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In vitro Propagation and *In vivo* Acclimatization of Three Coffee Cultivars (*Coffea arabica* L.) From Yemen

[1]Naji Ebrahim, [1]Rida Shibli, [1]Ibrahim Makhadmeh, [2]Mohamad Shatnawi and Abdallah Abu-Ein

[1]College of Agriculture, Jordan University of Science and Technology, Irbid, Jordan
[2]Department of Biotechnology, Faculty of Agriculture Technology,
Al-Balqa Applied University, Al-Salt 19117, Jordan

Abstract: Micropropagation of *Coffea arabica* cvs. Oudayni, Hammady and Dawaeiry from Yemen were initiated from seeds. Seeds were surface sterilized and inoculated into media supplemented with different salt strengths and germinated under dark. Seeds germinated on agar medium gave high hypocotyl length, high root length and full cotyledonary leaves expansion after 120 days of culture. Proliferation of these cultivars was experimented on MS media supplemented with different levels (0.0, 2.0, 4.0. 6.0 or 8.0 mg l^{-1}) of N6-Benzyladenine (BA), Thidiazuron (TDZ), 6-furfurylaminopurine (Kinetin), 6-(4-hydroxy-3-methyl-2-butenylamino) purine (Zeatin) or 6-(γ,γ-Dimethylallylamino) purine (2ip). Highest proliferation for all cultivars was obtained when BA was used at the highest level (8.0 mg l^{-1}). Satisfactory proliferation rate in the three cultivars was achieved at 8.0 mg l^{-1} kinetin and 6.0 mg l^{-1} TDZ. Zeatin and 2ip were both failed to promote proliferation at any used level. Rooting was experimented on half-strength MS media supplemented with different levels (0.0, 2.0, or 3.0 mg l^{-1}) of indole-3- butyric acid (IBA), indole-3- acetic acid (IAA) or 1-naphthaleneacetic acid (NAA). Highest root number and length was achieved at 3.0 mg l^{-1} IAA or IBA for all cultivars. Rooted plantlets were transferred to 1 peat: 1 perlite mixture and ex vitro acclimatization gave 100% survival.

Key words: Acclimatization · coffee · *in vitro* · micropropagation · root formation

INTRODUCTION

Coffee (*Coffea arabica*) is one of the most important agricultural products in the international markets. The world trade in coffee is an important contributor to the income of some fifty or more producing countries on widely differing scales There are many countries which depend in their economy on the coffee exportation [1] Commercially, only two out of more than 100 coffee species are cultivated: the *C. arabica* (Arabica) and *C. canephora* (Robusta) [1, 2, 3]. 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. On the other hand *C. canephora* is usually grown in tropical areas at lower altitudes which represent 80% of the African production. On a very reduced scale, *C. liberica* is grown in some African countries, *C. racemosa* is grown in Mozambique and *C. dewevrei* in Ivory Coast and Zaire. These later species produce beans of lower quality that are acceptable only in local markets [4]. In Yemen, there are many coffee cultivars and they are named according to their place of origin, for example Oudayni, Matiery, Hammady, Yavaeiy. In addition to the previous cultivars of arabica coffee which grow to a standard size, there is a dwarf cultivar which is known as Dawaeiry in Yemen and universally known as Mokka [5].

Botanically, coffee belongs to the family Rubiacea and is classified taxonomically under the genus *Coffea* which includes at least 64 species grouped into four sections [6]. The most important species is *C. arabica*. The coffee from Yemen gave rise to two distinct types: *C. arabica* var. arabica, usually called "typical", which was the earliest grown coffee in Asia and Latin America and *C. arabica* var. bourbon which came to South America through the island of La Reunion, formerly called Bourbon [7].

Corresponding Author: Dr. Mohamad Shatnawi, Department of Biotechnology, Faculty of Agriculture Technology, Al-Balqa Applied University, Al -Salt 19117, Jordan

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 [8]. Plant production via tissue culture is advantageous over traditional propagation methods because it leads to the production of disease and virus - free plants [8, 9]. Also, it allows the production of a high number of plants, in a short period of time and in a very limited propagation space [9]. In addition, rapid multiplication rate of plants that are difficult to propagate conventionally can be easily achieved via *in vitro* culture [10].

Various approaches have been considered for *in vitro* multiplication of coffee (*C. arabica*) apical meristem and axillary bud culture, induction and development of adventitious buds [10] and somatic embryogenesis [11].

C. arabica is predominantly self-pollinated [12] and thus, the progenies as arising from the seeds are very uniform. Therefore, seeds are considered a good starting material in the *in vitro* establishment for this plant. Therefore, the objective of this investigation was to develop a protocol for *in vitro* establishment, multiplication, rooting and acclimatization of some leading coffee cultivars (*C. arabica*) from Yemen.

MATERIALS AND METHODS

Establishment of plant material

Seeds: Healthy 10 years old coffee (*C. arabica*) trees in Yemen were used as a source for seeds. Fruits of the three coffee cultivars, Oudayni, Daweiry and Hammady, were harvested in September 2001. Seeds were obtained using mature and ripe fruits by depupling (natural fermentation for 24 hours) and washing. Seeds were then dried under shade until approximately 13% moisture content which is suitable for storage.

Surface sterilization: Seeds were surface sterilized by washing thoroughly under running tap water for 10 min. Afterwards seeds were immersed in Benomyl (fungicide) solution (10 mg l^{-1}) for 5 min. Then rinsed with autoclaved distilled water, for 5 min to remove traces of Benomyl. Seeds were immersed in the antiseptic solution of 1.25% sodium hypochlorite plus two drops of tween-20 for 20 min (under the laminar air-flow cabinet). Seeds were rinsed with sterile distilled water for three times (5 min each). Seeds were then soaked in 70% (v/v) ethanol solution for 30 sec and then rinsed with autoclaved distilled water three times (5 min each).

***In vitro* germination:** For *in vitro* germination of seeds, four treatments were used, Agar medium only, full strength medium MS [13], half strength MS medium and Quarter strength MS medium. In the different treatments, media contained 8.0 g l^{-1} agar (Difco-Bacto) and supplemented with 2 mg l^{-1} Gibberellic acid (GA_3). Sterilized seeds of the three cultivars were inoculated in 25 x 150 mm test tubes containing 25 ml of media. Each treatment consisted of twenty replications in a Completely Randomized Design (CRD). Cultures were kept under dark at 24±2 °C for 30 days until full germination. Total germination percentage and contamination % were recorded. Germinated culture were transferred to the growth chamber under 16 hours of artificial fluorescent light (photosynthetic photon flax density PPFD = (40-45 µmol/m^2/sec) and 8 hours of darkness at 26±2°C for 90 days. Different parameters, including hypocotyl length, root length, total seedling length and cotyledonary leaves expansion were recorded.

Micropropagation

Shoot multiplication: Microshoots (10 mm) were subcultured in Erlenmeyer flasks (250 ml) containing 150 ml of solid MS media. The following experiments were done to study the best cytokinin type and concentration for maximizing microshoot production for each cultivar. Different concentrations (0.0, 2.0, 4.0, 6.0 or 8.0 mg l^{-1}) of BA, TDZ, Kinetin, 2ip or Zeatin were studied in separate experiments. All treatments had 0.5 mg l^{-1} IAA. The cultured microshoots were incubated under 26±2 °C with 16 hours light (photosynthetic photon flux density; PPFD = (40-45)µmol /m^2 / sec) and 8 hours dark. Data were collected after 120 days on number of proliferated shoots, shoot height, number of leaves/explant. Callus and the plant performance were monitored. Experiments were arranged in a completely randomized design (CRD) with 10 replications.

Rooting: Rooting was carried out for each cultivar by subculturing microshoots (10 mm) in 25 x 150 mm test tubes containing 25 ml of solid half-strength MS media and sucrose (15 g l^{-1}). Media was supplemented with IBA, IAA or NAA at 0.0, 1.0, 2.0 or 3.0 mg l^{-1}. Experiments were arranged in a Completely Randomized Design (CRD) with 10 replications. The cultured microshoots were maintained under 26±2 °C with 16 hours light (PPFD) = 40 - 45µmol/m^2/sec) and 8 hours dark. Data were collected on number of roots, root length and shoot height after 60 days.

Ex vitro acclimatization: *Ex vitro* acclimatization was carried out by opening test tubes for 3 days before transferring plantlets outside of the growth chamber. *In vitro* rooted plantlets were extracted from test tubes and the agar was removed by washing with warm sterile water. The plantlets were transferred to 1 peat: 1 perlite mixture in 84 cell polystyrene trays covered with glass beakers for 3 weeks. Plantlets were acclimatized under 16 hours supplementary light of 40-45 µmol./m^2/sec/ and 8-hours dark at 26±2 °C. Survival percentage was recorded at the end of three weeks. Acclimatized plants were transferred to 12 x 23 cm polyethylene bags contain 1 soil: 1 perlite mixture and grown under the greenhouse condition 24±2 °C day, 20±2 °C night and overhead irrigation.

Statistical Analysis: Each experiment was set up as a completely randomized design. Collected data were statistically analyzed using Statistical Analysis System (SAS) [14]. Means were separated according to the least significant difference (LSD) test at 0.05 level of probability.

RESULTS AND DISCUSSION

***In vitro* germination:** Table 1 refers to the effect of different medium strength on the germination of the studied coffee cultivars. It is clear that the total germination percentage decreased as the MS medium strength increased. However, agar medium only resulted higher germination percentage for all studied cultivars than other MS medium strength. Lower germination percentage, resulted at high MS medium strength (full strength MS) could be due to high osmotic pressure of the germination solution which makes imbibitions more difficult and retards germination [14, 15]. Sterilization procedures resulted in low number of contaminated cultures (Table 1) and these results are in agreement with previous result by Quirzo *et al.* [16]. Furthermore, Table 2 shows the effect of different medium strength on *in vitro* growth of coffee seedlings. After 120 days of inoculation, seeds (in the agar medium) germinated into plantlets with higher root length, more hypocotyl length and full cotyledonary leaves expansion. While increased medium strength reduced the plantlets growthand cotyledonary.

Maximum hypocotyl length 6.5-7.3 cm was obtained on agar medium (Table 2). For all three cultivars significant differences were also obtained between media, as to root length. The maximum length of roots was 9.8-11.6 cm in the agar medium. There was not significant difference between agar medium and quarter - strength MS medium on root length of Oudayni seedlings which resulted maximum seedling length of 17.1-18.6 cm in the agar medium. Thus, the differences obtained between different media strength were significant.

Table 1: Influence of different types of medium strength on germination of some Yemeni *Coffea arabica* cultivars

Medium	Germination %	Contamination %
OudayniAgar	95	12
1/4 MS	82	12
1/2 MS	78	14
MS	66	18
Hammady		
Agar	98	13
1/4 MS	86	14
1/2 MS	64	16
MS	54	18
Dawaeiry		
Agar	96	10
1/4 MS	88	10
1/2 MS	72	12
MS	58	13

Table 2: Influence of different types of medium strength on in vitro growth of some Yemeni *Coffea arabica* cultivars seedlings after 120 days of inoculation

Medium	Hypocotyl length (cm)	Root length (cm)	Seedling length (cm)	Cotyledon leaves expansion (+/-) Oudayni
Agar	7.3 a[y]	9.8 a	17.1 a	+
1/4 MS	6.3 b	9.2 a	15.5 b	-
1/2 MS	2.7 c	2.4 b	5.1 c	-
MS	1.5 d	0.8 c	2.3 d	-
Hammady				
Agar	6.5 a	10.8 a	17.3 a	+
1/4 MS	6.1 b	8.7 b	14.8 b	-
1/2 MS	2.5 c	2.2 c	4.7 c	-
MS	1.4 d	0.7 d	2.1 d	-
Dawaeiry Agar	7.0 a	11.6 a	18.6 a	+
1/4 MS	6.7 b	10.3 b	17 b	-
1/2 MS	2.8 c	2.6 c	5.4 c	-
MS	1.6 d	1.0 d	2.6 d	-

y = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = Seedling without full cotyledon leaves expansion. (+) = Seedling with full cotyledon leaves expansion

***In vitro* shoot multiplication:** Table 3 shows the effect of BA concentrations in combination with 0.5 mg l^{-1} IAA on shoot multiplication of the studied coffee cultivars. It is clear that after 120 days of culture, multiplication

Table 3: Influence of BA on number of shoots, shoot length, number of leaves per explant and callusing of some *in vitro* grown Yemeni *Coffea arabica* cultivars

BA mg l^{-1}	Number of shoots	Shoot length (cm)	Number of leaves/explant	Callusing (+/-)
Oudayni				
C1 [y]	1.0 d[z]	1.4 bc	4.3 d	-
C2	1.0 d	1.5 bc	4.6 d	-
2.0	4.1 c	1.8 ab	28.0 c	-
4.0	7.7 b	1.6 b	36.0 bc	+
6.0	9.5 b	1.4 ac	41.8 b	+
8.0	16.8 a	2.0 a	89.0 a	++
Hammady				
C1	1.0 d	1.6 b	4.8 d	-
C2	1.0 d	1.6 b	4.9 d	-
2.0	3.7 c	1.7ab	27.0 c	-
4.0	7.1 b	1.5 bc	35.5 bc	+
6.0	8.3 b	1.5 bc	38.3 b	++
8.0	16.0 a	2.1 a	86.8 a	++
Dawaeiry				
C1	1.0 d	1.3 c	4.0 d	-
C2	1.0 d	1.5 bc	4.2 d	-
2.0	3.6 c	1.7 ab	26.3 c	-
4.0	6.5 b	1.3 c	34.2 bc	+
6.0	7.7 b	1.4 bc	37.4 b	+
8.0	15.4 a	1.9 a	85.1 a	++

y = C1 and C2 represent control treatments (with and without 0.5 mg l^{-1} IAA, respectively). z = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

Table 4: Influence of kinetin on number of shoots, shoot length, number of leaves per explant and callusing of some *in vitro* grown Yemeni *Coffea arabica* cultivars

Kinetin mg l^{-1}	Number of shoots	Shoot length (cm)	Number of leaves/explant	Callusing (+/-)
Oudayni				
C1 [y]	1.0[z] c	1.4 b	4.3 d	-
C2	1.0 c	1.5 b	4.6 d	-
2.0	1.3 c	1.8 ab	9.4 c	+
4.0	2.4 b	1.7 b	12.6 b	+
6.0	3.0 b	1.7 b	13.3 b	++
8.0	5.2 a	2.0 a	29.2 a	++
Hammady				
C1	1.0 c	1.6 b	4.8 d	-
C2	1.0 c	1.6 b	4.9 d	-
2.0	1.2 c	1.9 b	8.8 c	+
4.0	2.2 b	1.7 b	11.8 bc	+
bvv	2.7 b	1.6 b	12.6 b	+
8.0	5.0 a	2.4 a	29.0 a	++
Dawaeiry				
C1	1.0 c	1.3 c	4.0 d	-
C2	1.0 c	1.5 bc	4.2 d	-
2.0	1.1 c	1.9 b	8.6 c	+
4.0	2.0 b	1.8 b	11.3 bc	+
6.0	2.4 b	2.0 b	12.3 b	++
8.0	4.4 a	2.5 a	27.9 a	++

y = C1 and C2 represent control treatments (with and without 0.5 mg l^{-1} IAA, respectively). z = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus, (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

parameters and growth performance of these cultivars responded significantly to increased BA concentrations up to 8.0 mg l^{-1}. This concentration (8.0 mg l^{-} ^{1}BA) increased multiplication parameters significantly as compared to other BA used concentrations. Shoot length and number of leaves produced from microshoots cultured on MS medium supplemented with 4.0 mg l^{-1} BA was not significantly different from MS supplemented with 6.0 mg l^{-1} BA in the three evaluated cultivars. In this study, no significant differences appeared between control treatments, C1 (without 0.5 mg l^{-1} IAA) and C2 (with 0.5 mg l^{-1} IAA) during 120 days of incubation and also no proliferation was occurred in both controls.

Callusing occurred at the basis of microshoots at 4.0, 6.0 and 8.0 mg BA/l. Results showed that the 9best concentration of BA which enabled *C. arabica* microshoots to produce highest shoots number (15.4-16.8 shoots per explant) was 8.0 mg l^{-1} combined with 0.5 mg l^{-1} IAA and resulted in highest shoot length and highest number of leaves per ex-plant. The three studied cultivars exhibited similar response to BA concentrations (Table 3). Result of Kahia and Owuor [17] showed successful *in vitro* multiplication on solid MS media supplemented with 8.0 mg l^{-1} BA combined with 0.8 mg l^{-1} IAA. Furthermore, Haidar [4] reported that the combination of 6-benzylaminopurine with 0.5 mg l^{-1} IAA produced the proliferation rate.

The effect of different concentrations of kinetin combined with 0.5 mg IAA/l on *in vitro* shoot multiplication of some Yemeni coffee cultivars is shown in Table 4. It is obvious that the continuous increase in kinetin concentrations to 8.0 mg l^{-1} increased significantly the shoots number (4.4-5.2), shoot height (2.0-2.5 cm) and leaves number per ex-plant (27.4-29.2). Number of proliferated shoots and leaves produced from microshoots cultured on MS medium supplemented with 4.0 mg l^{-1} kinetin was not significantly different from MS medium supplemented with 6.0 mg l^{-1} kinetin in the

Table 5: Influence of TDZ on number of shoots, shoot length, number of leaves per explant and callusing of some *in vitro* grown Yemeni *Coffea arabica* L. cultivars

TDZ mg l^{-1}	Number of shoots	Shoot length (cm)	Number of leaves/explant	Callusing (+/-)
Oudayni				
C1 [y]	1.0 c [z]	1.4 a	4.3 d	-
C2	1.0 c	1.5 a	4.6 d	-
2.0	1.7 b	0.5 b	5.4 b	+++
4.0	2.2 ab	0.4 b	6.0 ab	++++
6.0	2.8 a	0.3 b	7.7 a	+++++
8.0	2.4 a	0.2 b	5.2 b	++++++
Hammady				
C1	1.0 c	1.6 a	4.8 bc	-
C2	1.0 c	1.6 a	4.9 bc	-
2.0	2.0 b	0.6 b	5.8 bc	+++
4.0	2.4 b	0.4 b	6.2 b	++++
6.0	3.1 a	0.3 b	8.2 a	+++++
8.0	2.0 b	0.3 b	4.1 c	++++++
Dawaeiry				
C1	1.0 b	1.3 a	4.0 bc	-
C2	1.0 b	1.5 a	4.2 bc	-
2.0	1.3 b	0.5 b	4.6 bc	+++
4.0	1.6 b	0.2 b	5.3 b	++++
6.0	2.6 a	0.4 b	8.8 a	+++++
8.0	1.5 b	0.2 b	3.5 c	++++++

y = C1 and C2 represent control treatments (with and without 0.5 mg l^{-1} IAA, respectively). z = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

Table 6: Influence of 2ip on number of shoots, shoot length, number of leaves per explant and callusing of some *in vitro* grown Yemeni *Coffea arabica* cultivars

2ip mg l^{-1}	Number of shoots	Shoot length (cm)	Number of leaves/explant	Callusing (+/-)
Oudayni				
C1 [y]	1.0 [z]	1.4 b	4.3 b	-
C2	1.0	1.5 ab	4.6 ab	-
2.0	1.0	1.7 ab	5.2 ab	+
4.0	1.0	1.8 a	5.4 ab	+
6.0	1.0	1.9 a	5.7 a	+
8.0	1.0	1.5 ab	4.3 b	+
Hammady				
C1	1.0	1.6 abc	4.8 ab	-
C2	1.0	1.6 abc	4.9 ab	-
2.0	1.0	1.9 ab	5.5 ab	+
4.0	1.0	2.0 a	5.8 a	+
6.0	1.0	1.7 abc	5.1 ab	+
8.0	1.0	1.4 c	4.4 b	+
Dawaeiry				
C1	1.0	1.3 b	4.0 b	-
C2	1.0	1.5 ab	4.2 b	-
2.0	1.0	1.6 ab	5.1 ab	+
4.0	1.0	1.7 a	5.5 a	+
6.0	1.0	1.8 a	5.6 a	+
8.0	1.0	1.4 ab	4.0 b	+

y = C1 and C2 represent control treatments (with and without 0.5 mg l^{-1} IAA, respectively). z = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

studied Yemeni cultivars. It was reported that multiplication rate was increased in *C. arabica* cv. Bourbon when MS medium was used and supplemented with 15 mg l^{-1} Kinetin [18].

All studied levels of BA except control treatments showed callus formation on the basal part of the proliferated shoots, the 6.0 and 8.0 mg l^{-1} kinetin showed higher callus formation in comparison to that of 2.0 and 4.0 mg l^{-1} kinetin. The effect of different concentrations of TDZ combined with 0.5 mg l^{-1} IAA on *in vitro* shoot multiplication of the three coffee cultivars is shown in Table (5). It is clear that 6.0 mg l^{-1} TDZ caused the highest significant increase in proliferated shoots and number of leaves/explant as compared to all other treatments. On the other hand the shoot length in the three studied cultivars decreased with increasing TDZ concentration (Table 5). At concentration above 6.0 mg l^{-1} TDZ, the number of proliferated shoots and the number of leaves/explant significantly decreased. These results are in disagreement previous finding by Arafeh [19] who found that TDZ failed to promote proliferation in *Origanum* spp at the used levels. TDZ was a callus inducing factor and these results are in agreement with the finding of Arafeh, [20], who reported that TDZ was a callus inducing factor in *Origanum vulgare* L. The effect of different levels of 2ip and zeatin combined with 0.5 mg l^{-1} IAA *on in vitro* shoot multiplication of the studied coffee cultivars are shown in Tables (6, 7). It is clear that both cytokinins failed to induced shoot multiplication at any of the used levels (2.0-8.0 mg l^{-1}) and the growth of mother stock (shoot length and number of leaves/explant) slightly increased in comparison to the control treatments. These results indicate that coffee did not responded to the use of zeatin and 2ip, at least in the studied cultivars at the used levels.

***In vitro* rooting:** Microshoots of the three studied cultivars were rooted on half-strength MS medium. The

Table 7: Influence of zeatin on number of shoots, shoot length, number of leaves per explant and callusing of some *in vitro* grownYemeni *Coffea arabica* cultivars

Zeatin mg l^{-1}	Number of shoots	Shoot length (cm)	Number of leaves/explant	Callusing (+/-)
Oudayni				
C1 [y]	1.0 [z]	1.4 b	4.3 c	-
C2	1.0	1.5 b	4.6 c	-
2.0	1.0	1.1 b	5.9 bc	+
4.0	1.0	2.3 a	10.1 a	++
6.0	1.0	2.4 a	7.3 b	++
8.0	1.0	2.1 a	7.5 b	+++
Hammady				
C1	1.0	1.6 bc	4.8 c	-
C2	1.0	1.6 bc	4.9 c	-
2.0	1.0	1.5 bc	7.1 b	+
4.0	1.0	2.5 a	10.6 a	++
6.0	1.0	2.1 ab	7.3 b	+++
8.0	1.0	1.5 c	8.7 b	+++
Dawaeiry				
C1	1.0	1.3 bc	4.0 b	-
C2	1.0	1.5 b	4.2 b	-
2.0	1.0	1.0 c	4.5 b	+
4.0	1.0	2.1 a	9.2 a	++
6.0	1.0	2.5 a	9.7 a	+++
8.0	1.0	2.2 a	9.0 a	+++

y = C1 and C2 represent control treatments (with and without 0.5 mg l^{-1} IAA, respectively). z = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

Table 8: Influence of IAA on number of roots, root length, shoot height and callusing of some *in vitro* grown Yemeni *Coffea arabica* cultivars

IBA mg l^{-1}	Number of roots	Root length (cm)	Shoot height (cm)	Callusing (+/-)
Oudayni				
0.0	0.0 d [y]	0.0 d	1.0 d	-
1.0	1.5 c	1.4 c	1.7 c	+
2.0	3.2.2 b	2.8 b	2.8 b	+
3.0	10.19 a	5.7 a	6.2 a	+
Hammady				
0.0	0.0 d	0.0 d	1.3 c	-
1.0	2.4 c	2.0 c	1.5 c	-
2.0	4.7 b	3.2 b	3.0 b	+
3.0	11.3 a	6.1 a	7.1 a	+
Dawaeiry				
0.0	0.0c	0.0 d	1.2 c	-
1.0	1.8 b	1.4 c	1.4 b	-
2.0	2.92b	2.4 b	1.5 b	+
3.0	7.3a	3.8 a	4.2 a	+

y = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

reduction of MS macronutrients has been previously reported [21-25], to promote rooting in many Rubiaceae plants. However, it is now considered that this effect is due to a reduction of the nitrogen sources in the medium [26]. Table 8 reflects the effect of different concentrations of IAA on rooting of the three studied Yemeni coffee cultivars. It is obvious that IAA had a significant effect on rooting of *in vitro* grown coffee microshoots. Increasing IAA to 3.0 mg l^{-1} gave significantly the highest number of roots, root length and shoot height on the three studied cultivars.

No callus formation was observed on the basal part of the microshoot when 1.0 mg l^{-1} IAA was used. On the other hand, the 2.0 or 3.0 mg l^{-1} IAA showed small callus formation on the basal part of the microshoots. Furthermore, Table 9 indicates the effect of IBA on rooting of coffee microshoots. This similar to previous study on *Prunus amygdalus* [27]. Better rooting in the three studied coffee cultivars was obtained when IBA auxin was used. Higher number of roots, root length and shoot height were obtained with IBA of 3.0 mg l^{-1}. Among the auxins tested for root initiation (*i.e*. IAA, IBA, NAA), IBA has been used for rooting microshoots of many Rubiaceae plants including *C. canephora*, *Lucelia* spp, *Rubia cordifolia*, *Gardenia Josminodies* and *Cephalis ipecacunha* [12, 13]. A recent study by Ganesh and Sreenath, [28] who reported that IBA to be suitable for the rooting of *C. arabica* and best rooting were achieved in half-strength MS medium supplemented with 1.0 mg l^{-1} IBA.

Table (10) shows the effect of different concentrations of NAA on rooting parameters of coffee. It is clear that NAA was the least suitable as it produced much callus and failed to promote good rooting parameters in comparison to other auxins. However, increasing NAA to 1.0 mg l^{-1} gave significantly the highest number of roots, root length and shoot height in comparison to all other treatments in case of Hammady and Dawaeiry cultivars. While in case of Oudayni cultivar, 2.0 mg l^{-1} gave the best results. Rooting parameters significantly d ecreased at 3.0 mg l^{-1} NAA for the three cultivars. This could be due to large callus mass (12-14 mm) around the basis of microshoots. Our results are in agreement with the finding of Kahia [21] who rooted coffee microshoots on half-strength MS media supplemented with 2.0 mg l^{-1} NAA. The damage of

Table 9: Influence of IBA on number of roots, root length, shoot height and callusing of some *in vitro* grown Yemeni *Coffea arabica* cultivars

IBA mg l^{-1}	Number of roots	Root length (cm)	Shoot height (cm)	Callusing (+/-)
Oudayni				
0.0	0.0 d [y]	0.0 d	1.0 d	-
1.0	2.5 c	2.8 c	2.2 c	+
2.0	4.2 b	4.5 b	3.2 b	++
3.0	9.0 a	7.1 a	5.3 a	++
Hammady				
0.0	0.0 d	0.0 d	1.3 c	-
1.0	3.5 c	2.9 c	2.4 c	-
2.0	4.7 b	4.0 b	3.7 b	++
3.0	10.2 a	7.2 a	5.5 a	++
Dawaeiry				
0.0	0.0 d	0.0 c	1.2 c	-
1.0	1.8 c	2.4 b	1.8 bc	-
2.0	2.9 b	4.6 a	2.4 b	++
3.0	7.8 a	4.8 a	4.1 a	++

y = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

Table 10: Influence of NAA on number of roots, root length, shoot height and callusing of some *in vitro* grown Yemeni *Coffea arabica* cultivars

NAA mg l^{-1}	Number of roots	Root length (cm)	Shoot height (cm)	Callusing (+/-)
Oudayni				
0.0	0.0 c [y]	0.0 c	1.0 b	-
1.0	2.8 b	2.7 a	0.9 ab	++++
2.0	4.1 a	3.1 a	1.1 a	+++++
3.0	2.1 b	1.6 b	0.7 b	++++++
Hammady				
0.0	0.0 c	0.0 c	1.3 b	-
1.0	2.9 a	2.9 a	1.9 a	+++
2.0	1.5 b	2.4 ab	1.3 b	++++
3.0	1.2 b	1.9 b	1.1 b	+++++
Dawaeiry				
0.0	0.0 c	0.0 b	1.2 b	-
1.0	3.2 a	1.9 a	1.7 a	+++
2.0	1.4 b	1.4 a	1.5 a	++++
3.0	0.8 b	1.5 a	1.0 b	+++++

y = Means within columns for the same cultivar having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability. (-) = no callus. (+, ++, +++, ++++, +++++, ++++++) = (callus with 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, mm) diameter, respectively

microshoots caused at high concentration of NAA (3.0 mg l^{-1}) in our study was in agreement with the finding of Pasqual and Barros [29] who reported that high concentration of NAA damaged coffee shoots *in vitro*.

Table 11: Total survival and survival percentage in different *in vitro* rooted Yemeni *Coffeaarabica* L. cultivar plantlets

Plant growth regulator	Survival Number	Survival Percentage
Oudayni		
IAA	30/30	100%
IBA	30/30	100%
NAA	10/30	33%
Hammady		
IAA	30/30	100%
IBA	30/30	100%
NAA	12/30	40%
Dawaeiry		
IAA	30/30	100%
IBA	30/30	100%
NAA	6/30	20%

***Ex vitro* acclimatization:** Rooted plantlets showed different survival percentage according to plant growth regulator used, Table 11. *C. arabica* plantlets rooted on IAA or IBA gave complete survival percentage (100%) under acclimatization. The lowest survival percentage was found when NAA was used. This could be due to the presence of short roots and large callus mass around the basis of rooted plantlets in case of NAA. The partial acclimatization protocol used by Zok [18] in micropropagation of *C. Arabica* resulted in good number of healthy acclimatized plants for the three cultivars. Plants that were transferred to the greenhouse after acclimatization had 100% survival and reached about 30 cm length after 5 months. The produced plants did not show abnormalities which might indicate variation or mutation occurrence during the micropropagation procedures and all plants appeared as true-to type.

Seeds of *C. arabica* can be considered a good starting material for *in vitro* establishment with high percentage of germination. Agar medium gave higher root length, hypocotyl length and full cotyledonary leaves expansion during in vitro germination of the three studied cultivars. High concentrations of BA and kinetin (up to 8.0 mg l^{-1}) gave higher proliferation and TDZ gave good results at 6.0 mg l^{-1}. BA was superior in the proliferation followed by kinetin and then TDZ. Better rooting was occurred at high concentrations of IBA or IAA in the three studied cultivars but NAA gave good results at 1.0 mg l^{-1} in both Hammady and Dawaeiry cultivars while Odayni cultivar responded positively at 2.0 mg l^{-1} NAA. Acclimatization of the three cultivars resulted in (100%) of acclimatized plants that remain healthy and showed normal growth in the greenhouse.

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