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Thin Cell Layer Culture of *Chukrasia tabularis* A. Fuss and *Coffea* sp.

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Abstract: Vietnam has planning in re-forest of *Chukrasia tabularis*, a special-use forest and re-culture of *Coffea* sp., an industrial crops. The first barrier is seedlings for supplying higher demand from farmers. Using thin cell layer culture method is one of the ways to resolve seedlings of woody species. Results gave capacities using thin cell layer of shoot-tips in regeneration and using them for far propagation. *Chukrasia tabularis* A. Juss: Shoot tip from the pot was used as material to establish in vitro plant and was cultured on medium MS + BA (0.1mg/l) to rise up 5.7 shoots/sample and shoot height 12mm after 30 days cultivation. In vitro plantlets were used as materials for thin cell layer cultivation. Samples of leaves and slices of shoot tip were cultured on medium MS + BA (0.1mg/l) + CW (10%) to initiate 2.2 shoots/leaf sample and 5.8 shoots/slices of shoot tip sample. Using 2 genotypes from Con Dao and Lang Son to show not different in shoot initiation of 5.4-5.6 shoots/sample. Shoots were regenerated and rooted well on medium MS + NAA (0.1mg/l). The thin cell layer culture of *Chukrasia tabularis* A. Juss was established. *Coffea* sp.: Shoot tip from the pot plant was used as material for set up in vitro plantlet by cultiavting on medium WPM + BA (0.1mg/l) after 45 days. In vitro plantlets were used as material for thin cell layer on medium MS + Adenin (20mg/l) + kinetin (1mg/l) + BA (3mg/l) and to rise up 5.2 shoots/leaf sample (1cm^2) and 6.2 shoots/shoot tip meristem sample after 45 days cultivation. Using 2 clones of *Coffea* sp. as *C. robusta* and *C. arabica* in cultivation to imitiate 4-6 shoots/robusta leaf sample and 3-5 shoots/arabica leaf sample. Plantlets were regenerated and rooted well on medium WPM + IBA (0.6mg/l). The thin cell layer culture of *Coffea* sp. was established.

Keywords: *Chukrasia tabularis* A. Juss, *Coffea* sp., Thin Cell Layer (TCL), *C. robusta*, *C. arabica*

1. Introduction

Mineral components and plant growth regulators play an important role in the growth and development of plants, especially in vitro plants [1]. Mineral nutrient composition in each environment has different nutrient composition and concentration. Each plant species is suitable for a certain composition and concentration of mineral nutrients. Plant growth regulators are commonly used in vitro to regulate homeostasis and control morphogenesis and organogenesis in vitro [2]. The balance of growth regulators determines the in vitro culture process [3]. Controlling the composition and concentration of growth regulators determines the direction of cell and tissue differentiation in vitro [4]. The cytoskeleton is a thin piece of tissue containing several layers of cells. Thin cell players (TCLs) are commonly cultured from leaves, stems, and inflorescences [5]. The thin cell layer usually contains many types of somatic cells, interspersed with cells capable of differentiating into shoots [6]. Using TCLs culture techniques to regenerate tissues and cells into complete plants for difficult-to-regenerate plants [7]. The thin cell layer is commonly cultured from leaves, stems, and inflorescences [10]. The thin cell layer usually contains many types of somatic cells, interspersed with cells capable of differentiating into shoot [6]. There are least successful in TCLs culture of *Cymbidium* [11], *Catleya* sp. [12], *Dendrobium* sp. [13], *Rhynchostylis* sp. [14], *Phalaenopsis* sp. [15], *Panax gingseng* [16], *Brassia napus* [17], *Petunia hybrida* and *Nicotina plumbaginifolia* [18], morphogenesis [19], embrygenesis [20] and soot regeneration [21]. This paper studies the technique of thin

cell layer culture on *Chukrasia tabularis* A. Juss and *Coffea* sp.

2. Materials and Methods

2.1. Materials

The shoots of *Chukrasia tabularis* A. Juss and coffee plants were collected at 2-month-old.

The stem, leaves of *Chukrasia tabularis* A. Juss and coffee plants were collected at 20 days old in vitro.

The apical shoot tip of *Chukrasia tabularis* A. Juss and coffee plants were used for thin cell layer cultrure.

2.2. Methods

Base medium: MS [8], WPM [9].

Added: BA (6-benzylaminopurine), 2iP (2-isopentyl adenine), IAA (β-indolacetic acid), IBA (β-indolbutyric acid), NAA (α-naphthaleneacetic acid), Adenine, B1 (10mg/l), CW coconut water (10%).

Culture conditions: room temperature 26+2°C, RH = 65%, lighting time 10 hours/day, light intensity 33.3µmol/m^2/s in room, 55.5µmol/m^2/s outside course.

The experiment was arranged in full block with 3 replicates, each time 3 triangle flasks, inoculating 3-5 samples/triangle flask. The data were statistically processed using MSTATC software.

Using TCLs techniques.

3. Results and Discussion

3.1. Chukrasia Tabularis A. Juss

3.1.1. Creating Material for Thin Cell Layer Culture

Effect of Na-Hypochlorite and $HgCl_2$ on sterility of *Chukrasia tabularis* A. Juss cultured in vitro: Hypochlorite-Na (Na-hypo) can neutralize fungi on the surface but has poor antiseptic ability. Addition of $HgCl_2$ disinfectant, which has good sterility ability on the surface, combined with Na-hypo to be disinfected on many plant objects put into in vitro culture.

The results showed that (Table 1): At the concentration of 3.5-5% Na-hypo, the infection rate was the lowest and the percentage of shoots was relatively high. At the concentration of 10%, although the sample rate was higher than 0.4%, the percentage of infected samples was much higher. Hypo concentration 5% for 5 minutes combined with $HgCl_2$ 0.05% for 10 minutes; The concentration of hypo 10% for 10 minutes combined with $HgCl_2$ 0.1% for 5 minutes had a high percentage of shoots (18.2-18.9%). Sample sterility depends on sampling season: In the dry season, when using hypo at a concentration of 3.5-5% for 10 minutes, the sample infection rate is low, but in the rainy season, it is unsuccessful. In the dry season, it is not necessary to use hypo concentration of 10% because at this concentration, although the sample is less infected, the percentage of samples shootding is not high, even causing sample death. But in the rainy season, water-borne microorganisms penetrate most parts of the plant, so hypo must be used at higher concentrations and sometimes combined with $HgCl_2$ to be successful.

Effect of the base medium to culture the plant in vitro: The growth tip was the meristem region containing pre-embryonic cells capable of division, differentiation and organogenesis. This is the starting area, cells are always in physiologically young form, strong morphogenesis ability, growth apical culture is a prerequisite for creating starting materials for in vitro culture and always keeping their characteristics of the parent tree. Thinly sliced young shoots were used as culture material on different mineral media: MS, 1/2MS, B5 and WPM supplemented with BA (0.1-0.5mg/l), kinetin (0.1mg/l) and CW (10%).

Research results show that (Table 2): After sterility sprouting samples were cultured in different environments, after 20 days of culture, they all developed as: In the control medium without adding bioregulators the explants had high shoot regeneration ability, good stem elongation (11mm height), high shoot regeneration ability and normal growth and development of shoots.

Effect of macronutrients MS and BA on the ability to rapidly multiply *Chukrasia tabularis* A. Juss in vitro: young shoots were thinly sliced as culture material. The principle of the method was to use growth regulator to stimulate and regenerate shoots. Cytokinin is necessary for lateral shoot development, for some plants, it is necessary to combine with auxin, and in the culture medium, coconut water is often added to stimulate the effect of cytokinin and auxin. Mineral growth medium MS-1/2MS supplemented with BA (0.05-0.1-0.2mg/l) and CW (10%).

Research results show that (Table 3): When the medium contains BA, after 45 days of culture, there was the ability to generate lateral shoots. In MS medium supplemented with BA (0.1mg/l) showed the highest ability to regenerate shoots (8.2 shoots/sample) but slow growth of shoot height. In 1/2MS medium supplemented with BA (0.05mg/l) showed the highest ability to regenerate shoots (7 shoots/sample) but less shoot growth (14mm). In MS and 1/2MS mineral medium supplemented with BA (0.05-0.1-0.2mg/l), the shoot regeneration ability of MS mineral medium was higher than ½ MS, but shoot extension was lower.

3.1.2. Sample Culture for Thin Cell Layer

Effect of basal medium on in vitro culturing: The growth apex is the meristem region containing pre-embryonic cells capable of division, differentiation and organogenesis. This is the starting area, cells are always in physiologically young form, strong morphogenesis ability, growth apical culture is a prerequisite for creating starting materials for in vitro culture and always keeping their characteristics of the parent tree. Thinly sliced young shoots were used as culture material on MS medium supplemented with BA and coconut water.

The results showed that (Table 4): After 30 days of culture: MS + BA medium (0.1mg/l) + CW (10%) stimulated shoot formation (5-7 shoots/sample), had shoot height was 12mm,

shoot regeneration was high and shoots grow and develop normally.

Table 1. *Effect of Na-hypochlorite and $HgCl_2$ on sample sterilization.*

Hypo-Na (%)	Time (minutes)	$HgCl_2$ (%)	Time (minutes)	Number of culture samples	Number of infected samples	Infection rate (%)	Number of shoot formation samples	Shoot formation rate (%)
-	-	0.1	5	20	16	83.7	0	0.0
4	5	0.1	5	27	25	92.8	0	0.0
5	3	0.05	5	34	22	64.7	1	9.6
5	5	0.05	10	11	7	61.3	2	18.4
10	8	0.1	5	13	12	92.2	1	7.8
10	10	0.1	5	106	60	56.6	20	19.0
CV%					15.3			

Table 2. *Effect of basal medium on ability to culture Chukrasia tabularis.*

Medium	BA (mg/l)	Kinetin (mg/l)	CW (%)	Shoot height (mm)	Number of shoots	Number of leaves	Number of nodes	Leaf length (mm)	Leaf Width (mm)	Leaf development (+/-)
MS	-	-	-	15	1.5	5.5	6.5	12	6	+
MS	0.1	-	10	11	6.7	4.0	5.0	12	10	+
MS	0.1	-	-	13	3.5	4.5	5.5	19	7	+
MS	0.5	-	10	6	1.7	5.5	6.5	27	13	+
1/2MS	0.1	-	10	10	1.5	4.5	5.5	17	8	+
B5	1.0	0.1	-	10	1.0	5.5	6.5	13	4	+
WPM	0.1	-	10	5	1.0	5.5	6.5	20	9	+
CV%				4	13.4	4.3	3.6	8	6	

Table 3. *Effects of MS and BA macronutrients on the rapid multiplication of Chukrasia Tabularis A. Juss.*

Medium	BA (mg/l)	CW (%)	Shoot height (mm)	Number of shoots	Number of leaves	Number of nodes	Leaf length (mm)	Leaf Width (mm)	Leaf development (+/-)
MS	0.1	10	13	8.2	3.0	4.0	8	3	-
	0.5	10	9	10.5	2.2	3.2	8	5	-
	1.0	10	13	17.5	3.5	4.5	13	6	+
1/2MS	0.1	10	14	5.7	3.2	4.2	11	5	+
	0.5	10	12	6.2	3.2	4.2	14	5	+
	1.0	10	9	5.5	2.5	3.5	16	6	+
CV%			4	5.6	10.5	7.8	4	6	

The results showed that (Table 4): After 30 days of culture: MS + BA medium (0.1mg/l) + CW (10%) stimulated shoot formation (5-7 shoots/sample), had shoot height was 12mm, shoot regeneration was high and shoots grow and develop normally.

3.1.3. Thin Cell Layer Culture

Effect of culture samples: the young leaves and shoot tip of in vitro shoots ($1cm^2$) were sliced thinly and used as culture materials. Culture medium MS + BA (0.1 mg/l) + CW (10%). Research results show that (Table 5): After 30 days of culture: On two types of culture samples: leaves in vitro shoots and thinly sliced shoots tip were produced shoots. The number of shoots arising on leaves (2.2 shoots/sample) was lower than the number of shoots arising on apical thin slices (5.8 shoots/sample).

Effect of genotype: There are two lines of *Chukrasia tabularis* A. Juss introduced into culture from Lang Son and Con Dao. Thinly sliced apical shoots were used as culture material. Culture medium MS + BA (0.1 mg/l) + CW (10%). The results showed that (Table 5): After 30 days of culture: Shoot formation did not depend on the origin of the cultured inflorescence line. Genotype does not affect shoot development. The number of shoots rising 5.4-5.6 shoots/sample.

Table 4. *Effects of macronutrients MS and BA on the ability to rapidly multiply Chukrasia tabularis in vitro.*

Treatment	Shoots/cultivars	Shoot height (mm)	Number of leaves/shoots	Number of nodes/shoots	Leaf length (mm)	Leaf width (mm)	Leaf development (+/-)
1	8.3	13.3	3.0	3.3	8.3	3.8	+
2	17.5	9.3	2.3	3.3	8.8	5.0	+
3	5.0	13.8	3.5	4.3	13.3	6.5	+
4	5.8	14.8	3.3	4.3	11.8	5.3	+
5	6.3	12.0	3.3	4.3	14.0	5.0	+
6	5.5	9.5	2.5	3.5	16.0	6.0	+
M	24.3	39.3	24.3	0.6	20.3	5.8	-
CV (%)	24.3	51.9	24.3	20.0	37.6	46.1	-
LSD (0.05)	7.4	9.5	1.1	1.1	6.8	3.6	-

Table 5. *Effect of cultures and genotypes on shoot growth.*

Culture medium	Culture samples	Number of shoots	Shoot height (mm)
MS + BA (0.1mg/l) + CW (10%)	Thin slices of top shoot	5.8	14
	Young leaves (0.5cm^2)	2.2	12
	Con Dao	5.4	13
	Lang Son	5.6	12
CV%		10.2	8

3.1.4. Shoot Multiplication from Thin Cell Layer

The apical shoots were used as culture material on 1/2 MS basal medium supplemented with BA (0.1-0.5-1mg/l) and IAA (0.1-0.5-1mg/l). Research results show that (Table 6): after 30 days, shoots grow strongly on MS + BA (0.5mg/l) + IAA (0.1mg/l) medium.

Table 6. *In vitro shoot multiplication.*

BA (mg/l)	IAA (mg/l)	Number of shoots	Shoot height (mm)
0.1	0.1	4.6	45b
	0.5	3.2	38b
	1.0	2.4	30c
0.5	0.1	12.5	58a
	0.5	6.5	42b
	1.0	3.8	38b
1	0.1	16.4	34b
	0.5	14.8	28c
	1.0	12.6	21c
CV (%)			12

3.1.5. Regeneration, Rooting and Acclimatization

Regeneration: The germinated shoots were cultured separately on MS medium without the addition of a growth regulator that stimulated root formation. The roots appeared after 15 days of culture. After 30 days, the in vitro shoots were ready to move to the domestication stage.

In vitro rooting: Rooting culture was the final stage in the micropropagation process, which was the preparation stage to bring the plant to the domestication stage. There were plants that were easy to root, when cultured on the medium without adding growth substances, they also give rise to roots such as potato, sweet potato, acacia hybrid, eucalyptus... However, there were also species that are difficult to root like trees, in the culture medium supplemented with auxin. The medium MS and 1/2MS were used for root growth medium.

The results showed that (Table 7): the 100% rooting ability on both culture media with different macronutrients MS and 1/2 MS. On MS medium, *Chukrasia tabularis* A. Juss grew and developed better than 1/2 MS mineral medium. *Chukrasia tabularis* A. Juss was in the same family as the *Chinaberry*, the ability to root in vitro was relatively easy, the results was similar to that of the *Chinaberry* in vitro.

Acclimatization: In natural light with diffused light, the light intensity was 55.5µmol/m^2/s. Acclimatizing time 30 days. The substract was coconut fiber (Table 8).

Table 7. *In vitro rooting of Chukrasia tabularis A. Juss.*

MS	Shoots/cultivars	Shoot height (mm)	Number of leaves/shoots	Number of nodes/shoots	Leaf length (mm)	Leaf Width (mm)	Leaf development (+/-)
MS	1.0	14.6	3.5	3.3	8.3	8.8	+
1/2xMS	1.0	10.2	2.2	3.3	6.8	5.0	+
T (0.05)	ns	*	*	ns	*	*	

3.1.6. Procedure for Regeneration of Chukrasia tabularis A. Juss from Thin Cell Player Culture

Table 8. *Procedure for Regeneration of Chukrasia tabularis A. Juss from Thin Cell Layer Culture.*

No.	Content	Time	Culture medium
1	Prepare materials	30	1/2MS + BA (0.1mg/l) + CW (10%)
2	Material Breeding	30	1/2MS + BA (0.1mg/l) + CW (10%).
3	Cultivation of thin slices to create shoots	45	1/2MS + BA (0.1mg/l) + CW (10%).
4	Shoot regeneration	45	MS
5	Rooting	30	MS + NAA (0.1mg/l)
6	Acclimatization	30	Coconut fiber

3.2. Coffee Robusta

3.2.1. Creating Material for Thin Cell Layer Culture

Sterilize the initial culture from coffee plants: Sample sterilization was a very important step. It determines the starting material of in vitro propagation. Almost all samples taken from the wild are not clean, containing mud, soil, pathogens... and especially microorganisms.

Plant tissue culture medium is rich in nutrients with high sugar, mineral salts and vitamins content, very suitable for microbial species such as fungi and bacteria to grow. In particular, the growth rate of fungi and bacteria is much faster than that of plant cells. If the culture medium is contaminated with bacteria or a few fungal spores, after a short time the entire surface of the culture medium and the explant will be covered with fungi and bacteria. The rapid growth of microorganisms causes many undesirable results during the experiment. Therefore, the objective of this experiment was to determine the optimal Na-hypo disinfectant concentration (3-9%) and sterilization time (5-9

minutes) to obtain a high percentage of sterile live samples. (Table 9).

The results showed that (Table 9): The highest percentage of sterile samples was achieved when sterilizing the cultures at 7% Na-hypo concentration for a period of 7-9 minutes. Sterilization time from 5-9 minutes corresponding to treatment 1-9 gives the sample rate but sterility increased proportionally with Na-hypo concentration (3-7%), from the 9th treatment onwards, the sample rate but sterility decreased inversely with Na-hypo concentration (7-9%). In treatments 1-6, the sample rate was very low, but sterility was very low because the low concentration of Na-hypo (3-5%) was not enough to kill microorganisms in 5-9 minutes. In treatment 10, the proportion of sterile samples decreased significantly. With a time of 5 minutes, the percentage of samples contaminated with microorganisms was still quite high, while the samples began to show signs of darkening and death after sterilization. The longer the sterilization time, the higher the ability to kill microorganisms, but the number of dead samples also increased proportionally with the sterilization time, which can be clearly seen in the last two treatments (11 and 12). So, the best sterilization condition for coffee plants was Na-hypo concentration of 7% in 7-9 minutes.

Table 9. *Effect of Na-hypo concentration and sterilization time on sample sterility.*

Treatment	Na-hypo concentration (%)	Time (minutes)	Aseptic survival rate (%)
1	3	5	0.33 ^{H}
2	3	7	1.33 ^{H}
3	3	9	4.33 ^{G}
4	5	5	6.67 ^{G}
5	5	7	10.67 ^{F}
6	5	9	25.33 ^{E}
7	7	5	46.67 ^{C}
8	7	7	63.67 ^{A}
9	7	9	60.67 ^{B}
10	9	5	31.33 ^{D}
11	9	7	23.67 ^{E}
12	9	9	6.33 ^{G}
CV (%)			6.52

The influence of the base medium for the growth of coffee plants in vitro: Each plant species has different physiology and nutritional requirements are also very different. There are plants that can only live in very nutrient-rich environments, but there are also plants that only grow well in very poor nutrient and mineral environments. Therefore, the selection of medium with appropriate mineral content in in vitro plant propagation is necessary, it affects the growth and development of plants, determines the rate of in vitro propagation of seedlings.

This experiment was arranged on three base mediums, MS, WPM and LV with the characteristic mineral concentrations varying from low to high. All three experimental mediums were supplemented with BA (0.1mg/l) to stimulate shoot growth of the samples.

Research results show that (Table 10): When culturing coffee samples on WPM medium, the best shoot height growth (20.44 mm). On MS medium, the sample showed growth, but the expression was worse than on WPM medium. On LV medium, shoot height growth was the lowest (12.44 mm) but leaf formation and growth were the best in all three experimental treatments. So, WPM + BA medium (0.1mg/l) was the optimal medium in 3 experimental environments. On WPM medium, plants have the best performance and WPM will be used for the following experiments.

Table 10. *Growth capacity of coffee plants on base mediums supplemented with BA (0.1 mg/l).*

Treatment	Medium	BA concentration (mg/l)	Shoot height (mm)	Leaf growth
1	MS	0.1	15.78 ^{B}	+
2	WPM	0.1	20.44 ^{A}	+
3	LV	0.1	12.44 ^{C}	++
	CV (%)		8.53	

3.2.2. Sample Culture for Thin Cell Layer

Effect of concentration of BA on the ability to regenerate shoots of coffee plants in vitro: the plants could synthesize and adjust plant growth regulators appropriate to each growth period, growing, with weather, climate, living conditions (Oparin, 1977; Maróti Mihaly, 1976). For tissue culture in heterotrophic state, the ability to synthesize and regulate growth regulators is limited, it is necessary to add these substances to the culture medium in an appropriate amount. Depending on the type of explant, the type of plant, the growth time of the explant and the purpose of culture, it was affected by different concentrations of growth regulators. WPM culture medium supplemented with BA (0-0.01-0.03-0.05-0.1-0.3mg/l).

Table 11. *Effect of BA on the shoot-elongation of in vitro coffee samples.*

Treatment	Culture medium	BA concentration (mg/l)	Shoot height (mm)	Total number of leaves/sample (leaves)	Coefficient Breeding	Root edema
1	WPM	0	8.78 ^{E}	3.11 ^{D}	1.55	-
2	WPM	0.01	11.89 ^{D}	4.44 ^{C}	2.22	-
3	WPM	0.03	14.22 ^{C}	4.89 ^{C}	2.44	-
4	WPM	0.05	18.78 ^{B}	6.67 ^{B}	3.33	-
5	WPM	0.1	21.78 ^{A}	9.11 ^{A}	4.45	+
6	WPM	0.3	14.00 ^{C}	4.44 ^{C}	2.22	++
CV (%)			9.54	18.2		

Research results showed that (Table 11): From treatment 1-5, shoot height of coffee samples increased proportionally with BA concentration. The sprouting process of the sample affected the ability to produce leaves of coffee plants in vitro,

the total number of leaves also tended to increase in direct proportion to the concentration of BA. In 2 treatments 5-6, BA's ability to stimulate shoot growth tended to decrease, the total number of leaves on the plant also decreased, and the explants showed root edema. At low concentrations, most growth stimulants promote plant growth. Treatment 1-5 corresponds to a concentration of BA from 0-0.1mg/l, the concentration of BA was relatively low, so BA stimulates coffee plants to develop shoot height, elongated shoots, causing young leaves to wrap around. The shoot opens and grows, forming nodules on the shoot. In treatment 5, there was a phenomenon of root edema, although not much, but this proves that BA at 0.1mg/l concentration causes the sample to grow in the direction of callus formation, which is not good for shoot multiplication. However, in treatment 5, shoot height (21.78 mm) and total number of leaves (8.22 leaves) were still the highest among treatments.

In treatment 6, the shoot height decreased significantly (14mm), the total number of leaves of the sample also decreased (4.44 leaves), accompanied by obvious root edema and underdeveloped plants. This phenomenon proves that the concentration of BA used in treatment 6 was too high. With BA concentration of 0.3mg/l, the sample in treatment 6 inhibited the growth of shoot height as well as the total number of leaves. In vitro propagation techniques of coffee trees, shoot height and total number of leaves were very important because it determines the propagation coefficient of the plant. The process of growing shoots entails the formation and development of leaves. The more leaves, the more nodes, the total number of nodes per sample was the propagation coefficient of the plant. Therefore, the concentration of BA capable of stimulating the growth of shoot height and total number of leaves in the sample was the most significant in this experiment (Table 11).

WPM + BA medium (0.1mg/l) was the most optimal medium, capable of stimulating coffee shoots to grow tall (21.78 mm) and form a large number of leaves (8.22 leaves). The leaves were used for thin cell layer culture.

3.2.3. Thin Cell Layer Cultures

Effect of BA on organogenesis of coffee plants through leaf culture: The cultured samples were in vitro coffee shoot leaves which were micropropagated for the purpose of physiological rejuvenation of cultured leaf samples. The cultured leaf samples were cut into thin strips measuring 0.5cm^2. Leaves were cultured on basic mineral medium: MS + Adenin (20mg/l) + kinetin (1mg/l) supplemented with BA (1-3-5mg/l). Research results show that (Table 12): After 45 days of culture: The appropriate culture medium for direct shoot regeneration from leaves was the medium: MS + Adenin sulfate (20mg/l) + kinetin (1mg/l) supplemented with BA (3mg/l). Shoots appeared after 45 days of culture; no somatic cells appeared on the cut. Regeneration frequency of 1-3 shoots per 1cm^2 leaf culture.

Effect of genotype: Using young shoots at the top of growth, thinly sliced and used as materials for culturing two lines of coffee with *C. robusta* and *C. arabica*. Culture medium: MS + Adenin (20mg/l) + kinetin (1mg/l) + BA (3mg/l). Research results show that (Table 12): After 45 days of culture: Shoots arising on two genotypes were put into culture: coffee with *C. robusta* and *C. arabica*. The number of shoots arising on *C.* robusta (4-6 shoots/acre) was higher than the number of shoots arising on *C. arabica* (3-5 shoots/acre) in absolute value. But there was not much difference in statistics.

Table 12. *Effect of BA on squamous cell shoot proliferation.*

Culture medium	BA (mg/l)	Culture samples	Number of shoots/samples
WPM + kinetin (1mg/l) + Adenin (20mg/l) + IBA (0.1mg/l)	1	Young leaves	0.0
	2	Young leaves	1.6
	3	Young leaves	5.4
	4	Young leaves	0.0
	5	Young leaves	0.0
	3	Young leaves of *Coffea arabica*	5.2
	3	Young leaves of *Coffea robusta*	4.4

Table 13. *Effect of coconut water (CW) on the growth of coffee plants in vitro.*

Treatment	Culture medium	BA concentration (mg/l)	CW (%)	Shoot height (mm)	Total number of leaves/sample (leaves)	Replication coefficient	Root edema
1	WPM	0.1	0	19.56 ^{C}	8.22 ^{C}	4.11	+
2	WPM	0.1	5	21.78 ^{B}	9.33 ^{B}	4.67	+
3	WPM	0.1	10	25.67 ^{A}	12.67 ^{A}	6.35	-
CV (%)				6.18	10.64		

3.2.4. Shoot Multiplication from Thin Cell Layers

Effect of coconut water (CW) on the in vitro propagation of *C. robusta*: Coconut water is a nutrient containing a full range of organic ions, nitrogen components, amino acids, enzymes, and inorganic acids. muscles, vitamins, sugars, especially alcohol-based simple sugars, growth regulators and several substances necessary for seedlings. Thus, coconut water is a very nutritious ingredient that can fully meet the needs of cells in plant tissue culture. The addition of coconut water in tissue culture is also significant in reducing the concentration or replacing the growth regulators, thereby reducing the variations caused by the growth regulators. The aim of this experiment was to study the effects of coconut

water supplementation with a combination of growth regulators on in vitro propagation of coffee. Culture medium WPM + BA (0.1mg/l) supplemented with CW (0-10%).

Research results (Table 13): showed that in vitro *C. robusta* were capable of sprouting on all 3 experiments with coconut water concentrations varying from 0-10%. There was a huge difference between the treatments with coconut water and without coconut water. The shoot height measured on the medium without coconut water was the smallest (19.56mm). The higher the concentration of coconut water, the stronger the shoot growth. The shoot height of coffee plants in treatment 3 was the highest (25.67 mm) and the best of all treatments, especially in this treatment, there was no sign of root edema. Total number of leaves per sample in this experiment tended to increase in proportion to the concentration of coconut water with the highest number of leaves in treatment 3 (12.67 leaves).

Coconut water has a great influence on the growth of coffee plants in vitro. The higher the concentration of coconut water added to the culture medium, the better the plant performance. The medium WPM + BA (0.1mg/l) + CW (10%) was suitable for the growth of coffee plants in vitro.

3.2.5. Regeneration, Rooting and Acclimatization

In vitro root culture of *C. robusta*: Roots are an important part of the plant. It has the effect of absorbing water, mineral salts and nutrients to provide plants with growth and development. Therefore, in creating a complete in vitro plant, we must pay attention to the root system of the plant, find a suitable environment for the formation and development of roots. In addition, giving plants a healthy root system is also very meaningful in increasing the survival rate of the plants when they go to the nursery because when transferring the seedlings from in vitro conditions to the nursery, the plants must endure the change of the plant. There are great changes in environmental conditions such as changes in nutrient environment, temperature, humidity, light, etc., so the tree is easy to die.

In plant tissue culture, most of the auxins play a role in stimulating the rooting ability of plants in vitro. WPM root growth medium supplemented with IBA (0-0.9mg/l).

Research results show that (Table 14): After 45 days of culture: IBA concentration (0-0.3mg/l) was completely incapable of stimulating coffee plants to create roots (-), at a concentration of 0.6 and 0.9mg/l, IBA could stimulate rooting (+; ++). However, there was a big difference between the two concentrations of IBA in the process of stimulating rooting of coffee plants in vitro.

With IBA (0.6mg/l) the stimulation of rooting on coffee plants in vitro was very good (++). Roots grow long, large, healthy, giving rise to many branched roots. With IBA 0.9mg/l proved to be less effective for rooting stimulation. The phylogenetic roots of the coffee plant in vitro are pale white, very young and weak, the root fibers were very small and thin, the parts exposed to the experimental environment have root edema, which was a sign of callus formation. WPM + IBA (0.6mg/l) was the medium with the best ability to stimulate rooting in coffee plants.

Acclimatization: In natural light with diffused light, the light intensity was 55.5µmol/m^2/s. Acclimatization time 30 days. The substrate was coconut fiber (Table 15).

Table 14. *Effect of IBA on in vitro coffee root development.*

Treatment	Culture medium	IBA concentration (mg/l)	Possibility of rooting	Root edema
1	WPM	0.0	-	-
2	WPM	0.3	-	-
3	WPM	0.6	+ +	-
4	WPM	0.9	+	+

3.2.6. Process of Regeneration of Coffee sp. from Thin Cell Layer Culture

Table 15. *Process of Regeneration of Coffee sp. from Thin Cell Layer Culture.*

No.	Content	Time	Culture medium
1	Prepare materials	30	WPM + BA (0.1mg/l)
2	Material Breeding	30	WPM + BA (0.1mg/l)
3	Thin slice culture produces	45	WPM + BA (3mg/l) + kinetin (1mg/l) + Adenin (20mg/l)
4	Regeneration	45	WPM + BA (0.1mg/l).
5	Rooting	30	WPM + IBA (0.6mg/l)
6	Acclimatization	30	Coconut fiber

4. Conclusion

Chukrasia tabularis A. Juss: MS + BA medium (0.1mg/l) was suitable for high shoot regeneration, shoot extension, normal growth and development of shoots. On MS + BA (0.1mg/l) + CW (10%) medium: In vitro thinly sliced apical shoots were generated shoot. Shoot formation was independent of the origin of the cultured sample. Genotype does not affect shoot development. The technology of culturing thin cell layer of *Chukrasia tabularis* A. Juss has been developed.

Coffea sp.: Young shoots of potting plants were used as in vitro shoot culture materials and cultured on WPM + BA medium (0.1mg/l). The suitable culture medium to regenerate shoots directly from tissue cultured shoots was the medium: MS + Adenin (20mg/l) + kinetin (1mg/l) + BA (3mg/l). The number of shoots arising directly on the leaves was lower

than those arising on thin slices of the apical shoots. Shoots arising on *C. robusta* was higher than the number of shoots arising on *C. arabica*. *Coffea* sp. with root regeneration on WPM + IBA. The technology of culturing thin cell layer of *Coffea* sp. has been developed.

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