Journal of Horticultural Science & Ornamental Plants 2 (2): 111-117, 2010
ISSN 2079-2158

Micropropagation of *Ixora undulata* Roxb by Tissue Culture

[1]*Salwa S. Sakr,* [1]*Afaf M. Habib,* [2]*M.A. El-Shamy, and* [2]*Heba B. Soliman*

[1]Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]El-Zohria Botanical Garden, Horticulture Research Institute,
Agriculture Research Cente, Ministry of Agriculture, Giza, Egypt

Abstract: This study was carried out in Plant Tissue Culture laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture from 2008 to 2010. The aim of this study was to reach a well-defined protocol for *in vitro* propagation of *Ixora undulata*. Shoot tips of *Ixora undulata* were effectively surface sterilized with 20 % clorox (sodium hypochlorite as a commercial bleach for 30 min. The longest shoots and greatest number of leaves had been obtained when explants were cultured on MS establishment medium with 0.5 mg/l NAA. MS medium supplemented with 0.1 mg/l Kinetin (kin) resulted in the highest number of leaves and longest shoots, however BA was more favorable for shoot formation than Kin. During the rooting stage, the medium containing 2.0 mg/l NAA was the most positively effective on root formation. NAA was better than IAA and IBA during the rooting stage. RAPD-PCR analysis based on DNA fingerprints gave no evidence of somaclonal variation during the *in vitro* propagation protocol. The RAPD profiles of tissue culture derived plantlets revealed high similarity to mother plants. The survival of the acclimatized plantlets reached 60% in plastic pots filled with a mixture of peatmoss and sand at a ratio of 1:1 v/v.

Key words: Micropropagation · *In vitro* · Tissue culture · *Ixora* · Shoot tips

INTRODUCTION

Malathy and Pai [1] illustrated a method for micropropagation of *Ixora singaporensis* Roxb, where stem segments (1 cm long) containing the nodal or apical region with a pair of dormant axillary or apical buds were placed in a basal medium of Murashige and Skoog (MS) medium or Gamborg's (B5) medium. MS medium was supplemented with adenine sulfate with or without benzyladenine (BA) and B5 medium was supplemented with indole butyric acid (IBA). For initiation of shoots, MS medium + 10 mg/l adenine sulfate was best, with shoots appearing after 15 days. Zeng *et al.* [2] obtained callus from nodal stem segments of *Ixora coccinea* L. on MS medium supplemented with 2.0 mg/l 2, 4-D. Shoot multiplication of *Ixora coccinea* L was increased 4-fold and little callus was induced in MS medium with 1.0 mg/l BA and 0.2 mg/l NAA. Only shoots but no callus was induced in MS medium with 0.2-2.0 mg/l NAA. Many shoot clusters were induced from callus subcultures in medium with 0.5 mg/l BA and 0.5 mg/l NAA.

Rabia *et al.* [3] reported that callus cultures were initiated from leaf (explants) of *Ixora chinensis* taken from expanding young leaves. MS salt mixture containing various concentrations of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) was used for callus initiation. It was significantly higher in MS medium containing 3mg/l 2, 4-D. Two media were used (WPM and MS) for embryogenesis of *Ixora chinensis*. No embryogenesis occurred but callus multiplication was observed. The best combination which showed excellent multiplication was both WPM and MS media supplemented with gibberellic acid at 0.15 mg/l.

Lakshmanan *et al.* [4] found that stem cuttings of *Ixora coccinea* were the most suitable explants for multiple-shoot proliferation and when cultured on woody plant medium (WPM) containing 0.1 mg/l BA, produced axillary shoots which branched repeatedly, yielding an average of 27 shoots per explant after 6 weeks of culture. The presence of indole acetic acid (IAA) in the multiplication medium was detrimental to shoot proliferation but did not affect shoot growth. Production of large amounts of basal callus and vitrification of shoots were the major problems in proliferating shoot cultures.

Corresponding Author: Salwa S. Sakr, Ornamental Horticulture Department,
Faculty of Agriculture, Cairo University, Giza, Egypt.

Malathy and Pai [1] found that rooting of *Ixora singaporensis* was best on B5 medium supplemented with IBA. Zeng *et al.* [2] obtained that the best results when the shoots of *Ixora coccinea* L were rooted in 1/2-strength MS with 0.5 mg/l (NAA) naphthalene acetic acid and achieved 93.5% survival rate of the *in vitro*-derived plantlets of *Ixora coccinea* L. was at transplanting.

Rabia *et al.* [3] found direct root hairs formation of *Ixora chinensis* from calluses induced on 2,4-D at 1 and 2 mg/l. Saifullah *et al.* [5] concluded that the most suitable medium for rooting *Ixora coccinea* was half-WPM enriched with IBA at a concentration of 0.05 mg/l.

The aim of this study was to establish a well-defined protocol for *in vitro* propagation of difficult propagated *Ixora undulata* plant.

MATERIALS AND METHODS

This study had been carried oout in Plant Tissue Culture Laboratory, Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out during the period from 2008 to 2010. The objective of this study was to investigate the most suitable protocol for micropropagation of *Ixora undulata* Roxb.

Plant Material: The mother plants were grown naturally at the open field condition at Zohria Botanical Garden. Shoot tips and auxiliary buds were used as explants.

Culture Room Condition: The cultures were kept under a 16 h photoperiod at 27±1 °C [6]. The pH of the medium was adjusted to 5.8 and solidified with 0.7% agar. Explants were then transferred to the growth room at 27±1 °C, 16 h photoperiod and 32 μmol m^{-2} s^{-1} light intensity for 120 days [7]. The cultures were maintained at 27±1 °C, 16 h photoperiod and 35 μmol m^{-2} s^{-1} light intensity for 60 days [8].

Experimental Design and Statistical Analysis: A factorial experiment in a complete randomized design was employed in all of the experiments. Analysis of variance was used to show statistical differences between the means using the L.S.D. at 5% probability level [9].

Experimental Treatments

Surface Sterilization of Explants: The explants were excised from the mother plants and then washed by a soapy water for 15 min. followed by two hrs under a running tap water. They were then sterilized by immersion in a clorox (commercial bleach) solution at the rate of 20, 25, 30 and 35% plus 2-3 drops of tween 20 for 20, 25 and 30min. Finally, they were washed 5 times with sterile distilled water. At the end of the experiments, the collected data included: Survival percentage, contamination percentage and mortality percentage.

Establishment Stage: In culture establishment stage, MS medium [10] at full, half and quarter strengths supplemented with NAA (0.0, 0.1, 0.5 and 1.0 mg/l) and their combinations were used for investigation of explants development. All treatments of medium were solidified and supplemented with 7.0 g/l agar. Sucrose at 30.0 mg/l was added as a source of carbohydrate. The pH was adjusted at 5.7. Twenty ml medium were poured in 100 ml jars and sterilized by autoclaving under steam pressure of 1.5 bar at 121°C for 20 min. Number of leaves and shoot length (cm) were recorded.

Multiplication Stage: At the multiplication stage, two experiments were initiated to determine the most suitable medium for this stage. An initial experiment was carried out using both thidiazuron (TDZ) at 0.0, 0.5, 1.0, 2.0 or 3.0 mg/l and IBA (0.0, 0.5, 1.0 and 2.0 mg/l). Twenty treatments were used in this experiment. The second experiment was initiated using BA (0.0, 0.5, 1.0 and 2.0 mg/l) and kinetin (kin) at 0.0, 0.1, 0.5, 1.0 and 1.5 mg/l giving twenty treatments. Each treatment consisted of three jars containing three shoots in each jar. Shoot length (cm), number of leaves and number of shoots were recorded.

Rooting Stage: The resulted shoots of *Ixora undulata* (3.5 cm in length with 6-7 leaves) were cultured *in vitro* on MS medium supplemented with different concentrations of IBA, IAA or NAA for rooting. Six treatments were established at the concentrations of 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l for each. Activated charcoal at 3.0 g/l was added to all media in order to improve root formation. Each treatment consisted of nine jars. Number of root, root length (cm), shoot length (cm) and number of leaves were recorded.

Isolating of Genomic DNA and RAPD Analysis: DNA of *Ixora undulata* was isolated using CTAB method of Doyle and Doyle [11].

RAPD-PCR Analysis: PCR amplification was performed in 20ul reaction mix containing 20 ng genomic DNA, 1 unit Taq polymerase (Gibco), 200 uM each of dATP, dCTP,

dGTP, dTTP, 20 p mole random primer (Operon) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed according to Williams *et al.* [12] for 45 cycles, using Biometera Uno thermal cycler, as follows:

One cycle at 92°C for 3 min and then 45 cycles at 92°C for 30 sec, 35°C for 60 sec and 72°C for 2 min (for denaturation, annealing and extension, respectively). Reaction was finally incubated at 72°C for 10 min and further 10 min at 62°C.

Electrophoresis: The amplification products were analyzed by electrophoresis according to Sambrook *et al.* [13] in 2% agarose in TAE buffer (for each litre of 50X TAE Stock solution: 242 g Tris Base, 57.1 mL glacial acetic acid and 100 mL 0.5 M EDTA), stained with 0.2 ug/ml ethidium bromide. Nucleic acids bands were photographed and detected under short wave UV light.

Acclimatization Stage: Rooted plantlets were pricked out singly into 10 cm plastic pots filled with 1: 0, 1: 1, 1: 2 and 1: 3 (v/v) peatmoss and sand, respectively. To maintain cultures at high humidity, pots were covered with clear transparent plastic sheets for three weeks. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions, after that survival capacity (%) was recorded.

RESULTS AND DISCUSSION

Effect of Clorox Concentrations and Soaking Periods on Surface Sterilization of *Ixora* Explants: Results demonstrated in Table 1 indicate that surface sterilization by clorox (NaOCl) was positively significant on survival explants of *Ixora undulata* shoot tips. This effect increased with the increase of clorox concentration, while the percentage of contaminated explants was decreased. No mortality (0%) was observed when explants were treated by 10, 15 and 20% clorox. The best concentration was 20 %, which gave 40% survived explants.

On the other hand, the data indicated that increasing the soaking period of explants increased the survival percentage of explants. The highest percentage of survival (25.00%) was recorded when soaking the explants for 30 min. The lowest percentage of contamination (55.00%) was obtained at soaking period of 25 min and the highest percentage of mortality explants (12.50%) was recorded when soaking the explants for 30 min.

The data of the interaction between the concentration of clorox and the soaking period of explants indicated that the best percentage of survival explants (70%) was obtained when explants immersed for 30 min in 20% clorox. In this respect, using 20% clorox for 30 min was necessary for increasing the survival percentage as well as reducing the contamination percentage and mortality percentage of *Ixora* explants. These results are in harmony with those obtained by El-Shamy *et al.* [14].

Effect of Different Strengths of MS Medium and Different Concentrations of NAA on Establishment Stage of *Ixora undulata*: Data in Table 2 showed that the shoots were the tallest (1.25cm) at full strength. Also, there were significant differences in shoot length between the different strengths of MS medium. Full strength of MS medium produced the largest number of leaves (7.17).

For the different concentrations of NAA, the tallest shoot length (1.17cm) and the largest number of leaves (7.11) were found when MS medium containing (0.5 mg/l NAA), compared to control treatment (gave the shortest shoots (0.61cm) and the lowest number of leaves (4.78)).

Table 1: Effect of clorox and soaking periods during surface sterilization on explants of *Ixora undulata*

	Survival (%)				Contamination (%)				Mortality (%)			
	Soaking Periods (min)				Soaking Periods (min)				Soaking Periods (min)			
Clorox (%)	20	25	30	Mean (A)	20	25	30	Mean (A)	20	25	30	Mean(A)
10.00	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
15.00	0.00	0.00	10.00	3.33	100.00	10.00	90.00	66.67	0.00	0.00	0.00	0.00
20.00	20.00	30.00	70.00	40.00	80.00	70.00	30.00	60.00	0.00	0.00	0.00	0.00
25.00	30.00	40.00	20.00	30.00	60.00	40.00	30.00	43.33	10.00	20.00	50.00	26.6
Mean (B)	12.50	17.50	25.00	-- -	85.00	55.00	62.50	---	2.50	5.00	12.50	---
$LSD_{0.05}$ Clorox (A)		6.88				5.62				3.44		
Periods (B)		5.96				4.87				2.98		
(AxB)		11.92				9.73				5.96		

Table 2: Effect of MS medium strength and NAA on *Ixora undulata* during establishment stage

	Shoot length (cm)					No. of leaves				
	NAA (mg/l)					NAA (mg/l)				
MS (strength)	0.00	0.10	0.50	1.00	Mean (A)	0.00	0.10	0.50	1.00	Mean(A)
Full	0.83	1.17	1.67	1.33	1.25	5.33	6.33	8.33	8.67	7.17
1/2	0.50	0.67	1.17	0.83	0.79	4.67	5.67	6.67	6.67	5.92
1/4	0.50	0.83	0.67	0.50	0.63	4.33	5.33	6.33	6.00	5.50
Mean (B)	0.61	0.89	1.17	0.89	---	4.78	5.78	7.11	7.11	---
$LSD_{0.05}$ MS (A)			0.21					0.47		
NAA (B)			0.24					0.54		
(AxB)			0.42					0.93		

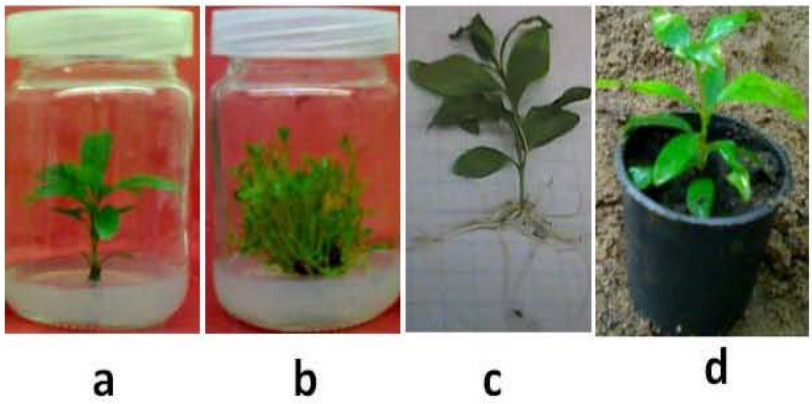

Fig. 1: Different stages of *Ixora undulata* micropropagation
a: Establishment stage, b: Multiplication stage, c: Rooting stage,d: Acclimatization stage

The combination between different concentrations of NAA and MS strengths (Fig. 1) resulted in the longest shoots (1.67cm) on MS full strength containing 0.5 mg/l NAA. The largest number of leaves (8.67) was recorded at 1.0 mg/l NAA followed by 8.33 leaves at 0.5 mg/l NAA. But the shortest shoots (0.50 cm) and the lowest number of leaves (4.33) were recorded when the explants cultured on quarter MS medium free hormones (control).

On the other hand, El-Shamy *et al*. [15] obtained higher number of leaves on all buds by using NAA in the culture media.

Effect of Different Concentrations of TDZ and IBA on Multiplication Stage of *Ixora undulata*: Data recorded in Table 3 demonstrated that the rate of proliferation, as identified by the number of shoots, was increased by increasing the concentration of TDZ when compared with the control in each case. The most shoots (1.83) were formed on medium supplemented with 1.0 mg/l TDZ. All concentrations of TDZ produced significantly shorter shoot length and lower number of leaves when compared to zero level (control).

For the different concentrations of IBA, the number of leaves and shoot length was increased by increasing the concentration level of IBA when compared with the control in each case. Number of shoots was decreased by increasing the concentration level of IBA.

Regarding the interaction between TDZ and IBA, the shoots cultured on MS medium supplemented with 1.0 mg/l TDZ produced the highest number of shoots (3.33). While the shoots on MS medium supplemented with 2.0 or 3.0 mg/l TDZ plus any concentrations of IBA produced the lowest number of shoots.

Effect of Different Concentrations of BA and Kin on Multiplication Stage of *Ixora undulata*: Results shown in Table 4 reveal that the multiplication of *Ixora undulata* was successfully achieved by culture on MS medium supplemented with different concentrations of BA and Kin.

For BA concentrations, after three subcultures in MS medium containing 1.0 mg/l BA, the mean higher number of shoots (30.00) giving significant effect as compared with 0.0, 0.5 and 2.0 mg/l BA. The highest values for shoot

Table 3: Effect of different concentrations of TDZ and IBA on multiplication stage of *Ixora undulata*

	Shoot length (cm)					Number of leaves					Number of shoots				
	IBA (mg/l)					IBA (mg/l)					IBA (mg/l)				
TDZ (mg/l)	0.0	0.5	1.0	2.0	Mean(A)	0.0	0.5	1.0	2.0	Mean(A)	0.0	0.5	1.0	2.0	Mean (A)
0.0	2.33	2.83	3.33	4.17	3.17	6.33	6.67	7.33	8.33	7.17	0.00	0.00	0.00	0.00	0.00
0.5	1.33	1.50	1.83	2.17	1.71	4.67	5.33	5.33	6.33	5.42	1.00	0.67	0.67	1.33	0.92
1.0	1.17	1.33	1.33	1.67	1.38	5.00	5.33	5.33	5.67	5.33	3.33	1.67	1.33	1.00	1.83
2.0	1.00	1.17	1.17	1.33	1.17	4.33	4.67	4.67	5.33	4.75	1.67	1.33	1.33	1.00	1.33
3.0	1.00	1.00	1.17	1.33	1.13	4.00	4.33	5.00	5.33	4.67	0.67	0.67	0.33	0.33	0.50
Mean (B)	1.37	1.57	1.77	2.13	---	4.87	5.27	5.53	6.20	---	1.33	0.87	0.73	0.73	---
$LSD_{0.05}$ TDZ (A)			0.21					0.44					0.39		
IBA (B)			0.19					0.40					0.35		
(AxB)			0.43					0.89					0.79		

Table 4: Effect of different concentrations of BA and Kin on multiplication stage of *Ixora undulata*

	Shoot length (cm)						Number of leaves						Number of shoots					
	Kin (mg/l)						Kin (mg/l)						Kin (mg/l)					
BA (mg/l)	0.0	0.1	0.5	1.0	1.5	Mean (A)	0.0	0.1	0.5	1.0	1.5	Mean (A)	0.0	0.1	0.5	1.0	1.5	Mean(A)
0.0	2.33	6.50	6.67	6.83	6.67	5.80	6.33	6.67	6.67	6.67	6.33	6.53	0.00	4.67	7.33	16.00	12.33	8.07
0.5	5.50	5.00	4.67	4.33	4.00	4.70	6.00	5.67	5.33	5.33	4.67	5.40	16.33	11.67	11.33	11.33	9.33	12.00
1.0	5.33	4.50	3.33	3.33	2.50	3.80	5.33	5.33	5.00	4.67	4.67	5.00	56.00	43.33	26.00	16.67	8.33	30.07
2.0	2.33	1.67	1.33	1.33	1.17	1.57	4.67	3.33	2.67	2.33	2.33	3.07	7.67	5.67	3.33	1.67	1.33	3.93
Mean (B)	3.88	4.42	4.00	3.96	3.58	---	5.58	5.25	4.92	4.75	4.50	---	20.00	16.33	12.00	11.42	7.83	---
$LSD_{0.05}$ BA (A)			0.27						0.41						0.83			
Kin (B)			0.30						0.46						0.92			
(AxB)			0.61						0.91						1.85			

Table 5: Effect of IBA at different concentrations on rooting stage of *Ixora undulata*

IBA (mg/l)	No. of roots	Root length (cm)	Shoot length (cm)	No. of leaves
0.00	6.00	5.89	4.22	9.33
0.10	0.78	1.16	4.11	7.11
0.50	0.56	0.66	3.67	6.67
1.00	0.00	0.00	3.61	6.11
2.00	0.00	0.00	3.28	5.44
4.00	0.00	0.00	3.11	5.22
$LSD_{0.05}$	0.27	0.45	0.22	0.30

length (5.80 cm) and number of leaves (6.53) had been obtained from the control treatment.

Reducing the concentration of Kin resulted in a significant increase in number of shoots (20.00) at zero-level. While the highest value of shoot length (4.42cm) was measured at 0.1 mg/L Kin. Whereas, the number of leaves was 5.58 for control treatment.

The interaction between the different concentrations of BA and Kin showed that the best concentration was 1.0 mg/l BA and 0.0 mg/l Kin on number of shoots giving 56.00 shoots. For shoot length, there were significant differences between all the different concentrations of growth regulators but the longest shoot was 6.83 cm at 1.0 mg/l Kin while the best number of leaves was 6.67 at 0.1, 0.5 or 1.0 mg/l Kin.

Effect of Different Concentrations of Auxins on Rooting Stage of *Ixora undulata*: The data shown in Table 5 reveal that IBA caused a decrease in number of roots, root length, shoot length and number of leaves of *Ixora*. This was true between the different concentrations of IBA and also when compared with the zero-level (control). The highest number of roots and root length was found when 0.1 mg/l IBA was used.

For IAA levels, data in Table 6 noted that only 0.0 mg/l IAA induced the formation of roots on *Ixora* shoots (6.0 roots) when compared with the other concentrations. Also results presented in Table 6 show that only MS-free gave positive plantlet height and number of leaves during *in vitro* rooting of *Ixora* results.

Table 6: Effect of IAA at different concentrations on rooting stage of Ixora *undulata*

IAA (mg/l)	Number of roots	Root length (cm)	Shoot length (cm)	Number of leaves
0.00	6.00	5.89	4.22	9.33
0.10	0.00	0.00	3.61	7.78
0.50	0.00	0.00	3.56	6.78
1.00	0.00	0.00	3.44	6.44
2.00	0.00	0.00	3.28	6.44
4.00	0.44	2.56	3.22	6.00
$LSD_{0.05}$	0.26	0.95	0.30	0.54

Table 7: Effect of NAA at different concentrations on rooting stage of *Ixora undulata*

NAA (mg/l)	Number of roots	Root length (cm)	Shoot length (cm)	Number of leaves
0.00	6.00	5.89	4.22	9.33
0.10	5.22	10.22	4.44	9.44
0.50	6.11	10.22	4.78	9.33
1.00	9.22	9.33	4.22	9.44
2.00	6.89	8.11	3.83	7.55
4.00	0.00	0.00	3.33	6.44
$LSD_{0.05}$	0.71	0.92	0.24	0.47

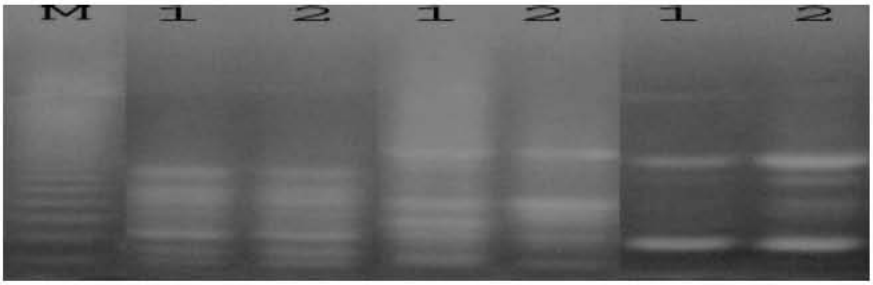

Fig. 2: Gel electrophoresis of RAPD fragments generated by primers A6, C12 and B6. Lane (1) represents *in vitro* produced plants; lane (2) represents *in vivo* plants and lane (M) indicates molecular weight DNA marker by bp.

For NAA concentrations, results in Table 7 indicate that *in vitro* rooting of *Ixora undulata* shoots commenced straight after three week from transformation of shoots to the rooting media treatments. In general, the addition of NAA to the rooting media led to increases in the number of roots and in the mean length of roots. By increasing the NAA concentration to 1.0 mg/l, there was a marked increase in the number of roots when compared with the zero-level control. It was found that 1.0 mg/l NAA gave the highest number of roots (9.22) and there were significant differences between it and the different concentrations (Fig. 1).

These results are in line with other studies recording that shoots of *Ixora coccinea* L were rooted in 1/2-strength MS with 0.5 mg/l NAA [2].

RAPD Analysis of *in vivo* and *in vitro Ixora* Plants: DNA of *in vivo* and *in vitro* plants was prepared from leaves and amplified by PCR amplification of the isolated DNA using random oligonucleotide primers obtained from Operon. The RAPD reaction was performed in a 25 µl reaction volume with 20 ng genomic DNA, 0.15 mM dNTPs 10 picomols primer, 2 mM $MgCl_2$ and 1U Taq DNA polymerase. The amplified products were separated on a 2 % agarose gel, visualized by staining the gel in 0.5 µg ml^{-1} ethidium bromide and documented with a gel documentation system to reveal band polymorphism. Data in Fig. 2 demonstrated the results of RAPD analysis of *in vivo* and *in vitro* Ixora plants.

Out of the 3 random primers screened, (sequences presented in Table 8) didn't found amplification products that were monomorphic across all the *in vivo* and *in vitro* plants.

Recently, RAPD fingerprints (Randomly Amplified Polymorphic DNA) showed several advantages as molecular marker. Employment of molecular marker technology including biochemical markers DNA-based markers were used for cultivar identification and screening of tissue culture derived plants for somaclonal variation (quality control test).

Table 8: Primers used and their annealing temperatures

Primer	Sequence 5'--------3'
A6	TGGCGACCTG
C12	GAGGCGTCGC
B6	CCCTACCGAC

Table 9: Effect of different mixtures of peatmoss and sand on survival (%) during acclimatization stage of *Ixora undulata*

peatmoss	Sand	Survival %
1	0	40.00
1	1	60.00
1	2	40.00
1	3	20.00
$LSD_{0.05}$		11.53

The RAPD profiles revealed by the bulked DNA samples of each explants (*in vivo* and *in vitro* plants) exhibited the same common bands previously identified in the individual samples for each explants. On the other hand, RAPD-based DNA fingerprints gave no evidence of somaclonal variation during the *in vitro* propagation protocol used. The RAPD profiles of tissue culture-derived plantlets revealed high similarity to mother plant (Fig.2).

Effect of different mixtures of peatmoss + sand on acclimatization stage of *Ixora undulata*: It is clear from the data in Table 9 that the various treatments caused a significant influence on acclimatization behavior.

During this stage of culture the plantlets grew vigorously and had healthy appearance. A high percentage of plant survival (60%) was achieved when peatmoss: sand (1:1 v/v) medium was used compared to other media. After four weeks, no abnormalities in physical appearance and growth habits were observed on the transplanted plants (Fig. 1).

REFERENCES

1. Malathy, S. and J.S. Pai, 1998. Micropropagation of *Ixora singaporensis* (Linn.) an ornamental shrub. Current Sci., 75: 545-547.
2. Zeng, S.J., S.C. Guo, X.M. Peng, J.L. Zhang and F.B. Zhao, 1999. Tissue culture and rapid propagation of *Ixora coccinea* L. J. Plant Resources and Environ., 8: 37-41.
3. Rabia, N., M.A. Khan, M.J. Jaskani and H. Nazir, 2001. Callogenesis and embryogenesis from leaf disks of *Ixora chinensis*. Intl. J. Agric. Biol., 3: 65-67.
4. Lakshmanan, P., C.L. Lee and C.J. Goh, 1997. An efficient *in vitro* method for mass propagation of a woody ornamental *Ixora coccinea* L. Plant Cell Reports, 16: 572-577.
5. Saifullah, K., I. Mariam and S. Bushra, 2004. An economical and efficient method for mass propagation of *Ixora coccinea*. Pakistan J. Botany, 36: 751-756.
6. Pasqual, M. and I. Barros, 1991. Effect of benzylaminopurine and naphthaleneacetic acid on shoot proliferation and *in vitro* growth of *Coffea arabica* L. Pesquisa Agropecuaria Brasileira, 26: 201-204.
7. Jesus, A.M.S., S.P. Carvalho, M. Pasqual, M. Carvalho and L.F. Dutra, 2002. Effect of BAP concentrations in pre culture medium and BAP and TDZ in subculture medium on coffee micropropagation. Revista Ceres, 49: 253-263.
8. Ribeiro, L.S., M. Pasqual, A.L.R. Maciel, E.A. Chagas and L.F. Dutra, 2002. *In vitro* multiplication of *Coffea arabica* L. cultivars in different culture medium. Cienciae Agrotecnologia, 26: 949-954.
9. Snedecor, G.W. and W.G. Cochran, 1989. One Way Classification, analysis of variance. In: Statistical Methods (8th Ed.). Iowa State Univ. Press, Ames, Iowa, U.S.A., Ch., 12: 217-236.
10. Murashig, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.
11. Doyle, J.J. and J.L. Doyle, 1990. Isolating of DNA from fresh tissue. Focus, 12: 13-15.
12. Williams, G., A. Kubelik, K. Livak, J. Rafalski and S. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18: 6532-6535.
13. Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor, NewYork.
14. El-Shamy, M.A., S.S. Sayed and S.A.A. Gomaa, 2009. Micropropagation and genetic stability of *Pyracantha fortuneana* Roem shrub. Egypt. J. Hort., 36: 149-161.
15. El-Shamy, M.A., A.E.H. El-Feky and N.Y. Eliwa, 2009. Propagation of Calla lily (*Zantedeschia aethiopica*) plants by tissue culture technique. Bull. Fac. Agric., Cairo Univ., 60: 99-105.