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IN VITRO MICROPROPAGATION PROTOCOL AND ACCLIMATIZATION OF COFFEE TREES *(Coffea arabica L.)*

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ABSTRACT

This study was carried out on coffee plants *(Coffea arabica L.)* during 2009/2010 season. This work was conducted to investigate the effect of different MS medium strengths (Full, ¾ and ½ strength) in starting stage on survival percentage of shoot tip explants, and the effect of different concentrations of benzyl adenine (2.0, 4.0 and 6.0 mg/l) and kinetin (2.0, 4.0 and 6.0 mg/l) on proliferation rate (shoot length, no. of shoots and leaves). In addition to study the effect of different IBA concentrations (1.0, 2.0 and 3.0 mg/L) on rooting (roots no. and length) and growth improvement (plantlets length and leaves no./ plantlets) of coffee plantlets. Also, Plantlets were acclimatized in acclimatization stage for 3 months in different growing media including sand, peat moss, sand : peat moss at 1:1 v/v and sand : peat moss at 1:2 v/v for producing good coffee offshoots.

At starting and multiplication stages, significant differences were observed on survival % of explants and proliferation rate as affected by different MS medium strengths and BA or Kinetin treatments. Full strength of MS Medium supplemented with 6 mg/L BA or 6 mg/L kinetin gave the best significant result for survival % of explants and multiplication rate (shoot length, no. of shoots and leaves).

At rooting stage, half strength MS medium with IBA (3 mg/L) gave the best significant result for coffee plantlets growth and root formation (length of plantlets and roots and no. of leaves and roots /plantlet). Thereafter, coffee Plantlets were hardened for 3 months during acclimatization stage and found that the growing media including sand : peat moss at 1:2 v/v recorded the best parameters measured in this stage.

Keywords: Micropropagation, Coffee, in vitro, MS medium strength, BA, Kinetin, Acclimatization.

INTRODUCTION

Coffee tree (*Coffea arabica* L.) is currently grown as a major crop in tropical and subtropical regions of Africa and Asia; south and central America, Papua New Guinea, the Caribbean and the Hawaiian Islands.

Coffee tree is one of the most important agricultural products in the international markets. Botanically, coffee belongs to the family Rubiaceae.

In Egypt, coffee trees is grown in EL-Kanater EL- Khairia Research Station farm, Kalubia governorate since year 1979 under mango trees shadow. Whereas, small areas of coffee trees is grown in private orchards in EL- Behaira and Giza governorates Abd Rabou *et al.*, (2011) .

Recently in vitro culture has played an important role in agriculture and plant science. This method allows the production of large number of genetically identical plants which can be produced from a single mother stock Shibli, *et al.*; (1997). Plant production via tissue culture is advantageous over traditional propagation methods because it leads to the production of disease and virus- free plants Shibli, (1995); Gowen, (1995); Oliveira *et al.*, (2000)

and Ibrahim , (2003). Various approaches have been considered for in vitro multiplication of coffee apical meristem and axillary bud culture Cameiro and Ribeiro, (1989). Multiplication stage significant differences were observed on the multiplication rate and shoot length as affected by BA, K and MS strenge Ibrahim, (2003).

In vitro vegetative propagation of cultivated coffee were reported by Staristsky (1970); Zok (1986); Carneiro and Ribeiro (1989); Santana *et al.*,(1989); Pasqual and Barros (1991); Haidar (1993); Kahia (1993); Ganesh and Sreenath (1997); Quirzo *et al.*;(2002) and Ebrahim *et al.*;(2007).

The ultimate goal of this investigation was to develop a micropropagation protocol and acclimatization for coffee trees.

MATERIALS AND METHODS

This study was carried out in tissue culture laboratory of horticulture Research Institute throughout season 2009/2010 on coffee plant (*Coffea arabica* L.)

Starting stage:

coffee explants mentioned above were taken and soaked in running water for 6 hours then soaked for 12 hours in solution of citric acid at 200 mg/L + ascorbic acid at 100 mg/L. Then the explants were sterilized for 15 min in 20% sodium hypo- chlorite solution (chlorox, 5.25 active ingredient NaOcl) with a few drops of tween- 20 were added as wetting agent followed by 10 min. in Iodine chloride 1.0% (w/v). The disinfected explants were rinsed three times with sterile distilled water.

Shoot tips of about (0.5cm) in length were cut from disinfected explants and cultured on different strengths from MS basal salts medium (Murashige and Skoog, 1962) (full- three- quarter or half) respectively, supplemented with thiamine-HCL 0.1 mg/l, pyridoxine-HCL 0.5 mg/l, nicotinic acid 0.5 mg/l, glycine 2 mg/l , myo-inositol 100 mg/l, citric acid 200 mg/l , sucrose 20g/L and solidified with 7 g/L agar, in addition to 1g/L activated charcoal. Media were autoclaved 121°C under pressure of 1.2 kg / cm^2 for 15 min.

Each treatment= 3 replicates and each replicate containing 10 culture jars and each jar containing 3 explants.

All culture jars in this research were incubated on the growth room at 25 ± 2°C, 2000 Lux for 16 hr /8 has light / dark day provided with cool white florescent lamps.

The survival % was recorded after four weeks of culturing date.

Proliferation stage:

The following experiments were conducted as follows:

1- Different strengths from MS basal salts medium (full- three- quarter or half) respectively.
2- MS medium supplemented with different concentrations of benzyl adenine (2.0, 4.0 or 6.0 mg/L).
3- MS medium supplemented with different concentrations of Kinetin (2.0, 4.0 and 6.0 mg/L).

The explants have been transferred every 4 weeks into fresh nutrient medium for 3 subcultures (proliferation stage).

Data were recorded proliferation rate (no. of shoots, shoot length and no. of leaves /shoot).

Rooting stage:

The following experiments were conducted in rooting stage:

MS medium (half strength) was supplemented with IBA (1.0, 2.0 and 3.0 mg/L). The plantlets length and roots formation (number and length) were recorded after six weeks of transferring coffee shoots to rooting media.

Acclimatization stage:

The plantlets were removed from the culture jars and then the plantlet roots were washed with tap water to remove the agar from the roots. The plantlets were individually transplanted to plastic pots (5 cm diameter) filled with different growing media including sand , peat moss, sand : peat moss at (1: 1) and sand : peat moss at (1: 2) which had been treated with fungicides and irrigated at need. Pots were covered with transparent polyethylene sheet during the first month then was gradually removed at the end of this month. In the beginning of second month, pots were transferred to the greenhouse and kept without polyethylene cover for two months. During this period, the plants were watered every 2 days with tap water and once per week with compound Rosasol fertilizer which consisted of N.P.K (19.19.19) + 1 MgO + micro elements (as 260 ppm Fe – 75 ppm Cu – 230 ppm Zn – 320 ppm Mn – 100 ppm B) at 1.0 g /l of irrigation water. Fifteen pots each contained one plantlet were represented in three replicates. The survival percentage, plant height (cm) and number of leaves / plant were recorded after two months from transplant date.

From the statistical point of view, the completely randomized design was adopted. The obtained data were statistically analyzed according to Snedecor and Cochran (1980). The mean were compared by using the method of new least significant differences (New L.S.D at 0.05%) described by Waller and Duncan (1969).

RESULTS AND DISCUSSION

Starting stage:

Effect of MS medium with different strengths (full, ¾ and ½) on survival percentage of explants was shown in Table (1) and Fig (1-a). MS salt strength significantly affected the survival percentage of explants. The best medium to increase survival % was MS basal medium at full salt strength (4.4 g MS/L), while the lowest one was half salt strength of MS medium (2.2 g /L). Similar results were obtained by Quirzo *et al.*, (2002) and Ibrahium (2003).

Table (1): Influence of different types of medium strength on survival in vitro coffee Arabica

Medium strength	Survival %
Full strength MS	52.33
¾ strength MS	44.00
1/2 strength MS	21.67
New LSD 5%	**2.39**

Multiplication stage:

Effect of MS medium different strength, BA, or Kin at different concentration on multiplication rate and shoot length:

Table (2) and Fig (1-b) represent the effect of various factors MS salt strength, BA, Kin at different concentrations through three subcultures. MS salt strength significantly affected multiplication rate. MS medium at full strength was the best medium to increase no. of shoots (1.33), shoot length (4.00 cm) and no. of leaves (3.33). On the other hand, the lowest no. of shoots (0.63), shoot length (2.61 cm) and no. of leaves (2.33) was found when explants were cultured on MS medium contained half-salt strength (2.2 g /L).

The obtained results are in agreement with those of Quirzo *et al.*; (2002) and Ibrahium (2003).

Table (2): Influence of different types of medium strength on shoot length, no. of leaves and no. of shoots in vitro *Coffee arabica*.

Parameters / Medium strength	No. of shoots	Shoot length (cm)	No. of leaves/ explant
Full strength MS	1.33	4.00	3.33
3/4 strength MS	1.33	3.72	2.67
1/2 strength MS	0.63	2.61	2.33
New LSD 5%	**0.18**	**0.84**	**1.51**

Data in **Table (3)** and **Fig (1-c)** show that proliferation rate were affected by BA concentration. Increasing BA concentration caused a significant increase in the multiplication rate. Multiplication rate was significantly increased by increasing BA concentration.

Table (3): influence of different BA concentrations on shoot length, no. of leaves and no. of shoots in vitro Coffee Arabica.

Parameters / BA Conc.	No. of shoots	shoot length (cm)	No. of leaves
2 mg/L	2.52	1.56	3.33
4 mg/L	2.22	3.22	5.67
6 mg/L	2.88	4.56	6.67
New LSD 5%	**0.32**	**0.34**	**1.11**

Multiplication rate no. of shoots, shoot length and no. of leaves was affected by BA concentration. The highest multiplication rate no. of shoots (2.88), shoot length (4.56), and no. of leaves/ explant (6.67) was noticed for MS medium supplemented with 6.00 mg/L BA. On the other hand, the lowest shoot length (1.56 cm), no. of shoots (2.52) and no. of leaves (3.33) were recorded for MS medium supplemented with 2.0 mg/ L BA. In agreement with obtained herein results Kahia, (1993) and Haidar, (1993).

Data in Table (4) reveal the effect of Kin concentrations (2.0, 4.0 and 6.0 mg/L) on multiplication rate. The highest value of shoots no. (1.64), shoot length (2.56 cm) and leaves no. (4.67) were obtained when Kin added to the medium at 6.0 mg/L. While the lowest ones were detected with 2.0 mg/L Kin treatment. Analogical results were obtained by Zok, (1986).

Table (4): influence of different kinetin concentrations on no. of shoots, shoot length and no. of leaves/ explant of coffee Arabica.

Parameters / kinetin Conc.	No. of shoots	shoot length (cm)	No. of leaves/ explant
2 mg/L	1.13	1.43	2.00
4 mg/L	1.41	2.41	2.83
6 mg/L	1.64	2.56	4.67
New LSD 5%	**0.41**	**0.59**	**0.90**

Rooting stage:

Effect of IBA concentrations on growth and rooting of coffee plantlet cultured in vitro. Data in Table (5)and Fig (1-d) observed that, increasing IBA concentration caused a significant increase in the plantlet length, number of leaves/ plantlet. Increasing IBA concentration gradually increased plantlets length (1.67, 3.22, 4.37 cm /plantlet) and no. of leaves/ plantlet (2.67, 4.33, 6.33) respectively. As well as, data presented in this table show that explants from shoots of coffee were cultured in vitro in MS rooting medium supplemented with IBA (1.0, 2.0, 3.0 mg/L). The highest significant average number of roots and root length/plantlets produced when shoots were cultured on MS medium supplemented with 3.0 mg/L IBA, while the lowest value was produced on MS supplemented with 1.0 mg/L IBA. In agreement with the obtained herein results Ganesh & Sreenath (1997) and Ebrahim *et al.*, (2007) on coffee plants.

Table (5): Influence of different IBA concentrations on plantlets length, no. of leaves/ plantlets and no. of nods in vitro *Coffee arabica*.

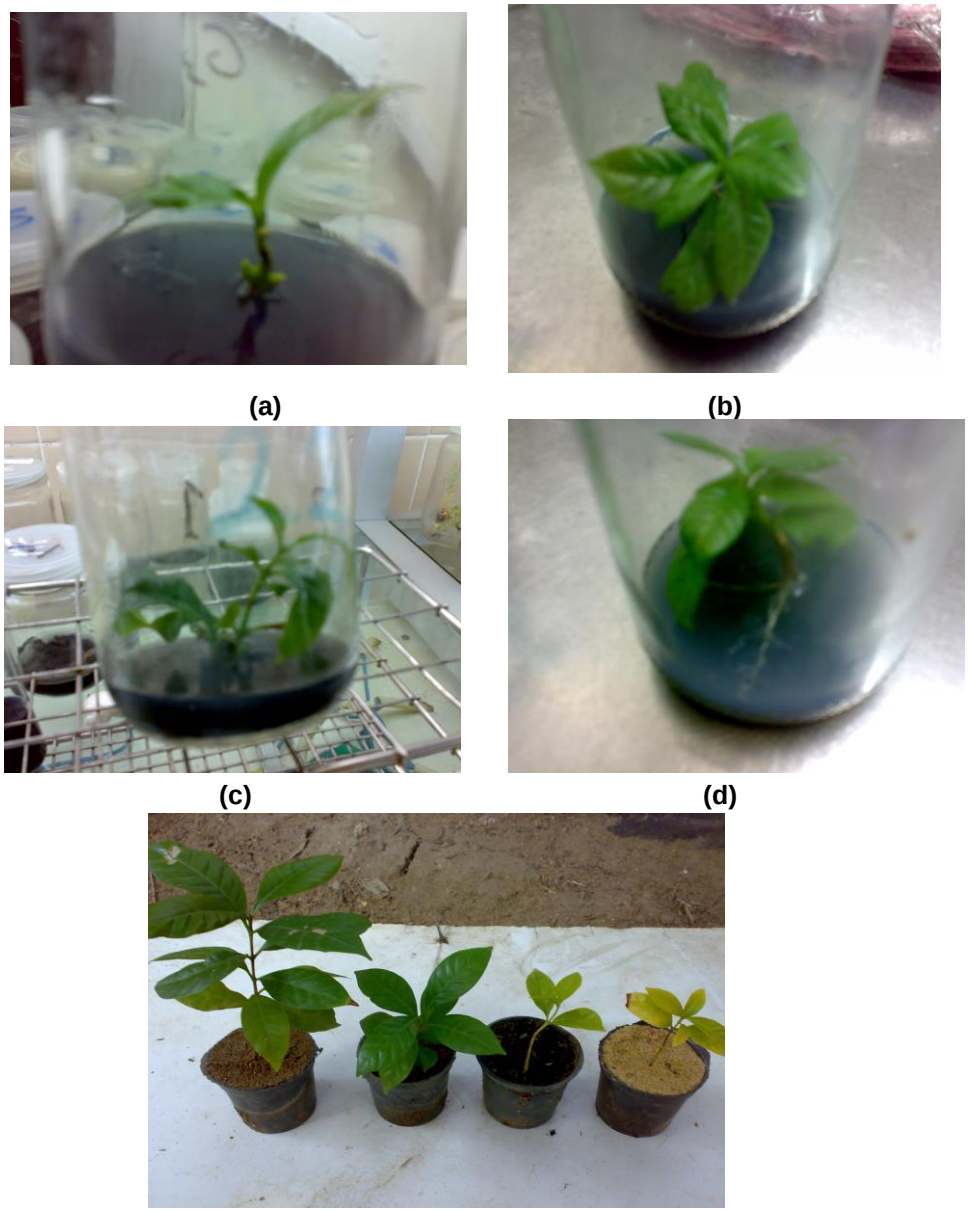
Parameters / IBA Conc.	Plantlets length (cm)	No. of leaves/ plantlet	no. of roots/plantlets	root length
1 mg/L	1.67	2.67	1.67	1.32
2 mg/L	3.22	4.33	2.33	2.57
3 mg/L	4.37	6.33	3.67	3.44
New LSD 5%	**0.54**	**0.76**	**1.51**	**0.30**

Acclimatization stage:

he results presented in Table (6) and Fig (1-e) show that, raising sand and peat moss mixture from 1:1 to 1:2 caused increase in survival % of plants, plant height (cm), plant thickness (cm) and number of leaves/ plant. Also, it can be noticed that the mixture of sand : peat moss (1:2 v/v) recorded the highest values of survivals % and plantlets height. These results go along with those of Arwa *et al.*,(2000), EL -Sherbini, (1991) on pineapple .

Table (6): Effect of culturing media on survival percentage, plant height (cm), and number of leaves/ plantlets of coffee Arabica plantlets:

Treatment / Culturing media	Survival %	Plant height (cm)	Plant thickness (cm)	No. of leaves/plant
Peat moss	48.33	5.00	0.40	3.67
Sand	51.00	4.24	0.33	3.33
Sand + peat 1:1(v/v)	71.67	6.34	0.47	6.67
Sand + peat 1:2 (v/v)	92	7.00	0.57	5.67
New LSD 5%	**3.90**	**0.06**	**1.30**	**0.15**



(a) **(b)**

(c) **(d)**

(e)

Fig. (1): In vitro micropropagation of coffee
(a) Starting stage (b) Multiplication stage (c) Multiplication stage
(d) Rooting stage (e) Acclimatization stage

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بروتوكول الأكثار الدقيق و الأقلمة فى اشجار البن (البن العربى)

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أجريت هذه الدراسة على نباتات البن العربى خلال موسمى ٢٠١٠/٢٠٠٩ و ذلك بهدف التوصل إلى بروتوكول لإنتاج نباتات البن العربي بواسطة تقنية الإكثار الدقيق. ففى مرحلة بداية الزراعة تم دراسة تأثير قوى مختلفة لبيئة موراشيج وسكوج (قوة كاملة ، ٤/٣ قوة ، ٢/١ قوة) على نسبة بقاء الأجزاء النباتية (قمم نامية) على قيد الحياة ووجد أن أفضل قوة بيئة في هذا الشأن هي القوة الكاملة من بيئة موراشيج وسكوج. أما في مرحلة التضاعف والتي تم فيها دراسة تأثير نوعين من السيتوكينينات هما البنزيل ادينين والكينتين بتركيزات (٢، ٤ ، ٦مللجم / لتر) على معدل تضاعف أفرخ البن الخضرية (طول الفرخ الخضري ، عدد الأفرخ الخضرية ، عدد الأوراق/فرخ خضري) ، فلقد وجد أن بيئة موراشيج وسكوج بقوة كاملة والمحتوية على إما البنزايل أدنين أو الكينتين بتركيز ٦ ملجم من أيا منهما/ لتر بيئة أعطت أفضل القيم للصفات المدروسة في هذا المجال. أما فيما يتعلق بمرحلة التجذير فلقد تم دراسة تأثير اندول حمض البيوتريك بتركيزات ١ ، ٢ ،٣ ملجم /لتر على معدل تجذير وتحسين نمو نبتات البن وتبين من النتائج المتحصل عليها أن استخدام اندول حمض البيوتريك بتركيز ٣ ملجم/ لتر بيئة قد حقق أفضل النتائج بالنسبة لمعدل تجذير ونمو نبتات البن الناتجة. وأخيرا في مرحلة الأقلمة تم إجراء عملية تقسية لنبتات البن الناتجة بزراعة الأنسجة خلال ثلاثة أشهر على بيئات نمو مختلفة تتضمن الرمل منفردا والبيت موس منفردا ومخلوط من الرمل : البيت موس بنسبة (١ : ١) وكذلك مخلوط من الرمل : البيت موس بنسبة (١ : ٢) ولقد تبين من النتائج المتحصل عليها أن بيئة النمو المحتوية على خليط من الرمل : البيت موس بنسبة (١ : ٢) قد أعطت أفضل النتائج المسجلة خلال هذه المرحلة.

قام بتحكيم البحث

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