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Studies on Micropropagation of *Begonia Rex* Putz Plants

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ABSTRACT

This study was intended to find out the protocol easily for *in vitro* propagation of *Begonia rex* Putz. due to considered as taking a step toward meeting the increased demand for economically important ornamentals. This study was carried out in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out through 2022 years. In this respect, shoot tips of the plant were effectively surface sterilized using mixture of 1.5 % sodium hypochlorite and 1.0 g/l mercuric chloride. Medium containing 3.0 mg/l IBA was better NAA for shoot length and number of leaves during establishment stage. For further multiplication, medium supplemented with 2.0 mg/l Kin formed the highest shoot length, number of leaves and number of shoots. Thus, half-strength of MS medium supplemented with 2.0 mg/l IBA gave the highest number of roots and root length. Nevertheless, the best survival percentage of plantlets at acclimatization stage (90 %) was observed when plantlets cultured in a mixture of peatmoss and Sand at 3/1(v/v).

Keywords: Micropropagation, *In Vitro*, Tissue culture, Begonia, Shoot tips.

1. Introduction

Jain, (2002) noted that Begonia (*Begonia rex*) is an ornamental plant belongs to Begoniaceae family, Originating in Brazil. Plant tissue culture method is a part of biotechnology that was used for massive propagation. Many factors such as growth regulators, plant species, explants type, environmental conditions (temperature and light) influence organogenesis and *in vitro* multiplication. However, growth regulators have the most effective influence for growth of plants *in vitro*. Kim *et al*., (2003) demonstrated that the success of the clonal propagation method depends on numerous factors like genotype, media, plant growth regulators and type of explants, which should be tested during the process. Fang *et al*., (2006) reported that Begonia montaniformis plants are difficult to grow. The germinated seedlings rarely survive and were extremely sensitive to environmental changes. If seedlings were grown satisfactorily, the leaves last for long periods.

Toma and Ahmed (2019) propagated Begonia rex by tissue culture technique. Two types of explants were tested at establishment stage including leaf discs and petioles after being disinfested with NaOCl for 15 minutes and the survival rates reached 70% and 60% respectively. Jelaska (2001) studied the effects of thidiazuron (TDZ) on adventitious shoot bud formation. Explants of Begonia were exposure to 40 pM TDZ in liquid MS medium for 5 to 120 min, followed by culture on agar-solidified MS medium without growth regulators. Sixty min exposure to TDZ resulted in the highest mean number of regenerated shoots. During the third and fourth week in culture shoots developed small roots. The sucrose concentration in the basal medium influenced the efficiency of shoot organogenesis. The shoot formation tended to increase when the medium was supplemented with 2% sucrose instead with 3%.

Lai *et al*. (2018) determined that the optimal conditions for the micropropagation of a Begonia montaniformis 3 Begonia ningmingensis var. bella F1 progeny by using various concentrations of plant growth regulators (PGRs) and varying light spectra in half-strength Murashige and Skoog (1/2 MS) medium. Also, the explant regeneration was optimal when the lamina was incubated in a medium supplemented with 2.0 mM N6 -benzylaminopurine and 0.8 mM a-naphthaleneacetic acid (NAA).

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However, under such conditions, 98% of the explants regenerated adventitious shoots after 8 weeks, and 41 buds were produced per explant on average. The applied light spectrum significantly influenced shoot regeneration and optimal results were achieved under an equal distribution of blue, red, and infrared light. Toma and Ahmed (2019) reported that the shoots were successfully multiplied by the addition of BA and kinetin to the culture media. Also, Ghatas. (2016) that using 2.0 mg/L BAP is recommended for maximizing proliferation of *Paulownia tomentosa*, Kinetin was more effective than BA at the same or higher levels added to the multiplication media.

Lai *et al*. (2018) reported that the adventitious shoots were transferred into rooting medium consisting of 1/2 MS and various NAA concentrations. The shoots subcultured in this medium showed root induction. The rooting adventitious shoots were subcultured in PGR-free medium for 8 weeks. Toma and Ahmed (2019) noted that the shoots were rooted on MS medium supplemented with NAA and giving the highest number of roots and the longest roots which were higher than the control treatment. NAA performed better than IAA and IBA at rooting stage of begonia micro-shoots. The highest rooting percentage was achieved from the culture of micro-shoots on half strength MS salts which was higher than obtained from both full and quarter MS salt strengths. Lai *et al*. (2018) showed that the plantlets were successfully acclimated 4 weeks after being transferred to soil and bloomed after 11 months in a greenhouse.

This study basically aimed to determine the response of begonia explants to various culture media components, test the effects of growth regulators including cytokinins (BA and Kinetin) and auxins (IBA and NAA) on explant establishment of begonia, different strength of MS on shoots rooting, peatmoss and sand on plantlets acclimatization to develop a procedure for mass production for the micro propagated begonia plantlets will considered as taking a practical step toward meeting the increased local demand for economically important ornamentals.

2. Materials and Methods

2.1. Location and duration

This study was carried out in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out through 2022 years.

2.2. Plant material

The mother plants of *Begonia rex* Putz. was grown at pot plant in greenhouse condition. Shoot tips excised from the plant were used as explants.

2.3. Culture medium

Murashige and Skoog (MS) medium were used for culturing the explants. Medium were supplemented with 30 g/l sucrose and 7 g/l agar. The medium was adjusted to pH 5.7±0.1 and autoclaved at 121°C (1.5 kg/cm^2) for 20 min before using.

2.4. Experimental treatments

2.4.1. Surface Sterilization of Explants

Explants were washed by soapy water for 15 min followed by two hour under running tap water. Then explants were transferred to sterilize at the air laminar flow by immersion in a sodium hypochlorite (NaOCl) at 0.5, 1.0, 1.5 and 2.0 % for twenty min and mercuric chloride ($HgCl_2$) at 1.0, 1.5 and 2.0 g/l for five min. Finally, they were washed 5 times with sterile distilled water. At the end of the experiments, the collected data included survival percentage, mortality percentage and contamination percentage of explants was recorded.

2.4.2. Establishment stage

Basal MS medium was supplemented with NAA or IBA at 0.0, 1.0, 2.0, 3.0, 0.4 and 5.0 mg/l to the establishment medium treatments. Data recorded for shoot length (cm) and number of leaves/explant was calculated after four weeks.

2.4.3. Multiplication stage

For multiplication stage, shoots were cultured on MS medium supplemented with BA or Kin at 0.0, 1.0, 2.0 and 3.0 mg/l. This stage was repeated three times every 4 weeks by sub-culturing on the same freshly prepared media of each treatment.

2.4.4. Rooting stage

At the rooting stage, shoots were cultured on MS medium at different strength (full, half and quarter) supplemented with different concentrations of IBA (0.0, 1.0, 2.0 and 3.0 mg/l). Activated charcoal at 3.0 g/l was added to all media to improve root formation.

2.4.5. Acclimatization stage

Rooted plantlets were cultured singly into 8 cm plastic pots filled with peatmoss at 1, 2, 3 or 4 and sand at 0, 1, or 2 (v/v) under plastic tunnel at plastic house condition. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions.

2.4.6. Culture condition

Cultures were incubated in a growth chamber under controlled conditions at 24 ± 2 °C. All cultures were exposed to a 16-h photoperiod/day (24 h cycle) at an intensity of 2000 lux from white fluorescent tube lamps.

2.5. Experimental design and statistical analysis

A complete randomized design was employed in all experiments. Analysis of variance was used to show statistical differences between treatments using the L.S.D. at probability level (5%) (Snedecor and Cochran, 1989).

3. Results and Discussion

3.1. Effect of sodium hypochlorite and mercuric chloride on surface sterilization

Data presented in Table (1) demonstrate that the effect of sodium hypochlorite as was positive for surface sterilization of Begonia buds. Number of survived explants was increased with the increase of sodium hypochlorite concentration to 1.5 mg/l, when mercuric chloride was present.

Regarding, the use of mercuric chloride (Hg_2Cl) on surface sterilization of explants, survived explants was decreased with increasing mercuric chloride when sodium hypochlorite was supplemented with 1.5% to the solution of surface sterilization.

Table 1: Effect of different concentrations of sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) on surface sterilization of explants.

$HgCl_2$ (g/l)	Survival (%)				Contamination (%)				Mortality (%)			
NaOCl (%)	1.0	1.5	2.0	Mean (A)	1.0	1.5	2.0	Mean (A)	1.0	1.5	2.0	Mean (A)
0.5	40	50	50	46.7	50	50	50	50.0	10	00	00	3.3
1.0	70	70	80	73.3	20	20	00	13.3	10	20	20	16.7
1.5	100	80	70	83.3	00	20	20	13.3	00	00	10	3.3
2.0	60	70	60	63.3	10	00	00	3.3	30	30	40	33.3
Mean	67.5	67.5	65.0		20.0	22.5	17.5		12.5	12.5	17.5	
LSD at 0.05												
$HgCl_2$ (A)			1.39			1.44			1.33			
NaOCl (B)			1.21			1.25			1.15			
(AxB)			2.41			2.50			2.30			

The interactions between sodium hypochlorite and mercuric chloride were significant with the highest value of disinfected explants (100%) when the mixture of 1.5 % sodium hypochlorite and 1.0 g/l mercuric chloride was used.

Results obtained here are in harmony with those obtained elsewhere when clorox and soaking

period were used on its own at *Pyracantha fortuneana* (El-shamy *et al.,* 2009).

3.2. Effect of IBA and NAA on establishment stage

For the establishment stage, data in Table (2) and Plate (1) showed that different concentrations of IBA and NAA at 0.0, 1.0, 2.0 or 3.0 mg/l had a significant effect on shoot length and number of leaves/explant.

The longest shoots (2.5 cm) and highest number of leaves/explant (3.0 leaves) had been obtained on medium containing 3.0 mg/l IBA. Using medium containing 3.0 or 4.0 mg/l NAA produced the longest shoot (1.5 cm) and highest number of leaves/explant (2.0 leaves). But the explants were cultured on medium with IBA higher than medium with NAA.

Table 2: Effect of different concentrations of auxin on explant establishment.

Auxins (mg/l)	Shoot length (cm)			Number of leaves/explant		
	NAA	IBA	Mean (A)	NAA	IBA	Mean (A)
0.0 mg/l	0.5	0.5	0.50	1.0	1.0	1.00
1.0 mg/l	1.0	0.5	0.75	1.0	1.0	1.00
2.0 mg/l	1.0	0.5	0.75	2.0	1.0	1.50
3.0 mg/l	2.5	1.5	2.00	3.0	2.0	2.50
4.0 mg/l	1.5	1.5	1.50	2.7	2.0	2.35
5.0 mg/l	1.5	1.0	1.25	2.0	1.0	1.50
Mean (B)	1.33	0.92		1.4	1.33	
LSD at $_{0.05}$						
Concentrations (A)		0.23			0.91	
Auxins (B)		0.14			0.52	
(AxB)		0.33			1.28	

3.3. Effect of BA and Kin on multiplication stage

Regarding subculture effect, it was found that all studied parameters were positively increased with the subculture progress. Also, the greatest number of leaves, longest shoot and highest number of shoots (3.52 leaves, 3.17 cm and 3.81 shoots, respectively) were obtained after the third subculture as compared to the other subcultures.

The data in Table (3) and Plate (1) illustrate that BA concentrations decreased number of leaves, shoot length and number of shoots. The highest number of leaves (2.56 leaves), shoot length (2.17 cm) and number of shoots (1.78) had been obtained on medium control as compared with BA treatments.

The obtained results showed that Kin at different concentrations increased the number of leaves, shoot length and number of shoots giving the highest number of leaves (6.33 leaves), shoot length (6.33 cm) and number of shoots (8.67 shoots) at 2.0 mg/l Kin after the third subculture as compared with all treatments.

Results of Kin treatments giving the highest value of all studied parameters were higher than BA treatments.

A similar observation has also been reported in other Begonia species. It was found, using BA alone resulted relatively unsatisfactory mean shoot elongation and high explant necrosis (Godo *et al*., 2008; Kumari *et al*., 2017; Mendi *et al*., 2009 and Nada *et al*., 2011).

These results seemed to be in harmony with those obtained on begonia shoot multiplication from both leaves and petioles that kinetin was more effective than BA except of kinetin when added at 7.0 mg/l (Toma and Ahmed, 2019).

3.4. Effect of MS-strength and NAA on rooting stage

Results presented in Table (4) and Plate (1) demonstrate that the MS-strength under study clearly affected number of roots and root length during the rooting stage of Begonia rex. The MS medium at half-strength was superior than other MS-strength in the number of roots and root length formed (4.17 roots and 2.50 cm, respectively). Whereas, full and quarter MS strength decreased the formation of roots on Begonia shoots when compared with the others treatments.

For IBA concentrations, results were a steady increase in number of roots and root length with

increasing of IBA concentrations. Thus, it was found that the highest number of roots and root length was obtained at half-strength of MS medium supplemented with 2 mg/l IBA (8.33 roots and 4.67 cm, respectively) when compared to the others IBA concentrations. In this context, Ghatas (2020) reported that the highest rooting response of *Myrtus communis* L. was observed with 2 mg/l IBA.

The reason behind the superiority of half MS salt strength on the full MS salt strength in rooting traits is due to the higher C/N ratio which means increasing energy source (carbohydrate), considered necessary source for rooting since the same sucrose was used with both salt strength (Hartmann *et al*., 2002).

Table 3: Effect of different concentrations of BA or Kin and subcultures on shoots multiplication.

Cytokinins (mg/l)	No. of leaves				Shoot length (cm)				No. of shoots			
	Subcultures			Mean	Subcultures			Mean	Subcultures			Mean
	1	2	3	(A)	1	2	3	(A)	1	2	3	(A)
0.0	1.33	2.33	4.00	2.55	1.67	2.17	2.67	2.17	1.33	1.67	2.33	1.78
1.0 BA	1.00	1.67	2.67	1.78	1.50	1.67	2.17	1.78	1.33	2.00	2.67	2.00
2.0 BA	1.00	1.67	2.33	1.67	1.50	1.50	1.67	1,56	1.67	2.00	2.67	2.11
3.0 BA	1.00	1.33	1.67	1.33	1.50	1.67	1.00	1.39	1.00	1.33	1.33	1.22
1.0 Kin	2.33	3.00	3.00	2,78	2.17	2.83	3.67	2.89	2.33	2.67	3.33	2.78
2.0 Kin	3.33	4.00	6.33	4.55	2.33	3.67	6.33	4.11	3.00	4.67	8.67	5.45
3.0 Kin	3.00	3.67	4.67	3.78	3.33	4.33	4.67	4.11	3.00	3.67	5.67	4.11
Mean (B)	1.86	2.52	3.52		2.00	2.55	3.17		1.95	2.57	3.81	
LSD at $_{0.05}$												
Cytokinins (A)			0.44			0.34			0.49			
Subcultures (B)			0.29			0.22			0.32			
(AxB)			0.76			0.58			0.84			

Table 4: Effect of different strength of MS and IBA on shoots rooting.

MS Strength	Number of roots				Mean (A)	Root length (cm)				Mean (A)
	IBA (mg/l)					IBA (mg/l)				
	0.0	1.0	2.0	3.0		0.0	1.0	2.0	3.0	
MS	0.00	1.00	2.33	3.33	1.67	0.00	0.67	1.67	1.83	1.04
$^1/_2$ MS	1.00	1.67	8.33	5.67	4.17	0.67	1.67	4.67	3.00	2.50
$^1/_4$ MS	0.33	1.00	2.67	4.67	2.17	0.17	0.83	2.17	1.67	1.21
Mean (B)	0.44	1.22	4.44	4.56		0.28	1.06	2.83	2.17	
LSD at $_{0.05}$										
MS strength (A)				0.42					0.20	
IBA (B)				0.48					0.23	
(AxB)				0.83					0.39	

3.5. Effect of peatmoss and sand on acclimatization stage

The well rooted Begonia rex plantlets were taken and gradually moved from lab aseptic conditions and washed with water. The plantlets then were transferred to pots containing a peatmoss (1, 2, 3 or 4 V) and sand (0, 1 or 2 v) suitable to grow begonia plant and covered by polyethylene sheets in greenhouse. After four weeks, the sheets were removed and left for two weeks under plastic house condition. The survival percentages of plantlets after six weeks were calculated. No abnormalities in physical appearance and/or growth habits were observed on the transplanted plantlets (Plate:1). The best percentage of survival plantlets (90 %) was observed when plantlets cultured in a mixture of peatmoss and sand at 3/1(v/v).

The plants of begonia were irrigated with a nutrient solution containing 1/4 strength of MS salts. After 8 to 10 days, the bags were opened and after further 8 to 10 days, the bags were removed and plants were grown under regular greenhouse conditions. The results of survival percentage reached to

75% of plantlets were survived to grow under greenhouse conditions (Toma and Ahmed, 2019).

Table 5: Effect of peatmoss and sand on survival percentage of plantlets acclimatization.

Peatmoss	Sand			
	0	1	2	Mean (A)
1	20.00	40.00	10.00	23.33
2	20.00	50.00	40.00	36.67
3	20.00	90.00	50.00	53.33
4	20.00	30.00	50.00	33.33
Mean (B)	20.00	52.50	37.50	

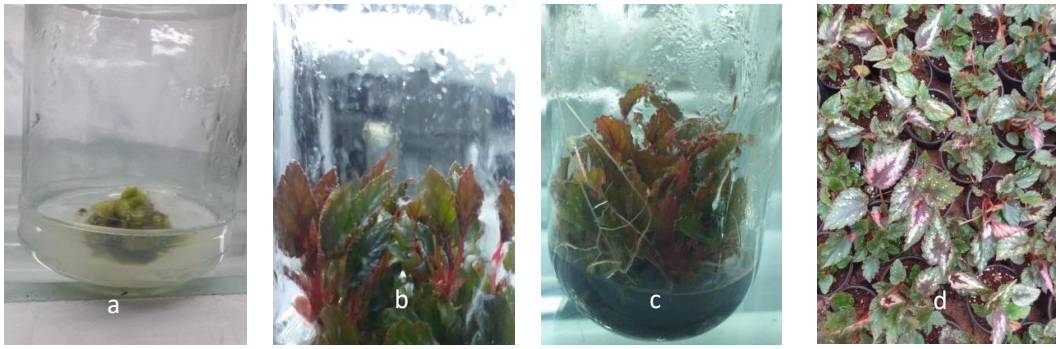

Plate 1: *In vitro* micropropagation of *Begonia rex*
(a) Establishment stage, (b) Multiplication stage, (c) Rooting stage, (d) Acclimatization stage.

Abbreviations

MS = Murashige & Skoog medium, Kin= Kinetin, IBA = Indol Butric Acid, NAA = Naphthalene Acetic Acid.

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