



Original Article

Micropropagation protocol for *Antigonon leptopus* an important ornamental and medicinal plantZenna Fawzia Ghareeb^a, Lobna S. Taha^{b,*}^a Botanical Garden Research Dept., Horticulture Res.Inst., Agricultural Research Center, Egypt^b Ornamental Plants and Woody Trees Dept., National Research Centre, Dokki, Giza, Egypt

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ABSTRACT

The effect of some factors on *in vitro* consecutive micropropagation behavior of *Antigonon leptopus* was examined including those of culture establishment, shootlets multiplication, rooting and acclimatization stages. The highest percent of aseptic cultures and survival of explants (100%) were obtained as a result of using Clorox 10% for 3 min followed by MC 0.1% for 2 min while, using each of them individually (Clorox 20% or MC 0.1%) for 5 min caused the highest percent of shoot formation. During the multiplication stage, the highest percent of shoot formation was reached to 100% with repeating culture of explants (two times) on MS medium supplemented with 2ip at 1.0 and IBA at 0.2 mg/l. The highest numbers of shootlets/explant were obtained when 2.0 mg/l of BAP or 0.5 mg/l BA + 0.2 mg/l of IBA were added to MS culture medium. Culturing the explants on MS medium supplemented with 2ip at 0.5 or 1.0 mg/l each combined with 0.2 mg/l of IBA showed the longest shootlets. Reducing the strength of culture media to ½ or ¼ had promotion effect on rooting formation of shootlets. The best results of plant acclimatization (survival percent, plant height and root length) were obtained by using sand or peat moss soil. The amplified DNA fragments using B7, B9 and C19 primers for mother and micropropagated plants showed that the produced pattern by primer B7 had a maximum number of 10 bands of DNA fragments with molecular size ranging between 1025.57 and 176.36 bp, micropropagated plants showed 95.2% similarity in relation to mother plant.

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1. Introduction

Corallita (*Antigonon leptopus*) is a member of Polygonaceae, has grown successfully worldwide in tropical climates as a perennial ornamental plant for its strong growth and abundant pink flowers or as a source of nectar to produce honey [1]. Many tubers at the end of inflorescence axes of the plant are used for climbing and plant base can be wooden with age [2]. The uses of corallita in homes decoration could be stated [3]. The vital role of this plant in both active and pure forms or in traditional herbal medicines is gradually popular for treating diseases because of its availability with no side effects and low prices. In many countries, the air dried parts of the plant can be used as tea that has been evaluated for lipid peroxidation (LPO) and cyclooxygenase oxidation (COX-1

and COX-2) enzyme inhibitory activities to relieve cold and pain [4].

The propagation of this plant is by seeds which produce small quantities relatively. The growing demand of this plant as a natural landscape for decorative raw materials or pharmaceuticals cannot be covered by traditional methods of propagation. For large-scale field cultivation, tissue culture technique can be an alternative to the continuous progress of plant supplies. Now, there are many types of ornamental and medicinal plants being propagated through *in vitro* culture techniques [5] such as *Centella asiatica* L. [6], *Hypericum perforatum* L. [7], and even woody medicinal plant, *Garcinia indica* [8], *Hibiscus sinencis* [9], *Hibiscus syriacus* [10] and *Khaya senegalensis* [11].

The role of cytokinin level is known to enhance the bud formation and shoots differentiation in cultured tissue [12]. It work on cell division mediated factors such as the development of shoots and the activity of the plant meristem as well as the arrangement of parenchyma and procambium necessary for the size of the apical meristem. Also, roots formation is an important step in the vegetative propagation of horticultural species [13]. Adjusting the

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cytokines to auxin ratio in the cultural medium can control the initiation of shoots and roots [14].

There are no reports on tissue culture of these plant species. Taking into account the economic, aesthetic and ornamental values, there is a need to spread the plant through *in vitro* culture techniques. Therefore, this work investigated the exact micropropagation protocol most suitable for this plant which can be widely used for multiplication and even transfer to greenhouse and mass production of *Antigonon leptopus* plants.

2. Materials and methodes

These investigations have been executed during seasons of 2016–2017 on *Antigonon leptopus* at Tissue Culture & Germplasm Conservation Research Lab., Horticulture Research Institute, Agricultural Research Center- Giza and Tissue Culture Technique lab., Central laboratories, Department of Ornamental Plants and Woody Trees, National Research Center (NRC), Egypt with the view to examine the effect of some factors on the behaviors of *in vitro* consecutive micropropagation stages including those of culture establishment, shootlets multiplication, rooting and acclimatization stages.

2.1. Plant materials

Stem nodal explants (2–3 cm) of *Antigonon leptopus* were taken from the unique climbing tree grown at Zohrya Botanical Garden, Zamalek, Cairo, Egypt as explant source for this work.

2.2. Culture establishment stage

Nodal explants (as starting plant materials that were taken from young branches of *Antigonon leptopus*) were rinsed in soapy water using septol soap with shaking for 20 min., then washed with running tap water for one hour. Under aseptic conditions in a laminar air-flow cabinet, the explants were then immersed in different solutions as follows: commercial Clorox (NaOCl, 5.25% free chlorine) at concentrations of 20% (v/v) for 5 min., mercuric chloride (MC) at concentrations of 0.1 (w/v) for 5 min or 10% Clorox for 3 min followed by 0.1 MC for 2 min with a few drops of Tween-20. Each treatment consisted of 7 jars, each jar containing five nodal explants. After receiving the disinfection treatments, the explants were rinsed three times with sterile distilled water. After receiving the different disinfection treatments, the explants were cultured on a basal MS medium [15] at full salt strength. Decontamination, survival and shoot formation percentages were recorded after 4 weeks of incubation for this stage.

2.3. Culture media and incubation conditions

MS culture medium was solidified by the addition of 0.7% agar prior to autoclaving at 1.2 kg/cm² for 15 min. The pH of the culture medium was adjusted to 5.8 by addition of 0.1 N KOH or 0.1 NHCL. Culturing was done in 200 ml glass jars containing 25 ml of the medium. All cultures were incubated for 4 weeks under controlled conditions in the growth chamber. The incubation temperature was 24 ± 2 °C controlled by a “Power” air conditioner. The photoperiod was 16 h light/8 h darkness, controlled automatically. Illumination intensity was 3000 lux from cool fluorescent lamps (120 cm Long).

2.4. Shootlet multiplication stage

The experiment of this stage was designed to study the effect of different concentrations of Benzyl adenine (BA) or 6-(γ , γ -

Dimethylallylamino)purine (2iP) (0.5 and 1.0 mg/L) separately or with 0.2 mg/L Indol Butyric Acid (IBA) on shootlet multiplication stage through three successive subcultures. The experiment included 8 treatments and each treatment combination was replicated 7 times (one jar/replicate) in a completely randomized design, and the explants were re-cultured three times, at 30 day intervals. The data after each subculture of this stage (shoot formation percentage, the number of shootlets/explant, shootlet length (cm) and number of leaves formed per shootlet) were recorded.

2.5. Rooting stage

In this stage, the trails were conducted to study the influences of various strengths of MS-medium ($\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and full strength) free hormones and MS full strength supplemented with 1.0 mg/L IBA on rooting behaviors of the grown shootlets. The produced uniform *in vitro* shootlets from the multiplication stage were individually separated and cultured in 7 replicates and incubated for 6 weeks. After that rooting ability, number of initiated roots and length of the formed roots were recorded.

2.6. Ex vitro acclimatization stage

An experiment was conducted to study the effect of six soil mixtures on the characteristics of the acclimatization stage. The mixtures were sand, clay, peat moss + sand, peat moss + clay and sand + clay (1:1 v/v). Plantlets (8–10 cm) coming from *in vitro* rooting stage were washed from agar and transferred into plastic pots (0.2 liter) containing soil mixtures after saturation with 0.2% Topsin-M70 fungicide in 7 replicates each replicate consists of 3 plantlets. The culture pots were covered by white transparent polyethylene bags and maintained in fiber-glass-house. After two weeks from culturing, one pore per polyethylene bag was performed, and after another two weeks the bags were gradually removed. The acclimatized plantlets were water irrigated twice a week for four weeks before transplanting out-door. At the end of this experiment, survival percentage, plantlet height and root length were recorded.

2.7. Randomly amplified polymorphic DNA (RAPD)

Plant tissues were ground under liquid nitrogen to a fine powder, then bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN). Polymerase Chain Reaction (PCR) amplification was performed using five random 10 mer arbitrary primers (synthesized by Operon biotechnologies, Inc. Germany) with the following sequences: OP-A02 (5' TGCCGAGCTG 3'), OP-B07(5' GGTGACGCAG 3'), OP-B09 (5' TGGGGGACTC 3'), OP-B11 (5' GTA-GACCCGT 3') and OP-C19 (5' GTTGCCAGCC 3').

Amplification was done in 25 μ l reaction “volume” containing the following reagents: 2.5 μ l of dNTPs (2.5 mM), 2.5 μ l of MgCl₂ (2.5 mM), 2.5 μ l of 10 x buffer, 3.0 μ l of primer (10 pmol), 3.0 μ l of template DNA (25 ng/ μ l), 1 μ l of Taq polymerase (1U/ μ l) and 10.5 μ l of sterile “dd” H₂O was conducted. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94 °C for 4 min followed by 45 cycles for 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C. The reaction was finally stored at 72 °C for 10 min [16]. Amplified products were size-fractionated (using 50 bp ladder marker) by electrophoresis in 1.5% agarose gel.

The relationships among mother plants and micropropagated plants were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix among lines [17].

2.8. Experimental design and data statistical analysis

The lay-out of the experiments achieved on shoot “establishment” rooting and acclimatization stages were designed as one factor in a completely randomized design. While that of experiments conducting on shootlets multiplication stage was arranged as a two factorials in a completely randomized design. The data of the three series of experiments were averaged and statistically analyzed using analysis of variance. The Duncan’s multiple range test was done for comparison among means at 5% level according to Steel and Torrie [18].

3. Results and discussion

3.1. Culture establishment

The data in Table 1 demonstrate the effect of two different disinfectants (Sodium hypochlorite and mercuric chloride) each alone or both of them on the percent of decontamination for cultures, survival of explants and the formation of shoots for *in vitro* *Antigonon leptopus* culture establishment. The highest percent of decontamination for cultures and survival of explants (100%) were obtained as a result from using the two disinfectants (Clorox 10% for 3 min followed by MC 0.1% for 2 min) while, using each disinfectant alone (Clorox 20% or MC 0.1%) for 5 min led to the highest shoot formation% (29.86 and 28.29%, respectively). From these data (Table 1), it seems that the use of two disinfectants retard the formation of adequate shoots to establish *in vitro* culture, and this may be attributed to the increased toxic effect of them on the explants tissue. However, Singh and Tiwari [19] confirmed results on jackfruit attributed the plant toxicity for the survival of explants to the use of heavy metals from mercury. Nest et al. [20] found that using 1.25% NaOCl (15 min) was suitable for Asiatic hybrid lily. Also, Taha et al. [21] noticed that the highest proportion of free contaminated and survival plants were obtained when 10% of sodium hypochlorite and 0.1% of mercuric chloride were used.

3.2. *In vitro* shooting capability

The tabulated data in Table 2, and Fig. 3, (A) indicate the *in vitro* shooting behaviors during multiplication stage of *Antigonon leptopus* under effect of two types of cytokinins (BA and 2ip) at two concentrations (0.5 and 1.0 mg/l) each alone or combined with IBA at 0.2 mg/l for three repeated subcultures. The results revealed the promotion effect of MS medium supplemented with 2ip at 1.0 mg/L when combined with IBA at 0.2 mg/L on the percent formation of shootlets/explant which was highest (89.67%) at the third subculture as a result of this treatment comparing with other treatments. The interaction effect of the above mentioned treatments and subcultures number on percent of shootlets formation was also recorded in Table 2. It can be observed that this percent was maximum (100%) with repeating culturing of explants on the same treatment (2ip at 1.0 + IBA at 0.2 mg/l) for two times (second subculture) with no significant differences between this

value and those for the same treatment in the third subculture (89%) or using the low concentration (0.5 mg/l) of 2ip alone for the second subculture which led to 85% of shootlets formation. For the effect of explants culture repeating for three times on the percent of shootlets formation, the results indicated that the capability of explants to form shoots as percent was in highest value (71.25%) for the second subculture with no significant difference between this value and that of the third subculture (67%) as compared to the first subculture which led to the lowest one (36.13%). Fig. 4.

The number of shootlets formed/explant as a result of the above mentioned treatments behaved another trend as shown in Table 2. It can be noticed that the highest numbers of shootlets/explant (2.24 and 2.25, respectively) were obtained when 1.0 mg/l of BAP alone or at 0.5 mg/l combined with 0.2 mg/l of IBA to the MS culture medium as compared to other treatments. When the effect of repeating cultures interacted with these treatments, the highest values of proliferated shoots (2.36 and 2.42, respectively) were appeared from using 1.0 mg/l of BAP for the second subculture or 0.5 mg/l BAP+ 0.2 mg/l of IBA in the first subculture. No significant variances were attributed to repeating subcultures for this character.

The behaviour of both *in vitro* shootlets length and number of leaves formed/shootlet was paralleled as revealed in Table 2. Culturing the explants on MS medium supplemented with 2ip at 0.5 or 1.0 mg/l each combined with 0.2 mg/l of IBA resulted in the longest shootlets (9.63 and 10.26 cm, respectively) as well as the highest number of leaves forms/shootlet (8.95 and 8.51, respectively). When the repeating subcultures interacted with these treatments, using the lower concentration (0.5 mg/l) of 2ip+ 0.2 IBA for the first subculture caused the best results that were represented in the longest shootlets (12.04 cm) and the highest number of leaves (10.22) as compared to other treatments. When the effect of culture repeating was investigated, it can be noticed that the first subculture had the best results for those characters (length of shootlets and number of leaves) which led to the highest values (6.95 cm and 7.91, respectively).

These results were in agreement with those found by Dello et al. [22] who mentioned that cytokinin is one of the important plant hormones for plant growth and development that is known to promote cell division and differentiation. The physiological role of cytokinin has been attributed to the activation of RNA, protein synthesis and enzyme activity as was reviewed by Kulaeva [23]. Different types of cytokinin can also stimulate lateral bud growth, and thus can cause multiple shoot formation through braking apical dominance of shoots [24]. Sercan [25] who worked on *Muscari aucheri* used various concentrations of two cytokinins (BA and 2ip) and detected that the optimum concentration for shoot multiplication was 1.5 mg/l but other concentrations of the two cytokines led to a decrease in this value. Cao Dinh and Stephen [26] carried out some studies on *Khaya senegalensis* and observed that the proliferated shoots were highest for over four subcultures when the culture media was containing 1.0 mg/l of BA.

Table 1
Effect of Clorox and/or Mercuric chloride (MC) on stem nodal explants of *Antigonon leptopus* during culture establishment stage.

Disinfectants	Measurements		
	Decontamination (%)	Survival (%)	Shoot formation (%)
Clorox 10% for 3 min + MC 0.1% for 2 min	100.0 a	100.0 a	4.71b
Clorox 20% for 5 min	57.14b	76.86c	29.86 a
MC 0.1% for 5 min	64.29b	90.29b	28.29 a
L.S.D	14.21	4.593	6.819

Clorox: Sodium hypochlorite, MC: mercuric chloride and Means within a column having the same letters are not significantly different according to Duncan’s Multiple Range Test (DMRT) at 5% level.

Table 2
Effect of plant growth regulators on *in vitro* growth behaviors during shoot multiplication stage of *Antigonon leptopus* plant.

	Growth regulators	Subculture			Mean
		Sub 1	Sub 2	Sub 3	
Shootlet formation (%)	0.5 mg/L BAP	60.00 e-h	45.00 h	52.00 gh	52.33 C
	1.0 mg/L BAP	45.00 h	70.00 c-f	55.00 f-h	56.67 C
	0.5 mg/L BAP + 0.2 mg/L IBA	60.00 e-h	80.00 b-d	69.00 c-f	67.67 B
	1.0 mg/L BAP + 0.2 mg/L IBA	45.00 h	50.00 gh	52.00 gh	49.00 C
	0.5 mg/L 2 iP	70.00 c-f	85.00 a-c	77.00 b-e	77.33 B
	1.0 mg/L 2 iP	75.00 b-e	65.00 d-g	70.00 c-f	70.00 B
	0.5 mg/L 2iP + 0.2 mg/L IBA	70.00 c-f	75.00 b-e	72.00 c-f	72.33 B
	1.0 mg/L 2iP + 0.2 mg/L IBA	80.00 b-d	100.00 a	89.00 ab	89.67 A
	Mean	36.13 B [*]	71.25 A [*]	67.00 AB [*]	
	L.S.D	Sub = 5.127, growth regulator = 8.373, A × B = 14.50			
Shootlet number	0.5 mg/L BAP	1.10 e	1.40 e	1.25 e	1.25 BC
	1.0 mg/L BAP	2.10 a-d	2.36 a	2.26 ab	2.24 A
	0.5 mg/L BAP + 0.2 mg/L IBA	2.42 a	2.16 a-c	2.16 a-c	2.25 A
	1.0 mg/L BAP + 0.2 mg/L IBA	1.30 e	1.24 e	1.32 e	1.29 BC
	0.5 mg/L 2iP	1.48 de	1.69 b-e	1.60 b-e	1.59 B
	1.0 mg/L 2iP	1.52 c-e	1.15 e	1.34 e	1.34 BC
	0.5 mg/L 2iP + 0.2 mg/L IBA	1.26 e	1.00 e	1.16 e	1.14 C
	1.0 mg/L 2iP + 0.2 mg/L IBA	1.62 e	1.35 e	1.40 e	1.46 BC
	Mean	1.60 A [*]	1.54 A [*]	1.56 A [*]	
	L.S.D	Sub = 0.2058, growth regulator = 0.3361, A × B = 0.5821			
Shootlet length (cm.)	0.5 mg/L BAP	3.62 gh	3.00 h	3.33 h	3.32 DE
	1.0 mg/L BAP	2.96 h	2.46 h	2.98 h	2.94 E
	0.5 mg/L BAP + 0.2 mg/L IBA	3.48 h	4.94 g	4.00 gh	3.97 D
	1.0 mg/L BAP + 0.2 mg/L IBA	3.38 h	3.12 h	3.32 h	3.31 E
	0.5 mg/L 2iP	9.38 cd	3.41 h	6.31 f	3.37 C
	1.0 mg/L 2iP	9.70 b-d	7.08 ef	8.30 de	8.36 B
	0.5 mg/L 2iP + 0.2 mg/L IBA	12.04 a	7.20 ef	9.64 b-d	9.63 A
	1.0 mg/L 2iP + 0.2 mg/L IBA	11.02 ab	9.53 cd	10.24 bc	10.26 A
	Mean	6.95 A [*]	5.09 C [*]	6.01 B [*]	
	L.S.D	Sub = 0.4743, growth regulator = 0.7746, A × B = 1.342			
Leaves number	0.5 mg/L BAP	5.86 g	5.66 g	5.78 g	5.77 D
	1.0 mg/L BAP	5.46 g	5.38 g	5.50 g	5.45 D
	0.5 mg/L BAP + 0.2 mg/L IBA	6.46 fg	4.02 h	5.94 g	5.47 D
	1.0 mg/L BAP + 0.2 mg/L IBA	6.72 eg	3.82 h	5.40 g	5.31 D
	0.5 mg/L 2iP	10.18 a-c	5.50 g	7.80 c-e	7.83 BC
	1.0 mg/L 2iP	9.10 a-c	5.48 g	7.92 c-e	7.50 C
	0.5 mg/L 2iP + 0.2 mg/L IBA	10.22 a	7.66 d-f	8.98 a-d	8.95 A
	1.0 mg/L 2iP + 0.2 mg/L IBA	9.28 ab	7.70 d-f	8.56 b-d	8.51 AB
	Mean	7.91 A [*]	5.65 C [*]	6.98 B [*]	
	L.S.D	Sub = 0.422, growth regulator = 0.6891, A × B = 1.194			

Sub1st = subculture, Sub2 = 2nd subculture and Sub3 = 3rd subculture. Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

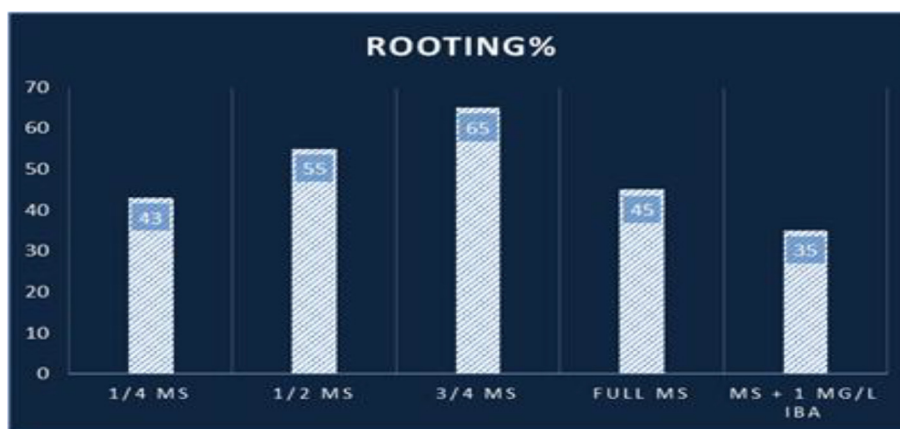


Fig. 1. Effect of MS medium salt strength and IBA on rooting percent of *Antigonon leptopus* according to Duncan's Multiple Range Test (DMRT) at 5% level.

3.3. *In vitro* rooting capability

The influences of various strengths of MS-medium ($\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and full strength) free hormones and MS full strength plus 1.0 mg/L for IBA on *in vitro* rooting capability *Antigonon leptopus* were

illustrated in Figs. 1, 2 and 3(B). The data showed that culturing the *in vitro* grown shootlets on both $\frac{1}{2}$ and $\frac{3}{4}$ strength of MS media free of hormones had the promotion effect on rooting percentage (Fig. 1), roots number and length of roots (Fig. 2). The highest

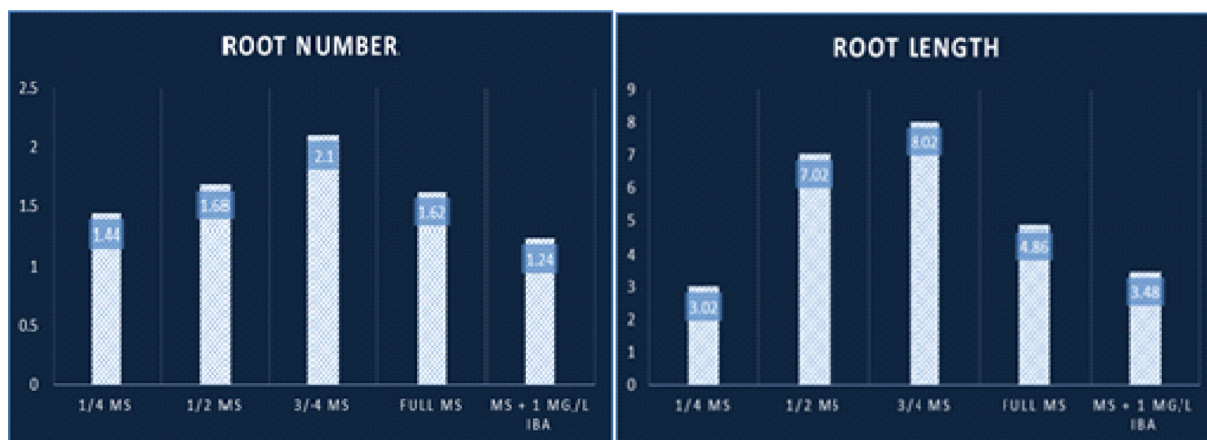


Fig. 2. Effect of MS medium salt strength and IBA on roots number and length of *Antigonon leptopus* according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 3

Effect of soil mixtures on *Antigonon leptopus* plantlets growth behaviors during *ex vitro* acclimatization stage.

Soil mixtures	Measurements		
	Survival (%)	Plant height (cm)	Root length (cm)
Sand	67.0 a	14.8 a	4.3 a
Peat moss	67.0 a	14.4 a	4.4 a
Clay	15.0c	8.8b	3.1b
Sand + peat moss (1:1)	33.4b	7.8b	4.0 a
Sand + clay (1:1)	14.2c	8.2b	2.9b
Peat moss + clay (1:1)	13.6c	8.6b	2.9b
L.S.D	6.894	2.445	0.9102

Means within a column having the same letters are not significantly different

values (65%, 2.10 and 8.02 cm, respectively) were produced with $\frac{3}{4}$ strength of MS medium with no significant differences between these values and those when the half strength of MS medium

was used as compared to other treatments. It can observe that adding 1.0 mg/l of IBA to the full strength of MS culture medium had inhibition effect on rooting capability and led to the lowest values of the mentioned root characters. Confirmed results were recorded by Nitishkumar and Reddy [27] who mentioned that the use of dilute media formulations has enhanced root formation, since the high concentration of salts may inhibit root growth, even in the presence of auxins in culture media.

3.4. *Ex vitro* acclimatization

The effect of six soil mixtures on the characteristics of the acclimatized plantlets was indicated in Table 3. and Fig. 3D and 3E. The data showed that the best results for plant survival, plant height and root length of acclimatized plantlets were obtained from using sand or peat moss soil. Also, the soil mixture of sand+ peat moss (1:1) appeared promotion effect on the length of roots formed per plantlet. Similar results were obtained by Taha et al. [28]. They

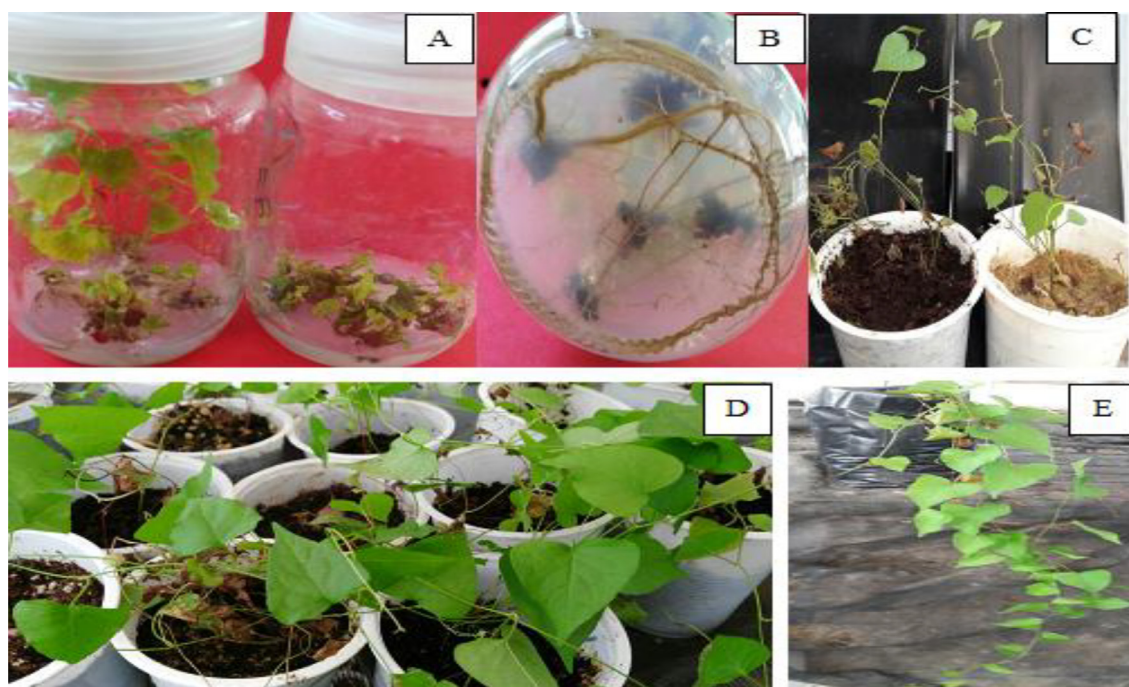


Fig. 3. Consecutive micropropagation stages of *Antigonon leptopus* plant. A: *In vitro* culture establishment stage, B: Rooting of *in vitro* microshoots, C, D and E: *ex vitro* acclimatization stage.

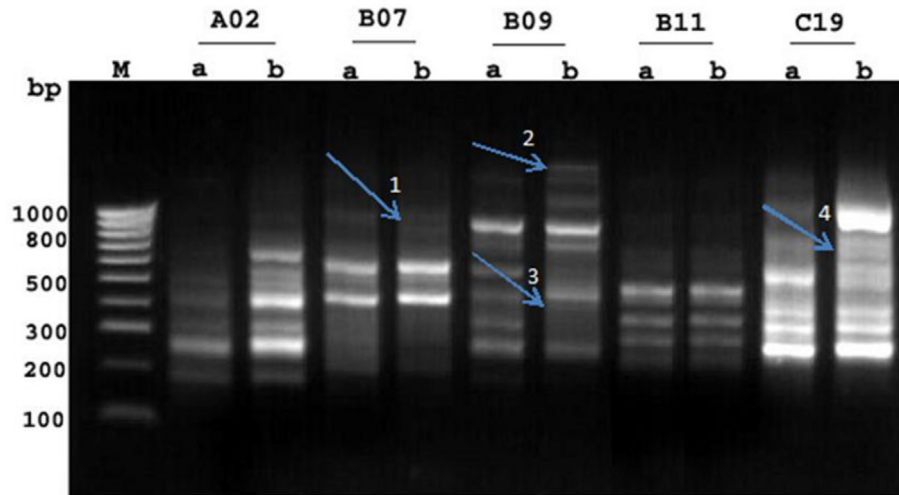


Fig. 4. RAPD-PCR using five primers (A02, B07, B09, B11 and C19) to compare between mother and micropropagated plants of *Antigonon leptopus*. M: DNA ladder, a: mother plant, b: micropropagated plant. (1) Band with molecular size 842.545 bp. (2) Band with molecular size 1701.65 bp. (3) Band with molecular size 370.24 bp. (4) Band with molecular size 682.38 bp.

Table 4

Total number of bands, number of polymorphic bands and polymorphism percentage for each used primer.

Primer	Sequence	Total number of bands	Number of polymorphic bands	Percentage of polymorphism
A02	5' TGCCGAGCTG 3'	7	0	0%
B07	5' GGTGACGCAG 3'	10	1	10%
B09	5' TGGGGGACTC 3'	11	2	18.182
B11	5' GTAGACCCGT 3'	8	0	0%
C19	5' GTTGCCAGCC 3'	8	1	12.5

observed that the root length was in highest value when Paulownia plantlets were cultured in mixture of peat moss and sand (1:1). Chan et al. [5] when they acclimatized *Gynura procumbens* (Lour.) Merr. plants, they observed that the addition of sand was a promotional effect on the survival rate of the acclimatized plantlets and this may be due to the sand water retention has been reduced and usually occur in organic soils that have caused root rot and gradually in all plant.

3.5. RAPD-PCR

As shown in Fig. 4 and Table 4, bands produced using A2 and B11 RAPD primers were monomorphic in both mother and micropropagated plants. Whereas, data of the amplified DNA fragments using B7, B9 and C19 primers for mother and micropropagated plants revealed that the pattern produced by primer B7 showed a maximum number of 10 bands of DNA fragments with molecular size ranging between 1025.57 and 176.36 bp, only one (842.545) polymorphic band was detected (10%). The result of primer B9 indicated the amplification of 11 DNA fragments with molecular size from 1701.65 to 161.13 bp. Two bands were polymorphic (1701.65 and 370.24) representing polymorphism percentage of 18.182%. Primer C19 exhibited 8 DNA fragments ranging in molecular sizes from 1072.251 to 216.158 bp. Only one polymorphic band was detected at 682.38 bp representing polymorphism of 12.5%. All polymorphic bands were detected in micropropagated plants, while they were absent in mother plant. According similarity index, micropropagated plants showed 95.2% similarity in relation to mother plant.

Although the tissue culture is an efficient method of clonal propagation, there are number of somaclonal variations in the obtained regenerated plants [29]. These variations are commonly caused by the generated mutations from tissue culture process

[30]. The mutation effects in tissue culture has been attributed to many stress factors such as wounding, exposure to sterilize methods, changing of media balance as a result of increasing the concentration of plant growth regulators, sugars as a replacement of leaves photosynthesis, light condition and imperfection the relationship between humidity and transpiration [31,32].

4. Conclusion

The micropropagation protocol was established for *Antigonon leptopus* with a rapid proliferation of shoots and facility of microshoots rooting as well as plantlets acclimatization to the external environment. It is useful for using this protocol to produce a uniform source for this plant and thus more applications.

Conflict of interest statement

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Significant statement

This study discovers the possible *in vitro* propagation of *Antigonon leptopus* and the synergistic effect of this protocol that can be beneficial for obtaining the highest numbers of shoots and leaves.

Thus, a new theory on these consecutive propagation stages may be arrived at tissue culture laboratories for decorative and pharmaceutical intents.

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References

- [1] Janelle Burke M, Antonio Tommaso Di. *Invasive Plant Sci Manage* 2011;4:265–73.
- [2] Pichardo JS, Vibrans H. *Antigonon leptopus*. *Malezas de México* 2009.
- [3] A Revision of *Antigonon* JEwing M.Sc. thesis. Bloomington, IN: University of Indiana; 1982. p. 63.
- [4] Vanisree M, Ruby L, Alexander L, David DeWitt L, Muraleedharan Nair G. Health-beneficial phenolic aldehyde in *antigonon leptopus* tea. evidence-based complementary and alternative medicine; 2009. Article ID 601249. 6 pages.
- [5] Chan Keng L, Lim Yee S, Pan Pin L. *J Med Plants Res* 2009;3:105–11.
- [6] Tiwari KN, Sharma NS, Tiwari K, B.D. *Plant Cell Tiss Org*, vol. 63; 2000. p. 179–85.
- [7] Santerém ER, Astarita LV. *Braz J Plant Physiol* 2003;15:43–7.
- [8] Malik SK, Chaudhury R, Kalia RK. *Sci Hort* 2005;106:39–553.
- [9] Hashish KHI, Lobna Taha S, Soad Ibrahim MM. *Int J ChemTech Res* 2015;8:131–6.
- [10] Metwally Sami A, Hashish KHI, Sawsan Sayed S, Lobna Taha S. *Int J Chem Tech Res* 2016;9:178–86.
- [11] Darwesh Mona A, Amal Naser A, Ebrahim Habba E, Lobna Taha S, Ahmed Gabr M, Ahmed Gabr M, Riham El-Assaly MB. *J Biol Sci* 2017;17:235–46.
- [12] Werner T, Schmülling T. *Curr Opin Plant Biol* 2009;12:527–38.
- [13] De Klerk GJ, Van Der Krieken WM, De Jong JC. *In vitro cell. Dev Biol* 1999;35:189–99.
- [14] Mosleh Duhoky MS, Layla Al-Mizory SM. *IOSR-JAVS* 2014;6:1–6.
- [15] Murashige T, Skoog F. *Bilogia Plantarum* 1962;15:473–97.
- [16] Rajapakse S, Belthoff LF, He G, Estager AE, Scorza R, Verde I, Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG. *Theor Appl Genet* 1995;90:503–10.
- [17] Yang X, Quiros C. *Theor Appl Genet* 1993;86:205–12.
- [18] Steel RGD, Torrie H, J.H. *Principle of statistics. Biometrical approach*. 2nd ed., McGraw-Hill Kogakusha, L.T.D; 1980.
- [19] Singh R, Tiwari JP. *Indian J Hort* 1998;55:213–7.
- [20] Nest B, Trinchello D, Lazzereschi S, Grassotti A. *HortScience* 2009;44:217–9.
- [21] Taha LS, Sawsan Sayed S, Farahat MM, Iman El-Sayed. *J Biol Sci* 2018;18:84–91.
- [22] Dello IR, Linhares FS, Scacchi E, Casamitjana-Martinez E, Heidstra R, Costantino P. *Curr Biol* 2007;17:678–82.
- [23] Kulaeva ON. In: Skoog F, editor. *Plant growth substances*. Heidelberg, New York: Springer-Verlag, Berlin; 1980. p. 119–28.
- [24] Yew CK, Balakrishnan B, Sundasekaran J, Subramaniam S. *J Med Plants Res* 2010;4:2641–6.
- [25] Sercan U. *Arch Biol Sci Belgrade* 2010;62:663–7.
- [26] Cao Dinh Hung, J Stephen Trueman, *New Forests* 2011;42:117–30.
- [27] Nitish kumar, Reddy MP. *J Forest Sci* 2011;27:61–72.
- [28] Taha LS, Soad MM, Ibrahim MM. *Aust J Basic Appl Sci* 2008;2:594–600.
- [29] Larkin P, Scowcroft W. *Theor Appl Genet* 1981;60:197–214.
- [30] Sato M, Kawabe T, Hosokawa M, Tatsuzawa F, Doi M. *Plant Cell Rep* 2011;30:229–39.
- [31] Joyce SM, Cassells AC, Jain SM, S.M. *Plant cell tissue organ cult*. 2003;74:103–21.
- [32] Smulders M, de Klerk G. *Plant Growth Regul* 2011;63:137–46.