Murashige et al, 1962). Asexual embryo-propagation system (Evans et al, 1981) addresses the above-mentioned problems with certain advantages such as the rapid multiplication in the form of asexual embryos which is a form of differentiation with a high regeneration coefficient, low cost of labor dynamic hence keeping the price down (Kurata et al, 1992). The starting material in asexual embryo culture plays a role in securing parental traits and maintaining a high regeneration coefficient for a long time (Lu et al, 2004). A suitable clonal culture medium plays an important role in embryonic growth and differentiation (Evans et al, 1981). Controlling the process of differentiation and regeneration of asexual embryos under the effect of growth regulators has become a rule (Jonkson et al, 1984) that arises in the cultivation of woody plants affected by cytokinin such as BA, 2iP and TDZ (Chung et al, 2005). Embryonic callus research, asexual embryo culture, and asexual embryo regeneration is the first barrier in somatic embryo technology (Jain et al, 1995; Sondahl et al, 1977). In this paper, we study the fast multiplication of *chukrasia tabularis* A.Fuss by somatic embryo technology.

Materials and Methods

The *Chukrasia tabularis* A.Fuss species of Con Dao was used as materials.

Culture sample: (vi) Young leaf buds in vitro 20 days old. (ii) Premature shoots.

The basal culture medium is MS (Murashige and Skoog, 1962). There are additional growth regulators: BA (6-benzylaminopurine), TDZ (thidiazuron), kinetin (6-furfurylaminopurine), 2,4D (2,4-dichlorophenoxy acetic acid), IAA (β -indolacetic acid), PVP (polyvinylpyrrolidone), coconut water (10%), sucrose (30g/l).

Culture conditions: room temperature of $26 \pm 2^\circ\text{C}$, RH = 65%, lighting time of 10 hours/day, lighting intensity of $33.3\mu\text{mol}/\text{m}^2/\text{s}$, shaking speed of 100rpm.

Experimental design: arranged in full randomized blocks, 3 times repeated, each time to re-cultivate 3 Erlenmeyer flashes (containing 60ml semi-solid medium or 50ml liquid medium). Initial cell biomass cultured to inoculate 10g/100ml suspension. Data were analyzed by MSTATC software ($t = 0.05$).

Cultivation and regeneration of callus

Apical Meristems of *Chukrasia Tabularis* A. Fuss Cultivation in Vitro: The young shoots are used as culture material on the MS growth peak culture medium containing BA (0.1mg/l) and coconut water (0; 10%).

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Shoot Multiplication of *Chukrasia Tabularis* A. Fuss: Rapid in vitro MS medium supplemented with BA (0.1, 0.5, 1mg/l) and IAA (0.1, 0.5, 1mg/l).

Cultivation of Embryonic Callus: Young leaf, stem and root buds in vitro 20 days old were used as culture materials. Culture medium for embryonic callus development: MS + BA (2mg/l) + PVP (500 mg/l) with 2,4D (0.5, 1, 2, 4mg/l) supplementation.

Proliferation of Embryonic Callus: Using clusters of embryonic calluses through the above study to continue biomass on agar medium, initial sample volume of 200mg/cluster. Culture medium increased biomass of embryonic callus: MS + BA (2mg/l) + PVP (500 mg/l) with 2,4D (0.5, 1, 2, 4 mg/l) supplementation.

Regeneration of Embryonic Callus: Embryonic callus cultured on the medium: MS + sucrose (45g/l) + CW (10%) supplemented with BA (0, 0.1mg/l) and kinetin (0, 0.1mg/l). The clusters of cells put into culture have a mass of 500mg/cluster.

Effect of Tissue and Genotype on Callus Regeneration

Effect of Cultured Samples on Embryonic Callus Regeneration: Young leaf, stem and root buds in vitro 20 days old were used as culture materials. Culture medium for embryonic callus development: MS + BA (2mg/l) + PVP (500 mg/l) with 2,4D (0.5, 1, 2, 4mg/l) supplementation.

Effect of Genotype on Embryonic Callus Regeneration: Using leaves of two species *chukrasia tabularis* A. Fuss of Con Dao and Lang Son as culture materials on culture medium of embryonic callus regeneration MS + kinetin (0.1mg/l) + sucrose (45g/l) + CW (10 %).

Formation and Proliferation of Callus Suspension

Proliferation of Embryonic Callus: Culture medium increased the suspension of embryonic callus MS supplemented with 2,4D (0.5, 1, 2, 4 mg/l) and BA (1, 2mg/l).

Effect of Initial Cell Density on The Proliferation of Embryonic Callus Suspension: Embryonic callus suspension is used in culture. Initial cell density was introduced into culture (2.47, 3.29, 4.91x10⁴ cell/ml) corresponding to the initial culture volume (15, 20, 30% v/v). Culture medium MS supplemented with 2,4-D (2mg/l) + BA (1mg/l).

Proliferation of Callus Suspension

Effect of Sucrose on Induction of Somatic Embryogenesis: Embryonic callus suspension is used as a culture material. Cultured volume 20% and culture medium: MS + TDZ (0.05mg/l) + 2,4D (1mg/l) with sucrose supplement (45, 55, 65g/l).

Effect of TDZ on Induction of Somatic Embryogenesis: Embryonic callus suspension is used as a culture material. Cultured volume 20% and the induction medium for embryogenesis MS + 2,4-D (1mg/l) supplemented with TDZ (0.05, 0.1, 0.3, 0.5mg/l). After 20 days of culture, spread the cells on agar with the same environmental components as cultured in liquid. The volume of cell suspension is 10ml/60ml semi-solid medium.

Regeneration of Somatic Embryo: Regeneration of embryos directly from suspension: suspension before transfer to regeneration stage on agar medium is transplanted on medium: MS + BA (2mg/l) + sucrose (45g/l) + CW (10%). The volume of cell suspension is 10ml/60ml semi-solid medium. The objective is to study the ability to create embryos directly in liquid medium to proceed to regenerate directly from suspension.

Regeneration of Transplanted Buds Stimulates Embryonic Induction: Culture medium: MS + sucrose (30g/l) + CW (10 %) supplemented with BA (0.1mg/l), kinetin (0.1mg/l)

Multiplication Of Buds Derived From Somatic Embryos: Based on the results from Le Xuan Ai's research shoot multiplication experiment in 2003, this experiment focused on examining the effects of IAA (0.1mg/l), kinetin (1mg/l) and coconut water (10%).

Results and Discussion

Cultivation and regeneration of callus

Apical Meristems of *Chukrasia Tabularis* A.Fuss Cultivation in Vitro:

The apical meristem is the meristem region containing pre-embryonic cells capable of dividing, differentiating and generating organs. This is the area of birth, the cell is always in the form of

physiology, and the ability to develop strong morphology, apical meristem culture is the promise for creating the starting material for culture in vitro and always keep the characteristics Genetics of mother plants. The results showed that (Table 1): After 45 days: On MS + BA (0.1mg/l) + CW (10%), regenerated shoot grew fast 11.2mm, generated 6.7 shoots/samples.

Shoot Multiplication of *Chukrasia Tabularis* A.Fuss

In order to build a system of in vitro buds as a material for biomass. Young shoots regenerated in vitro are used as culture materials. Here we use the method of cuttings in vitro to multiply buds. The principle of the method is to use growth regulators to stimulate lateral shoot regeneration. Cytokinin is necessary for lateral shoot development, for some plants that need to be combined with auxin, and in culture medium supplemented with coconut water stimulates increased effectiveness of cytokinin and auxin effects. The results showed MS + BA (1mg/l) + IAA (0.1mg/l) medium is suitable for in vitro propagation process (Table 2). Reaching 9 shoots/sample with growing leaf stems (7mm tall shoots).

Table 1: Effect of basal medium and growth regulators on apical meristem culture in vitro

Treatment	Medium	BA (mg/l)	CW (%)	Height of shoot (mm)	Number of shoots	Length of leaf (mm)	Width of leaf (mm)	Leaf development
1.1	MS	-	-	15.5	1.5	12.7	6.7	+
1.2	MS	0.1	10	11.2	6.7	12.7	10.7	+
1.3	MS	0.1	-	13.2	3.5	19.5	7.7	+
1.4	MS	0.5	10	6.5	1.7	27.5	13.2	+
CV%				4.5	13.4	2.6	6.3	

Source: Author

Table 2: Effects of BA and IAA on multiplication of *chukrasia tabularis a. fuss* buds in vitro

Treatment	BA (mg/l)	IAA (mg/l)	Height of shoot (mm)	Number of shoots	Length of leaf (mm)	Width of leaf (mm)	Leaf development
2.1	0.1	0.1	9.0	5.5	10.5	4.2	+
2.2	0.1	0.5	7.5	4.2	9.2	3.2	+
2.3	0.1	1.0	6.2	3.0	8.2	2.5	+
2.4	0.5	0.1	8.0	7.5	9.2	3.2	+
2.5	0.5	0.5	6.5	6.2	8.5	2.7	+
2.6	0.5	1.0	5.2	5.0	8.2	2.2	+
2.7	1.0	0.1	7.0	9.5	8.2	2.2	+
2.8	1.0	0.5	7.5	7.2	7.5	2.5	+
2.9	1.0	1.0	6.2	6.0	7.2	2.0	+
CV%			6.16	5.35	3.86	14.39	

Source: Author

Cultivation of Embryonic Callus

After 15 days, on the culture medium, an embryonic callus develops with additional 2,4D (2mg/l) (Table 3.1, 3.2). Embryonic callus arises on the cut of leaf and stem samples and soon reaches the maximum weight on the 20th day. With root samples right after 15 days, there is a phenomenon of callus development and proliferation which is also very slow. This shows that the root sample is completely unsuitable in cultivating callus cells. Between treatments it was also shown that the time to start callus development is not different. In terms of color, the embryonic callus appears in the treatments where the leaf sample is mainly opalescent, slightly yellow. The callus which arises from the stem and roots are golden brown, quite porous, without a clear structure; when cultured long on the environment, callus stiffens, turns black. Observation under a microscope, the callus contains cells with small nuclei and mainly vacuoles. During prolonged culture in vitro, shoots appear to be secreted when cultured on callus cells, causing browning and death of the sample. Adding PVP (500 mg/l) of a phenol absorber through a hydrogen ring resulted in the prevention of browning of the sample.

Proliferation of Embryonic Callus

The study shows that there is a difference between the induction of growth substances on the formation and the ability to increase callus biomass. The results showed the proliferation of embryonic

callus did not have a significant difference between treatments of 2,4D concentrations of 2 mg/l and 4 mg/l after 30 days (Table 4). However, compared to treatments with lower concentrations of 2,4D such as 0.5 mg/l and 1 mg/l, the significant difference in the volume of embryonic callus formed. Meanwhile, embryonic callus divides and differentiates well on MS + 2,4D (2mg/l) + BA (1mg/l). In this environment, somatic cells develop well 860mg/cluster, porous cells, are opalescent, or have little blue spots.

Table 3.1: Time of callus development in culture samples

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

(-): somatic cells have not yet appeared, (+): somatic cells appear, (++): many somatic cells appear, (+++): there are many somatic cells

Source: Author

Table 3.2: Effect of sample and media on the rate of callus development of the explants

Treatment	2.4-D (mg/l)	Rate of callus culture (%)		
		Leaf	Stem	Root
3.1	0.5	33.4c	13.4d	7.3b ⁽¹⁾
3.2	1	80.1a	73.5b	14.1ab
3.3	2	46.7b	53.3c	12.8ab
3.4	4	88.6a	86.6a	19.3a
CV%		6.62	9.30	17.76

Source: Author

Table 4: Effect of culture medium on somatic cell biomass on semi-solid medium

Treatment	2.4D (mg/l)	BA (mg/l)	Biomass (mg)	Biomass of factor
4.1	0.5	1	330e ⁽¹⁾	1.65
4.2	1	1	500cd	2.50
4.3	2	1	860ab	4.30
4.4	4	1	970a	4.85
4.5	0.5	2	260e	1.30
4.6	1	2	560bcd	2.80
4.7	2	2	770abc	3.85
4.8	4	2	850ab	4.25
CV%			11.68	10.4

Source: Author

Table 5: Effects of culture medium on embryonic callus regeneration

Treatment	BA (mg/l)	KIN (mg/l)	Number of shoot regeneration/clusters
5.1	-	-	2.6 b
5.2	0.1	-	2.0 d
5.3	-	0.1	3.2 a
5.4	0.1	0.1	2.2 c
CV%			12.4

Source: Author

Regeneration of Embryonic Callus

In all treatments (Table 5), regeneration buds on MS + kinetin medium (0.1mg/l) + sucrose (45g/l) were started. + CW (10%) after 3 weeks. However, during the follow-up process, the regeneration only occurred at 3 weeks after culture, then no more regenerative shoots appeared. The highest number of regenerated shoots reached 3.2 buds/cluster.

Effect of Tissue and Genotype on Callus Regeneration

Effect of Cultured Samples on Embryonic Callus Regeneration

On the culture medium, embryonic callus developed with additional 2,4D (2mg/l) after 15 days (Table 6). Embryonic callus arises on the cut of leaf and stem samples and soon reaches the maximum weight on the 20th day. With root samples right after 15 days, there is a phenomenon of callus development and proliferation which is also very slow. This shows that the root sample is completely unsuitable in cultivating callus. Between treatments also showed that the time to start callus development is not different. In terms of color, embryonic callus appears in the treatments where the leaf sample is mainly opalescent, slightly yellow. Callus arises from the stem and roots are golden brown, quite porous, without a clear structure; when cultured long on the environment, the callus stiffens and turns black. Observed under a microscope, the callus contains cells with small nuclei and mainly vacuoles. During prolonged culture in vitro, shoots appear to be secreted when cultured on callus, causing browning and death of the sample. Addition of PVP (500 mg/l) to a hydrogen-absorbing phenol, results in the inhibition of browning of the sample. However, only callus from leaves is able to regenerate on MS + kinetin medium (0.1mg/l) + sucrose (45g/l) + CW (10%).

Table 6. Effects of callus origin on embryonic callus regeneration			
	Origin of callus	Regeneration rate (%)	Number of shoots/clusters
	Stem	-	-
	Leaf	48.2	3.4
	Root	-	-

Source: Author

Effect of Genotype on Embryonic Callus Regeneration

Capacity of callus formation on two varieties from Con Dao and Lang Son was 44.6-42% and regenerated buds per cluster of callus was 3.6 – 3.1 shoots/clusters (Table 7).

Table 7: Effect of genotype on embryonic callus regeneration			
	Genotype	Regeneration rate (%)	Number of shoots/clusters
	<i>Chukrasia tabularis</i> A.Fuss of Con Dao	44.6	3.6
	<i>Chukrasia tabularis</i> A.Fuss of Lang Son	42.0	3.1

Source: Author

Formation and Proliferation of Callus Suspension

Proliferation of Embryonic Callus

The effect of culture medium in liquid conditions is completely different from their impact in culture conditions on agar medium. In the solution, the direct contact of the environment on all cells plus the state of being suspended and moved continuously causes the proliferation of cells to increase significantly, and the induction of substances growth also has large differences (Ziv, 2001). Research results show on (Table 8): the proliferation capacity of embryonic callus in combination 2,4D (2mg/l) + BA (1mg/l), suspension after 15 days of culture opaque with high cell density (16.47×10^4 cell/ml), many cells attach to the walls of the flask. In a liquid environment, the effect of BA is shown more clearly in significant differences on concentrations of 1mg/l and 2mg/l. MS medium supplemented with 2,4D (2mg/l) + BA (1mg/l) is an appropriate environment in multiplication of somatic suspension.

Table 8: Effect of culture medium on the proliferation of callus suspension			
Treatment	2,4-D (mg/l)	BA (mg/l)	Cell density after 15 days of culture (cellx10 ⁴ /ml)
1	0.5	1	8.43e
2	1	1	8.68e
3	2	1	16.47a
4	4	1	13.22c
5	0.5	2	12.96c
6	1	2	15.03b
7	2	2	15.28b
8	4	2	9.45d
CV%			9.92
Source: Author			

Effect of Initial Cell Density on Proliferation of Embryonic Callus Suspension

There was a significant difference in the proliferation of cell suspension in the treatment of 3.29×10^4 cell/ml compared with the treatment of 3.29×10^4 cell/ml and 4.91×10^4 cell/ml (Table 9). After 2 weeks of culture, embryonic callus were slowly proliferated for treatments with a low initial cell density of 2.47×10^4 cell/ml (3.7 fold increase) and 4.91×10^4 cell/ml (increase 3.5 times). While the average density of 3.29×10^4 cell/ml (corresponding to the volume of 20% of the initial environment) was more appropriate for somatic cell proliferation due to the increase in cell density of 5.7 times with the same period of culture.

Table 9: Effect of initial cell density on proliferation of cell suspension				
	Treatment	Cell density (cellx10 ⁴ /ml)		Proliferation factor
		Initial	After culture (15 days)	
	1	2.47	9.14	3.7b
	2	3.29	18.78	5.7a
	3	4.91	17.34	3.5b
	CV%			4.2
Source: Author				

Proliferation of Callus Suspension

Effect of Sucrose on Induction of Somatic Embryogenesis

For each crop and at different stages of embryo development, the demand for sugar and osmotic pressure varies. After 45 days of culture: The formation of somatic embryos at the concentration of 65g/l compared with the concentration of 45g/l and 55g/l is significant (Table 10). With a high concentration of sucrose (65g/l), the results of embryo production were higher than those at two lower sugar concentrations (45, 55g/l). Along this environment, blue somatic embryos, which are embryos with high regeneration potential, are formed more. Research on *chukrasia tabularis* A. Fuss somatic embryogenesis achieved the best results (37 embryos/treatment) on the medium of MS + TDZ (0.05mg/l) + 2,4D (1mg/l) + sucrose (65g/l) + CW (10%).

Table 10: Effect of Sucrose on somatic embryo induction			
Treatment	Sucrose (g/l)	Embryos/10ml cell suspension	
1	45	27.3b	
2	55	26.3b	
3	65	37.7a	
CV%		3.67	
Source: Author			

Effect of TDZ on Induction of Somatic Embryogenesis

One week of drying the cell solution then following in the next 4 weeks, the smooth cell layer covered the surface of the agar, appeared the same embryo-like structure (Table 11). Observing under a stereo microscope, it can be seen that these forms have somatic and milky somatic embryos in spherical and elongated forms. The results of embryogenesis in different treatments were significantly different, at

low TDZ concentrations (0.05; 0.1 mg/l) for high embryo production results (27.3 and 22.3 embryos/treatment). For other TDZ concentrations (0.3; 0.5mg/l) despite low embryo production results (9.3 and 10.7 embryos/treatment) but compared with control treatments (without TDZ supplementation) only 2.3 embryos are created/treatment, there are still big differences. Appropriate concentration of TDZ was 0.05 mg/l for culture of somatic embryogenesis on agar medium.

Table 11: Effects of TDZ on somatic embryogenesis			
	Treatment	TDZ (mg/l)	Number of embryos/10ml cell suspension
	1	0	2.3 d
	2	0.05	27.3a
	3	0.1	22.3b
	4	0.3	9.3 c
	5	0.5	10.7c
	CV%		5.95
Culture medium: MS + 2,4-D (1mg/l) + CW (10%) + Sucrose (45g/l)			
Source: Author			

Regeneration of Somatic Embryo

In all treatments (Table 12), regeneration buds began after 3 weeks. However, during the follow-up process, the regeneration only occurred 3 weeks after culture, then no more regenerative shoots appeared. The highest amount of regenerated buds is only 10.2 buds in a triangle. Regeneration directly from suspension had low efficiency.

Table 12: Effect of culture medium on regeneration of somatic embryos (not via activated embryo culture)				
	Treatment	BA (mg/l)	KIN (mg/l)	Number of embryos/10ml cell suspension
	1	-	-	7.4a
	2	0.1	-	3.4a
	3	-	0.1	10.2a
	4	0.1	0.1	5.3a
	CV%			22.63
Source: Author				

Regeneration of Transplanted Buds Stimulates Embryonic Induction: At this stage, embryonic cells develop into morphologies that clearly observe the states of spherical, elongated, heart and cotyledons under a microscope stereoscope. The results showed that the number of regenerated shoots on each cluster of somatic cells was significantly different in treatments. With regeneration ability in all treatments, flower slices proved to be plants capable of regenerating in vitro from somatic embryos. The results showed that (Table 13): The treatments all had regenerative buds, despite the control medium without supplementary growth stimulants. Shoots when regenerating do not appear mutation or degeneration. With the addition of growth stimulant BA (0.1mg/l), the highest regeneration results (5.89 shoots/clusters or 29.4 shoots/treatment) and long stem shoots were observed. We acquired 2,940 buds per liter of somatic embryo suspension.

Table 13: Effects of culture medium on regeneration of somatic embryos				
Treatment	BA (mg/l)	KIN (mg/l)	Number of shoots/clusters	Shoot regeneration/10ml cell suspension
1	-	-	0.32d	1.6
2	0.1	-	5.89a	29.4
3	-	0.1	2.87b	14.3
4	0.1	0.1	1.53c	7.6
CV%			14.3	12.4
Culture medium: MS + Suc (30g/l) + CW (10 %)				
Source: Author				

Multiplication of Buds Derived from Somatic Embryos: After regenerating directly from somatic embryos, multiplication of buds derived from regenerated embryos to obtain higher buds, and observe

and compare morphology with plants directly from the buds in vitro. The results showed that (Table 14): Simultaneous supplementation of BA (1mg/l), kinetin (1mg/l) and CW (10%) resulted in high multiplication of flower buds (16.3 shoots/clusters) and good stems (21.4 mm). Poor shoots (5.3 and 9.7 shoots/clusters) in treatments 9.3.1 and 9.3.2 showed that the effect of IAA in shoot shoots was unclear. Addition of IAA to the environment for root formation is not necessary when the flower plant is regenerated in vitro and easily rooted even in an environment without auxin similar to the results of Le Xuan Ai (2003). In terms of morphology, comparing with in vitro plants from buds and buds regenerated from somatic embryos, they do not have different morphology and development ability compared to buds from buds in vitro. In all treatments, shoots developed good leaves.

Table 14: Effect of culture medium on shoot regeneration from embryos						
Treatment	IAA (mg/l)	KIN (mg/l)	CW (%)	Shoot/cluster	Height of shoot (mm)	Root (+/-)
1	0.1	-	-	5.3c	17.1b	+
2	0.1	-	10	9.7b	15.3b	+
3	-	1	-	11.3b	21.8a	+
4	-	1	10	16.3a	21.4a	+
CV%				12.8	16.2	
Culture medium: MS + BA (1mg/l) + Suc (30g/l)						
Source: Author						

Conclusion

Stems and leaves of tissue culture are used as culture material: The callus is cultured on MS + BA (2mg/l) medium + sucrose (30 g/l) + CW (10%) + PVP (500 mg/l) with 2,4D supplement (2mg/l) and proliferation on MS + BA (2mg/l) + sucrose (30 g/l) + CW (10%) + PVP (500 mg/l) with 2,4D (2mg/l) supplementation. In all treatments, after 3 weeks, regenerative shoots were started on MS + kinetin medium (0.1mg/l) + sucrose (45g/l) + CW (10%). However, during the follow-up process, the regeneration only occurred at 3 weeks after culture, no more regenerative shoots appeared. The highest number of regenerated shoots reached 3.2 buds/cluster.

After 15 days of culture: On the culture medium, embryonic callus with MS + BA (2mg/l) + PVP (500 mg/l) + 2,4D (2mg/l) is added. Embryonic callus arises on the cut of leaf and stem samples and soon reaches the maximum mass on day 20. However, only callus from leaves is able to regenerate on MS + kinetin environment (0.1mg/l) + sucrose (45g/l) + CW (10%).

On MS regeneration medium + kinetin (0.1mg/l) + sucrose (45g/l) + CW (10%): After 45 days of culture: Cultured specimens are leaves for callus development and regeneration of shoot buds 42.8% and 3.2 buds/clusters of callus. Con Dao's *Chukrasia tabularis a. fuss* genotype has a higher regeneration rate and regenerative buds than Lang Son' *Chukrasia tabularis a. fuss* in 44.6-42% and 3.6-3.1 buds/callus clusters.

The proliferation of embryonic callus in MS + 2,4D (2mg/l) + BA (1mg/l), suspension after 15 days of culture is cloudy with high cell density (16.47×10^4 cell/ml), many cells attach to the walls of the flask. In a liquid medium, the effect of BA is shown more clearly in significant differences on concentrations of 1mg/l and 2mg/l. MS medium supplemented with 2,4D (2mg/l) + BA (1mg/l) is an appropriate medium in multiplication of somatic suspension. There was a difference in the proliferation of cell suspension in the 3.29×10^4 cell/ml treatment compared to the treatment of 3.29×10^4 cell/ml and 4.91×10^4 cell/ml. After 2 weeks of culture, embryonic cells were slowly proliferated for treatments with a low initial cell density of 2.47×10^4 cell/ml (3.7 fold increase) and 4.91×10^4 cell/ml (increase 3.5 times). While the average density of 3.29×10^4 cell/ml (corresponding to the volume of 20% of the initial environment) was more appropriate for somatic cell proliferation due to the increase in cell density of 5.7 times in the same period of culture.

Embryonic callus suspension is used as a culture material. Culture volume of 20% in culture medium: MS + TDZ (0.05mg/l) + 2,4D (1mg/l) + sucrose (65g/l) + CW (10%) for somatic embryogenesis achieved best results (37 embryos/treatment) after 45 days of culture. The induction medium for MS + 2,4-D embryo production (1mg/l) supplemented with TDZ (0.05mg/l) for somatic embryogenesis on agar medium resulted in high embryo production (27.3 embryos/treatment). On MS + BA medium (0.1mg/l), the highest regeneration results (5.89 shoots/clusters or 29.4 shoots/treatment) and

elongated shoots were observed (Table 9.2). We acquired 2,940 buds per liter of somatic embryonic suspension.

Acknowledgment

Thanks to the Office of Key State Programs and National Biotechnology Program KC04 for funding the implementation of the project KC04.15/06-10 (2006-2010) "Application of thin cell layer, somatic embryo and bioreactor techniques in micropropagation of economic crops".

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