Micropropagation of Four Coffee Cultivars (*Coffea arabica* L.) from Yemen through Shoot Tip Culture

Ameen, A. AL-Azab[1]; Sanad, S. M. Habib[2]; Mohammed, A. Hussein[3] and Fadia El-Sherif[4*]
[1,2,4] Horticulture Dept., Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt
[3] Botany Dept., Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt.
[*] Department of Biological Sciences, Faculty of Science, King Faisal University,
P.O. Box. 380, Al-Ahsaa 31982, Kingdom of Saudi Arabia.

Received: *20/11/2015*

Abstract: The aim of this study is to optimize *in vitro* multiplication of *Coffea arabica* cvs 'Benan',' Burai',' Odayni' and 'Odayni- Bayat'. Seeds germinated on modified quarter MS medium with 1.0 g l^{-1} activated charcoal gave the highest germination percentage of the four cultivars under study. BA at 8.0 mgl^{-1} was the most effective concentration with an average of 14 shoots per explant, resulting in better and morphologically superior microshoots in 'Benan' cv. However 'Burai' ,'Odayni' and 'Odayni- Bayat' cultivars, showed the highest number of shoots when they were grown on MS medium supplemented with BA at 4.0, 2.0 and 6.0 mgl^{-1} respectively. Root formation was 100% in response to the application of IAA, IBA and /or NAA to the culture media for the four cultivars. Half MS supplemented with NAA at 2.0, 1.0 and 3.0 mgl^{-1} was found to be a suitable medium for root induction in excised micro-shoots, of 'Odayni', 'Burai' and 'Odayni-Bayat' cultivars, respectively. The plantlets were successfully acclimatized in the greenhouse; consequently the survived plants reached 100%.

Keywords**:** *In vitro*, Benzyladenine, *Coffea arabica*. 'Benan', 'Burai', 'Odayni' and Odayni-Bayat, micropropagation,

INTRODUCTION

Coffee belongs to the family Rubiacea. The genus Coffee includes at least 64 species grouped into four sections (Carvalho and Monaco, 1969). Commercially, only two out of more than 100 coffee species are cultivated, the *C. arabica* (Arabica) and *C. canephora* (Robusta) (Pierson *et al*., 1983; Juma *et al*., 1994; Carneiro, 1997). The most important species is *C. arabica*. In Yemen, there are many coffee cultivars and they are named according to their place of origin (Robinson and Brian, 1993). There are many countries which depend in their economy on the coffee exportation (Juma *et al*., 1994). Quality beverage is produced from *C. arabica* which is cultivated at higher altitudes. This species represent 70% of the commercial coffee of the world and about 99% of Latin American production.

Traditionally the propagation of this species is made through cuttings and seeds, both of these methods of propagation have disadvantages and limitations in production of clones in large number (Ismail *et al*., 2003). Seed propagation involves different problems such as, low germination rate, slow initial growth and short life span while vegetative cuttings guarantees uniformity seeds (Monaco *et al*., 1995; Ismail *et al*., 2003).

In vitro cultivation has relevance in the clonal propagation of valuable or endangered plant germplasm, and in the production of transgenic plants (Johnson and Emino, 1979; Smith *et al*., 1991; Frederic *et al.,* 2007). To date, micropropagation techniques are employed to produce a large number of new and true-to-type plants in a relatively short period. It has already proved to be successful for several members of the Rubiacea family (Pierson *et al*., 1983; Carneiro and Ribeiro, 1989; Haidar, 1993; Ismail *et al*., 2003; Ebrahim *et al*., 2007; Almeasary, 2008). However, genotypic difference in shoot forming capacity in relation to the cytokinin level in the medium is not well documented.

The main aim of the present study is to find an efficient and simple method of *in vitro* clonal propagation using shoot tip explant for producing large numbers of Yemeni coffee cultivars,*Coffea arabica* cvs 'Benan', 'Burai', 'Odayni' and 'Odayni- Bayat'.

MATERIALS AND METHODS

Plant material and seed germination

Seeds: Healthy 12 years old coffee (*C. arabica*) trees grown in Yemen Republic were used as a source for seeds. Fruits of the four, *Coffea arabica* cvs 'Benan', 'Burai', 'Odayni' and 'Odayni-Bayat' were harvested in October, 2013. Seeds were obtained from mature and ripen fruits. The fruit crusts were separated from the pulp and seeds were washed with running water to remove the article horny. Seeds were then air dried under shade until having approximately 13% moisture content, which is suitable for storage.

The experiments were conducted on March 1st 2014 until May1st 2015 at the Tissue Culture Lab, Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia - Egypt. The *Coffea arabica* cvs 'Benan', 'Burai', 'Odayni' and 'Odayni-Bayat' were used in this study.

Surface sterilization

Seeds were soaked in sterilized tap water for 24 hours, surface sterilization went through rinsing seeds with 70% ethanol solution for 30 s, and then were rinsed in 20% (v/v) sodium hypochlorite solution for 25 min. The seeds were then washed three times with sterile tap water under laminar air-flow hood to remove all the traces of sodium hypochlorite.

Sterilized seeds were cultured into 40 ml capacity jars containing 10 ml quarter strength MS (Murashige and Skoog, 1962) basic salts and vitamins,

Corresponding author: Felsherif@Fku.edu.sa

supplemented with 20% (w/v) sucrose, 7 gl^{-1} agar, 1.0 gl^{-1} charcoal and the pH was adjusted to be 5.7 before autoclaving at 121°C and 1.2–1.3 kg/cm^2 pressure for 20 min. One hundred seeds were cultured, one seed per jar. The cultures were incubated under growth room conditions (22 ± 2°C, at dark). After germination, the seedlings were transferred into 16 h photoperiod with a light intensity of 4000 lux provided by florescent lamps (Phillips TLM 40W/33RS). The rate of germination was determined after 8 weeks from seed culture.

Effects of BA on shoot induction

After 12 weeks of seedling growth, shoot tips with the height of (0.5 cm) from *in vitro* culture explants were cultured onto 200 ml capacity jars containing 40 ml MS medium supplemented with 3% (w/v) sucrose, 7.0 gl^{-1} agar and different concentrations of benzyl adenine (BA) viz, (0.0, 1.0, 2.0, 4.0, 6.0 and 8.0 mgl^{-1}). Medium was adjusted to pH 5·7 prior to autoclaving at 121°C and 1.2–1.3 kg/cm^3 pressure for 20 min. Every jar contained one explant and each treatment had ten replicates. The cultures were grown for three months before data recording based on number of shoots, plant height, number of leaves/shoot clump as well as the culture fresh weight.

Rooting of shoots

For rooting, six-months-old *in vitro* shoots (0.5–1.0 cm in length) were excised and transferred to 200 ml capacity jars containing 40 ml half strength MS medium supplemented with 15 gl^{-1} sucrose, 7.0 gl^{-1} agar and different concentrations of indole 3-acetic acid (IAA), Indolebuteric acid (IBA) or NAA (0.0, 0.5, 1.0, 2.0 and 3.0 mgl^{-1}) each. The cultures were placed under the same previously mentioned growth room conditions for root formation. After 8 weeks of culture, data were recorded based on the number of roots, root length, plant fresh weight and plant height.

Acclimatization of plantlets

The rooted shoots were carefully removed from the culture jars, then washed with running tap water to remove residual agar around the roots. Followed by washing with fungicide (Rizolex 1%) to reduce the fungal contamination. The plantlets were transferred to a greenhouse for acclimation in plastic tray (209 cells) containing with a moist mixture of (1:1) sand and peat moss, then, maintained inside a plant growth chamber and irrigated with a fine mist of water for 3 weeks. The percentage of survived plants was determined after 4 weeks.

Statistical analysis

Experiments were set up in completely randomized design. Data were statistically analyzed using ANOVA\MANOVA of Statistica 6 software (Statsoft, 2001). The significance of differences among means was carried out using the Least Significant Test (L.S.D) at = p 0.05.

RESULTS AND DISCUSSION

***In vitro* germination:**

Data in Figs. (1 and 2) showed that using quarter MS medium with and without activated charcoal was able to induce germination in coffee seeds for the four cultivars. Modified quarter MS medium with 1.0 g l^{-1} activated charcoal gave the highest germination percentage (85-87%) as compared to 70 –75% without charcoal. of the four cultivars under study. The choice of medium that affect the germination, as has been reported for coffee plant and other species (Chen *et al*., 2015; Ebrahim *et al.,* 2007).

3-2. Shoot multiplication

Explants were directly capable of developing multiple shoots on MS basal medium containing different concentrations of BA. Data in Table (1) and Fig. (2) showed that BA with the different concentrations, significantly increased the shoot number per explant compared to control treatment in all coffee cultivars under study. BA at the highest concentration (8.0 mgl^{-1}) gave the highest number of shoots, shoot length, number of leaves and fresh weight of shoot per explant (14.6, 2.64, 127.2 and 0.92), respectively, compared to control in case of *Coffea arabica* cv 'Benan' cultivar (Table 1 and Fig. 2 D). BA at 2.0, 4.0 and 6.0 mgl^{-1} increased multiplication parameters significantly as compared to other BA concentrations in the case of *Coffea arabica* cvs 'Odayni', 'Burai' and 'Odayni-Bayat' respectively, with increment of BA concentration, the number of shoot per explant decreased in the three cultivars (Table 1). From this experiment, it was evident that best clump fresh weigh was obtained from MS medium amended with BA (2.0, 4.0 and 8.0 mgl^{-1}) which produced 0.56, 0.64 and 0.57 g fresh weight of shoots/explant from *Coffea arabica* cvs 'Odayni', 'Burai' and 'Odayni-Bayat', respectively. The positive effect of BA on the potential to induce plant regeneration in coffee plants has been reported previously by (Kahia and Owuor, 1990; Haidar, 1993; Ebrahim *et al.,* 2007; Almeasary, 2008) who used BA in combination with different concentrations of auxin to induce *in vitro* multiplication in other coffee species. Comparing the present results of this species with the previous studies, it was seen that each species needs appropriate culture medium, with appropriate concentration of growth regulator.

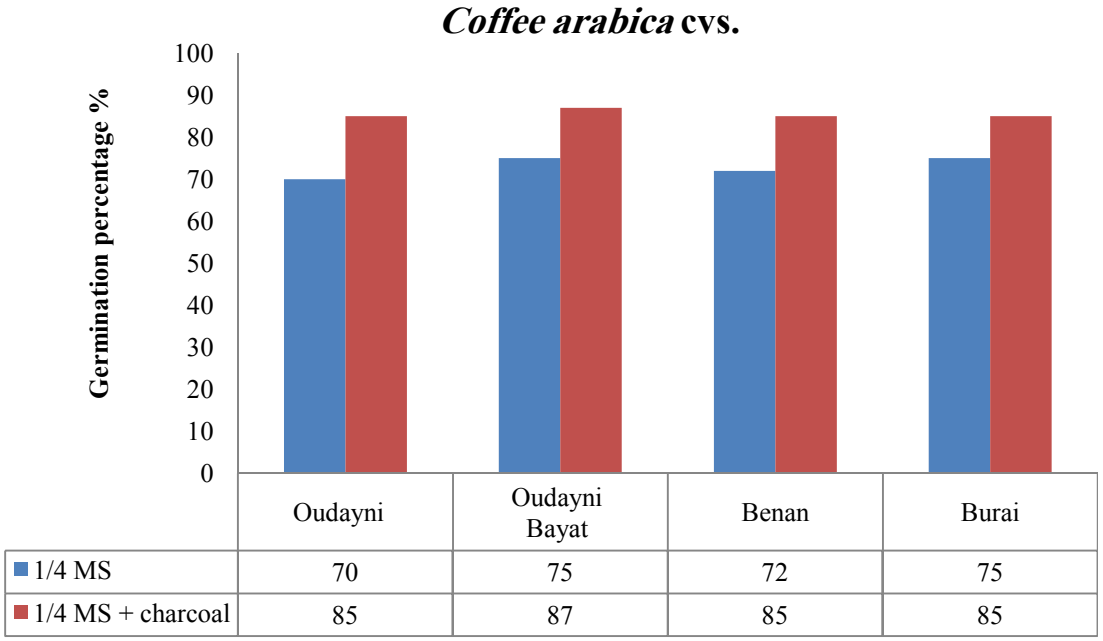
Root formation

Data presented in Table (2) show the effect of IAA, IBA and/or NAA on the *in vitro* rooting of proliferated shoots of the four *Coffea arabica* cvs 'Benan', 'Burai', 'Odayni' and 'Odayni-Bayat.'. Root formation was different according to cultivars in response to the application of IAA, IBA and/or NAA to the culture media. The MS basic medium without IAA, IBA and/or NAA also revealed root formation. MS medium supplemented with the three kinds of auxins induced callus on the rooting stage in all the studied cultivars except at the tested concentrations of IBA and IAA in the case of *Coffea arabica* cv 'Odayni' (Fig. 2 E). The MS medium with NAA (2.0, 1.0 and 3.0 mgl-1) provides the highest number of roots per explant (3.39, 3.35 and 3.40 roots/explants) in *Coffea arabica* cv 'Odayni', 'Burai' and 'Odayni-Bayat' respectively, (Table 2 and Fig. 2 E). However, the highest number of roots per plantlet (3.44) was recorded with IBA (1.0 mgl^{-1}) in the case of Benan cultivar. Further increase in the concentrations of NAA and IAA had no effects on the

Table (1): Effect of different BA concentrations on multiple shoot induction of four Coffee cultivars after 12 weeks from culture *in vitro*.

BA mgl^{-1}	*Coffea arabica* cv 'Benan'				*C. arabica* cv 'Burai'				*C. arabica* cv 'Odayni'				*C. arabica* cv 'Odayni-Bayat'			
	No of shoots/ explant	Length of the longest shoot (cm)	No of leaves/ explant (n)	explant fresh weight (g)	No of shoots/ explant (n)	Length of the longest shoot (cm)	No of leaves/ explant (n)	explant fresh weight (g)	No of shoots/ explant (n)	Length of the longest shoot (cm)	No of leaves/ explant (n)	explant fresh weight (g)	No of shoots/ explants (n)	Length of the longest shoot (cm)	No of leaves/ explant (n)	Explant fresh weight (g)
0.0	1.0d	2.0 c	6.8 e	0.11 d	1.0d	3.4 a	8.1 e	0.36 bc	1.0f	1.3 d	6.4 f	0.18 ij	1.0f	3.3 a	7.2 f	0.22 e
1.00	5.6 c	2.1 c	33.2 d	0.30 bc	2.6 c	2.9 b	14.3 d	0.27 d	6.6 c	1.5 bc	37.3 a	0.34 b	5.6 e	2.7 b	44.8 c	0.60 a
2.00	5.8 c	2.3 b	36 c	0.29 c	5.5 bc	2.6 c	32.9 bc	0.48 a	8.9 a	1.9 a	36.6 ab	0.56 a	6.4 d	2.1 d	39.6 e	0.37 d
4.00	8.8 b	2.3 b	50 b	0.37 b	6.9 a	2.5 cd	40.3 a	0.46 ab	7.4 b	1.6 b	35.3 b	0.24 d	7.2 c	2.1 d	42.0 d	0.36 d
6.00	8.8 b	2.2 bc	50 b	0.36 b	6.1 ab	2.6 c	28.6 c	0.32 c	5.9 d	1.4 c	30.4 c	0.28 c	9.6 a	2.2 d	62.8 a	0.49 c
8.00	14.6 a	2.64 a	127.2 a	0.92 a	5.75 b	2.9 b	37.1 b	0.38 b	2.7 e	1.6 b	12.4 d	0.13 f	8.2 b	2.4 c	53.2 b	0.57 b

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test



	Oudayni	Oudayni Bayat	Benan	Burai
1/4 MS	70	75	72	75
1/4 MS + charcoal	85	87	85	85

Fig. (1): Influence of different types of medium on germination of four Yemeni *Coffea arabica* cultivars.

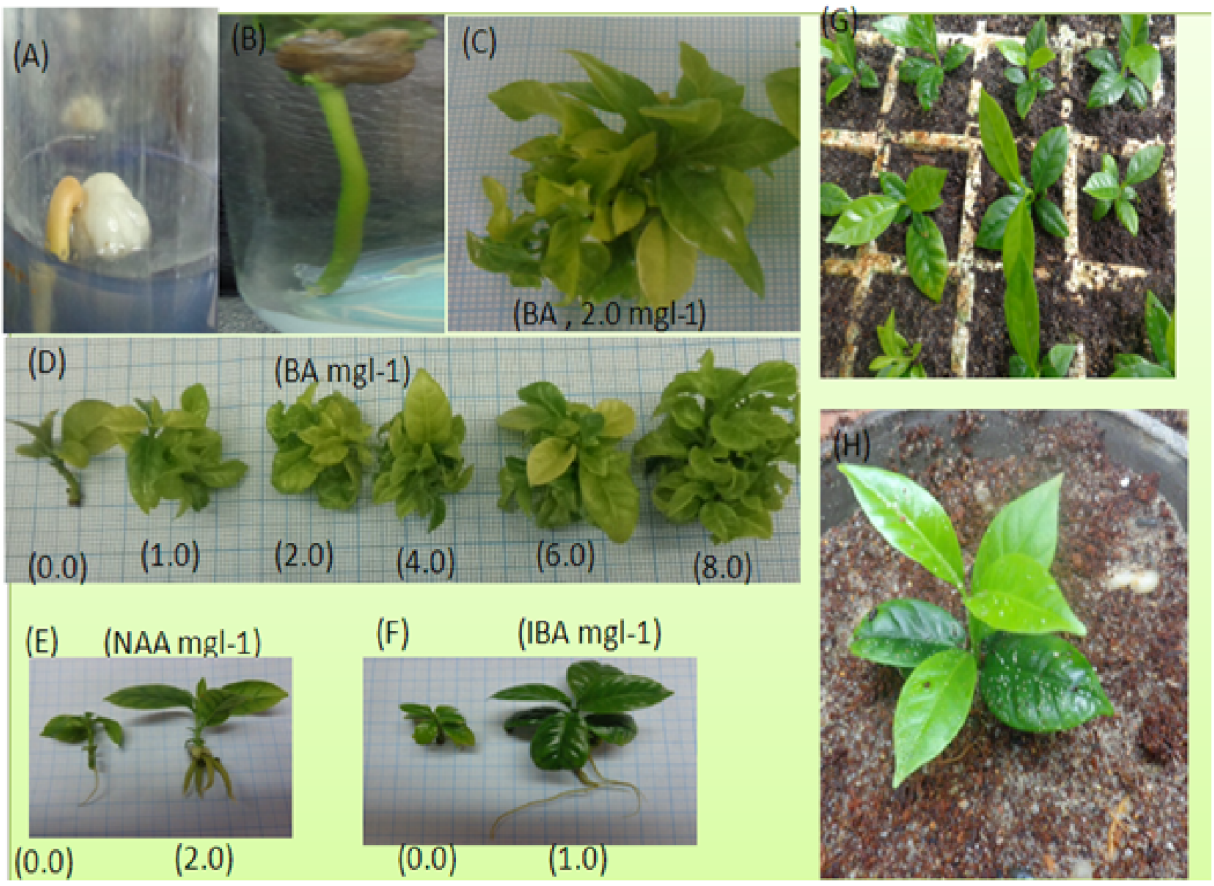


Fig. (2): Modified micropropagation method of *Coffea arabica* cultivars (A and B) germinated seed of *C. arabica* cv 'Oudayni – Bayat'. (C and D) multiple shoots generated from shoot tip explant of *C. arabica* cvs 'Oudayni' and 'Benan', respectively. (E) root formation of *C. arabica* cv 'Oudayni'. (F) root formation of *C. arabica* cv 'Benan'. (G and H) plants of *C. arabica* cvs 'Burai' and 'Benan' in greenhouse after 4 and 8 weeks of acclimatization, respectively.

Table (2): Effect of different concentrations of IAA, IBA and NAA on the root formation of four coffee cultivars after 8 weeks *in vitro*

Growth regulators mgl^{-1}			*Coffea arabica* cv 'Benan'				*C. arabica* cv 'Burai'				*C. arabica* cv 'Odayni'				*C. arabica* cv 'Odayni-Bayat'			
IAA	**IBA**	**NAA**	**Root number (n)**	**Length of the longest root (cm)**	**Plant height (cm)**	**Plant fresh weight (g)**	**Root number (n)**	**Length of the longest root (cm)**	**Plant height (cm)**	**Plant fresh weight (g)**	**Root number (n)**	**Length of the longest root (cm)**	**Plant height (cm)**	**Plant fresh weight (g)**	**Root number (n)**	**Length of the longest root (cm)**	**Plant height (cm)**	**Plant fresh weight (g)**
0.0	**0.0**	**0.0**	0.22e	0.10 h	1.98 ef	0.12 gh	0.21g	0.40 c	2.42 k-def	0.18 c-f	0.80c	0.82 c	2.14 d	0.15 c	0.00f	0.00 h	1.70 g	0.14 f
0.5	**0.0**	**0.0**	1.6 3bc	2.26 b-e	3.18 a	0.26 c	3.0 0ab	2.88 ab	2.90 bc	0.20 b-f	1.82abc	2.68 a	3.20 a	0.18 c	2.02b	2.00 ef	3.48 ab	0.24 d
1.0	**0.0**	**0.0**	1.03cd	2.02 c-f	2.7 bc	0.21 de	2.62abc	2.68 ab	2.86 bcd	0.30 b	3.23ab	2.84 a	3.28 a	0.20 bc	1.21cde	2.74 cd	3.00 c	0.19 e
2.0	**0.0**	**0.0**	1.03cdl	1.08 g	2.14 def	0.18 ef	0.81efg	2.44 b	2.14 f	0.14 f	1.63abc	0.96 c	2.88 ab	0.18 c	0.82e	1.22 g	2.18 ef	0.17 ef
3.0	**0.0**	**0.0**	0.82de	1.56 efg	1.82 f	0.15 cg	0.63fg	2.16 b	2.68 b-e	0.15 def	2.24abc	1.38 bc	2.84 abc	0.19 c	1.0 2de	1.90 ef	1.96 fg	0.18 e
0.0	**0.5**	**0.0**	1.42bcd	1.76 d-g	2.40 cde	0.23 cd	1.82cde	2.46 b	2.32 ef	0.12 bcd	1.42bc	2.00 abc	3.12 a	0.19 c	2.82 a	1.68 fg	2.36 de	0.18 e
0.0	**1.0**	**0.0**	3.40 a	4.30 a	3.14 a	0.49 a	1.62c-f	2.76 ab	2.42 def	0.17 def	2.23abc	1.82 abc	2.68 a-d	0.24 bc	3.23 a	4.30 b	3.02 c	0.44 b
0.0	**2.0**	**0.0**	1.6 3bc	2.96 b	2.22 def	0.18 ef	2.01bcd	3.70 a	2.44 c-f	0.27 bcd	1.42bc	2.28 ab	2.68 a-d	0.22 bc	1.84bc	4.10 b	2.16 ef	0.17 ef
0.0	**3.0**	**0.0**	1.03cd	1.26 g	1.98 ef	0.10 h	2.22bcd	2.82 ab	2.48 c-f	0.27 bcd	1.43bc	0.82 c	2.44 bcd	0.17 c	1.23cde	1.60 fg	2.60 d	0.18 e
0.0	**0.0**	**0.5**	1.22bcd	2.62 bc	2.70 bc	0.26 c	2.01bcd	2.04 b	4.00 a	0.45 a	2.63abc	1.68 abc	2.16 d	0.18 c	1.63bcd	1.66 fg	1.98 efg	0.18 e
0.0	**0.0**	**1.0**	1.83b	2.26 b-e	3.12 ab	0.38 b	3.35 a	2.64 ab	3.08 b	0.51 a	2.4 2abc	1.16 bc	2.92 ab	0.30 b	1.25cde	2.48 de	2.28 def	0.24 d
0.0	**0.0**	**2.0**	1.22bcd	2.40 bcd	2.59 cd	0.25 c	1.62c-f	3.20 ab	2.82 bcd	0.30 b	3.39 a	1.94 abc	2.92 ab	0.56 a	1.43b-e	3.20 c	3.28 bc	0.28 ch
0.0	**0.0**	**3.0**	1.04cd	1.46 fg	2.70 bc	0.21 de	1.43def	2.24 b	2.52 c-f	0.15 def	3.23ab	1.12 c	2.25 cd	0.53 a	3.40 a	5.4 a	3.70 a	0.60 a

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

number of roots in the case of *Coffea arabica* cvs 'Odayni', 'Burai' and' Benan' (Table 2). The maximum root growth was recorded on MS medium with 1.0 mgl^{-1} IBA, 2.0 mgl^{-1} IBA, 1.0 mgl^{-1} IAA and 3.0 mgl^{-1} NAA in the case of *Coffea arabica* cvs 'Benan' 'Burai', 'Odayni' 'Odayni-Bayat', respectively. Although excessive auxin in the medium is commonly characterized by callus formation, no callus formation was detected in rooting stage of all concentrations of IBA and IAA in the case of *Coffea arabica* cv 'Benan' (Fig. 2 F). The absence of callus at shoot base is an important observation because it can be excluded that auxin treatments were supplied in improper high supplements (Nerman *et al*., 2009).The obtained results are similar to the previous studies reported on many species by (Wamatu, 1990; Kahia and Owuor, 1990; Ganesh and Sreenath, 1997; Tiwari *et al*., 1999; khattab, 2011).

Acclimatization

The success of any *in vitro* micropropagation protocol largely depends on the survival and growth performance of the propagated plantlets *ex-vitro* (Joshi and Dhar, 2003). In the present study, the acclimation procedures applied was successful using the previously mentioned experimental conditions. Almost 90% of the regenerated plants of the four cultivars survived and showed vigorous growth (Fig. 2 G and H). *In vitro* derived plants did not display any phenotypic variation during subsequent vegetative development.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Salah Khattab Department of Horticulture, Faculty of Agriculture, Suez Canal University for his assistance. All thanks to all staff member of Tissue Culture Lab, Suez Canal University, Ismailia, Egypt.

REFERENCES

Almeasary, M.A.S. (2008). Micropropagation in Coffee (*Coffea arabica* L). Ph.D. Dissertation, Faculty of Agriculture, Assiut university.

Carneiro, M.F. and T.M.O. Ribeiro (1989). *In vitro* micropropagation of *Coffea arabica* L. cv Caturra by means of axillary buds. Broteria Genetica, 85: 139-152.

Carneiro, M.F. (1997). Coffee biotechnology and its application in genetic transformation. Euphatica, 97: 167-172.

Carvalho, A. and L.C. Monaco (1969). The breeding of Arabica Coffee. In: Ferwerda, F.P. and F. Wit (eds.). Outlines of Perennial Crop Breeding in the Tropics, Veenman and Zonen NV., Wageningen, pp. 198-216.

Chen,Y., M.G. Uromi, Fan Xu-Li and G. Jiang-Y. (2015). Asymbiotic seed germination and *in vitro* seedling development of *Paphiopedilum spicerianum*: An orchid with an extremely small population in China. Global Ecology and Conservation, 3: 367–378.

Ebrahim, N., R. Shibli, I. Makhadmeh, M. Shatnawi and A. Abu-Ein (2007). *In vitro* Propagation and *In vivo* acclimatization of three coffee cultivars (*Coffea arabica* L.) from Yemen. World Applied Sciences Journal, 2(2): 142-150.

Frederic, N., D. Yingshan and L. Bao (2007). Somaclonal variation at the nucleotide sequence level in rice (*Oryza sativa* L.) as revealed by RAPD and ISSR markers, and by pairwise sequence analysis. J. Appl. Genet., 48: 329–336.

Ganesh, D.S. and H.L. Sreenath (1997). Clonal propagation of coffee through apical bud culture, J. Plant. Crops, 25: 169-174.

Haidar, A.K.A. (1993). Studies on the micropropagation of *Coffea arabica* L. M.Sc. Thesis, Zagazig University, Egypt.

Ismail, S., B. Naqvi, N. Anwar and R. Zuberi (2003). *In vitro* multiplication of *Coffea arabica*. Pak. J. Bot., 35(5): 829-834.

Johnson, J.L. and E. R. Emino (1979). *In vitro* propagation of *Mammillariae longata.* HortScience, 14: 605–606.

Joshi, M. and U. Dhar (2003). *In vitro* propagation of *Saussurea obvallata* (DC.) Edgew, an endangered ethnoreligious medicinal herb of Himalaya. Plant Cell Rep., 21: 933–939.

Juma, C., J. M. Magmbo and H. Monteith (1994). Tissue culture for coffee: The case of Uganda. Biotechnology and Development Monitor, 20: 19-20.

Kahia, J.W. and J.B.O. Owuor (1990). *In vitro* propagation of the disease resistant *Coffea arabica* L. Cultivar-Ruiru 11. Kenya-Coffee, 55: 901-905.

Khattab, S. (2011). Effect of Different Media and Growth Regulators on the *in vitro* Shoot Proliferation of Aspen, Hybrid Aspen and White Poplar Male Tree and Molecular Analysis of Variants in Micropropagated Plants. Life Science Journal, 8(1): 177-184.

Monaco, L.C., M.R. Sundahl, A. Carvalho, O.J. Crocomo and W.R. Sharp (1995). Application of tissue culture in the improvement of coffee. In: Reinert, J. and Y.P,S. Bajaj (Eds). Applied and Fundament Aspects of Plant Cell Tissue and Organ Culture. Narosa publ., India.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473–497.

Nerman, M., E. Souad and T. Hussein (2009)*: In vitro* mass propagation of the endangered Sainai Hawthorn *Crataecus* Sinaica boiss. International Journal of Academic Research, 1(1): 24-29.

Pierson, E.S., A.A.M. Van Lammeren, J.H.N. Schell and G. Startisky (1983). *In vitro* development of embryoids from punched leaf discs of *Coffea canephora*. Protoplasma, 115: 208-216.

Robinson, J. and B. Brian (1993). Coffee in Yemen, Practical guide .Technical Cooperation Federal Republic of Germany, GTZ -Yemen.

Smith, R.H., P.J. Burdick, J. Anthony and A.A. Reilley (1991). *In Vitro* Propagation of *Coryphanthama cromeris*. HortScience, 26(3): 315.

Statsoft, Inc. (2001). STATISTICA for Windows (software-system fur Datenanalyse) Version 6. http:\www.statisoft.com.

Tiwari, K.P., S.K. Tiwari, M.C. Sharma and E.A. Siril (1999). Preliminary studies on micropropagation of *Rubia cordifolia,* a medicinal herb from central India.Vaniki- Sandesh, 23: 5-8.

Wamatu, J.N. (1990). Vegetative propagation of Ruiru11: a review. Kenya-Coffee., 55: 983-985.

الإكثار الدقيق لأربعة أصناف من البن العربي اليمنية من خلال زراعة القمة النامية

أمين علي العزب[١] - سند السيد حبيب[٢] – محمد عبد الجواد حسين[٣] ـــ فادية محمد الشريف[٤*]

[١، ٢، ٤] قسم البساتين – كلية الزراعة – جامعة قناة السويس – ٤١٥٢٢، الإسماعيلية - مصر

[٣] قسم النبات الزراعي – كلية الزراعة – جامعة قناة السويس – ٤١٥٢٢، الإسماعيلية - مصر

[*] قسم علوم الحياة – كلية العلوم – جامعة الملك فيصل - المملكة العربية السعودية – ص.ب ٣٨٠ الإحساء

أجريت هذه الدراسة خلال الفترة من ٢٠١٤ إلى ٢٠١٥ وذلك على أربعة أصناف يمنية من نبات البن العربي وهي (البنن – البرعي – العديني – العديني البياض) وذلك في معمل زراعة الأنسجة قسم البساتين – كلية الزراعة – جامعة قناة السويس. وكان الهدف من الدراسة هو معرفة أنسب بيئة لإحداث التضاعف لهذه الأصناف معملياً، كما تم دراسة أنسب بيئة من أجل إنبات البذور حيث تم استخدم ربع بيئة موراشيج وسكوج (MS) مع إضافة فحم نشط بمعدل ١جرام/اللتر وبدون فحم وقد أعطت البيئة المضاف لها الفحم النشط أعلى نسبة إنبات مقارنة بتلك الخالية من الفحم النشط. وجد أن استخدام ٨ ملليجرام/لتر من البنزيل أدنين BA مضاف إلى بيئة موراشيج وسكوج، أعطى أعلى نسبة من التضاعف وذلك مع الصنف البنن، أما بالنسبة لبقية الأصناف وهي (البرعي، العديني، العديني البياض)، فقد تم الحصول على أفضل النتائج عند استخدام بيئة موراشيج وسكوج (MS) مضاف لها البنزيل أدنين بالتراكيز التالية (٠.٤، ٠.٢، ٠.٦) على التوالي. أما بالنسبة لإحداث التجذير معملياً، فكان بإستخدام منظمات النمو أندول حامض الخليك (IAA)، أندول حامض البيوتريك (IBA) ونفثالين حامض الخليك (NAA) أعطت نسبة ١٠٠% تجذير للأربعة أصناف، وقد وجد أن استخدام نصف بيئة موراشيج وسكوج (MS) مضافاً لها ٠.٢، ١.٠، ٣.٠ ملليجرام/ لترمن منظم النمو NAA، أعطت أفضل عدد للجذور وذلك للأصناف (العديني، والبرعي، والعديني البياض) على التوالي. ومن أجل إحداث الأقلمة تم استخدام بيئة مكونة من البيت موس والرمل بنسبة ١:١ وكانت نسبة النباتات الحية بعد أربعة أسابيع من الزراعة في الصوبة ١٠٠% لكل الأصناف.