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In vitro Propagation of *Polyscias fruticosa* Plant

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this work was to develop a well defined protocol for *in vitro* propagation of *Polyscias fruticosa* plant.
A factorial experiment in a complete randomized design was employed in all experiments. Analysis of variance as described to compare statistical differences between treatments using L.S.D at 5% probability level by Snedecor and Cochran.
This study was carried out during the period from 2008 to 2011 aiming to achieved the most suitable protocol for micro propagation of *Polyscias fruticosa* Harms in Laboratory of Tissue Culture, Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center.
The young shoots were sterilized by immersion in a clorox (commercial bleach 5.25% sodium hypochlorite) solution at the rates of 20, 25 and 30 % for 10, 15, 20 and 25 min. Shoot tips were cultured on (Murashige and Skoog) MS medium supplemented with (Benzylaminopurine) BA at 0.0, 1.0, 3.0, or 5.0 mg/l and (Kinetin) kin at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and 3 g/l (activated charcoal) AC. The shoots obtained from establishment stage were cultured on multiplication media. Three sets of experiments with combination of different phytohormones for shoot regeneration were performed. The first experiment multiplication media consisted of MS medium supplemented with BA at 0.0, 1.0, 3.0 or 5.0 mg/l and kin at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and their combinations. In the second experiment multiplication media consisted of (Woody Plant medium) WPM basal nutrient medium supplemented with BA at 0.0, 1.0, 3.0 or 5.0 mg/l and kin at 0.0, 0.5, 1.0, 2.0 or

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4.0 mg/l, and their combinations. In the third experiment multiplication media consisted of (Gamborg medium) B5 basal nutrient medium supplemented with BA at 0.0, 1.0, 3.0 or 5.0 mg/l and kin at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and their combinations. Shoots were cultured on MS basal rooting medium supplemented with (Naphthalene acetic acid) NAA at 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l or (Indole butyric acid) IBA at 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l for 45 days. Rooted plantlets were singly picked out into 5 cm plastic pots filled with 1:0, 1:1, 1:2 or 1:3 (v:v) peatmoss and sand, respectively.
The interaction between 25% clorox for 15 minutes resulted in the highest value for survival. For the establishment stage, 3.0 mg/l BA and 2.0 mg/l kin showed the tallest shoots. For multiplication stage, the highest shoot length, number of shoots, number of leaves and callus formation was obtained at B5 medium supplemented with 5.0 mg/l BA and 2.0 mg/l kin. For NAA on rooting, the highest number of roots and root length was obtained on medium supplemented with 1.0 mg/l NAA. The highest percentage of plant survival was achieved by transplanting of the plantlets to pots containing sand and peatmoss at the ratio of 1:1(v/v).
This study was carried to develop the most suitable protocol for micropropagation of *Polyscias fruticosa* Harms, while *ex vitro* acclimatization need further work to increase establishment at greenhouse.

Keywords: Micropropagation; tissue culture; explants; In vitro; callus; Polyscias.

1. INTRODUCTION

Polyscias fruticosa (family araliaceae) leaves are used as tonic, is a perennial dicot evergreen shrub or dwarf tree native to India. The plant grows fairly slowly but can reach up to 1 to 2 meters in height. The leaves are of a dark green pigment, glossy in texture, and are tripinnate and appear divided. Individual leaves vary from narrowly ovate to lanceolate and are about 10 cm long, anti-inflammatory, antitoxin, and an antibacterial ointment native to Asian countries. They have also been proved to be an aid in digestion. The root is also used as diuretic, febrifuge, anti-dysentery, and is employed for neuralgia and rheumatic pains. Alongside with medicinal purposes, *Polyscias fruticosa* is also used as an ornamental plant and a spice. Ribas et al. [1] concluded that apical shoots sterilization from *Aspidosperma-polyneuron* was achieved with NaOCl (0.25% for 10 minutes) or HgCl2 (0.05% for 10 minutes); survival rates were 72.89 % and 84.10 %, respectively. Granilshchikova and Kopper [2] found that shoots of *Gentiana lutea* L. were sterilizated by ethanol (70%) for 5 min., Danchlorix (20%) for 5 min and HgCl2 (0.2%) for 5 min. Mantovani et al. [3] investigated that regeneration rate of *Didymopanax morototoni* and the shoot elongation were highest in the WPM medium with 1.0 mg/l BAP. Zhao et al. [4] illustrated that adventitious bud of *Panax ginseng* was induced on MS medium supplemented with 2.0 mg/l BA and 0.2 mg/l IBA, 1.0 mg/l BA and 0.2 mg/l IBA, and 2.5 mg/l BA and 0.2 mg/l IBA. Choi and Yun [5] stated that *in vitro* propagation using shoot tip culture in gold tree [*Dendropanax morbiferathe*] number of shoots was the maximum on the medium containing 0.1 or 1.0 mg/l BA and 0.5 or 1.0 mg/l NAA. Chu and Sun [6] showed that the optimum regeneration medium was WPM+ 1.0 mg/l 6BA + 0.1 mg/l NAA germination rate was 90%; the propagating medium was WPM+ 0.5 mg /l 6BA + 0.05 mg/l NAA for *Acanthopanax senticosus*. Marcinek et al. [7] inducted that rooting was achieved from *Hedera helix* on MS medium supplemented with the addition of 0.5 mg/l NAA in the culture media. Chu and Sun [6] reported that rooting medium was White + 0.5 mg/l IBA + 1.5 mg/l IAA, the rooting rate was 85.2% from *Acanthopanax senticosus.* Dai et al. [8] showed that plantlets 4-5 cm in

length were transferred to soil (1:1 v/v of peat moss and sand) and the survival rate of *Aralia elata* was 89% after 4 weeks under greenhouse conditions.

This study was carried to develop the most suitable protocol for micropropagation of *Polyscias fruticosa* Harms. There is continually growing concern about loss of genetic diversity of this precious material, as many of this species are endangered. This is why the research on *in vitro* propagation of *Polyscias fruticosa* Harms, which are known source of bioactive chemicals with highly valued medical properties are very important.

2. MATERIALS AND METHODS

Young shoots (1-3 months) were taken from mother plants of *Polyscias fruticosa* were grown under green house condition from 1990 at Zohria Botanical Garden, explants were obtained during spring from mother plants.

2.1 Culture Room Condition

Explants were incubated at growth room under light intensity of 3000 lux, 16h photoperiod provided by white fluorescent lamp at average temperature of 25+2ºC.

2.2 Surface Sterilization of Explants

The young shoots were sterilized by immersion in a clorox (commercial bleach 5.25% sodium hypochlorite) solution at the rates of 20, 25 and 30 % for 10, 15, 20 and 25 min, then rinsed in a sterilized distilled water (five times) to remove all traces of the disinfectant. Shoot tips of about 0.5-1 cm were excised from sterilized young shoots.

2.3 Establishment Stage

Uncontaminated shoot tips were cultured on (Murashige and Skoog1962 [9]) MS medium supplemented with Benzylaminopurine (BA) at 0.0, 1.0, 3.0, or 5.0 mg/l and Kinetin (kin) at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and 3 g/l activated charcoal (AC).

Callus formation was recorded as scores between other observations according to Pottino, 1981 [10].

2.4 Multiplication Stage

For multiplication stage, shoot tips were cultured on MS basal nutrient medium supplemented with BA at 0.0, 1.0, 3.0 or 5.0 mg/l and kin at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and their combinations. In the second experiment, (Woody Plant medium) Lloyd & McCown [11] (WPM) basal nutrient medium supplemented with BA at 0.0, 1.0, 3.0 or 5.0 mg/l and kin at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and their combinations were investigated. In the third experiment, (Gamborg medium) Gamborg et al. [12] (B5) basal nutrient medium supplemented with BA at 0.0, 1.0, 3.0 or 5.0 mg/l and kin at 0.0, 0.5, 1.0, 2.0 or 4.0 mg/l, and their combinations were investigated.

2.5 Rooting Stage

Shoots were cultured on MS basal nutrient medium supplemented with different concentrations of Naphthalene acetic acid (NAA) at 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l or Indole butyric acid (IBA) at 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/l.

2.6 Acclimatization Stage

Rooted plantlets were singly picked out into 5 cm plastic pots filled with 1:0, 1:1, 1:2 or 1:3 (v/v) peatmoss and sand, respectively.

2.7 Experimental Design and Statistical Analysis

Snedecor and Cochran, [13] 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%). Each treatment consisted of three jars containing three shoots in each jar.

3. RESULTS AND DISCUSSION

The micropropagation for *Polyscias fruticosa* was needed to be addressed in the previous studies were lacked. The aim of this work was to develop a well defined protocol for *in vitro* propagation of this plant.

3.1 Effect of clorox Concentration (%) and Soaking Periods on Survival (%), Mortality (%) and Contamination (%) at Surface Sterilization of Shoot Tip Explants

The data presented in Table 1 showed that the interaction between the concentration and period showed that using clorox 25% for 15 minutes resulted in the highest value for survival, the least value for contamination and the lowest values for mortality. Results were agreement with that found on *Magnolia grandiflora* by El-Shamy et al. [14] showed that Clorox was most suitable for surface sterilization of explants.

3.2 Effect of MS Medium Supplemented With Different Concentrations of BA and Kin on Shoot Length (Cm), Number of Leaves and Callus Formation at Establishment Stage

The data exhibited in Table 2 indicated that the tallest shoots were measured at 3.0 mg/l BA and 2.0 mg/l kin. These results are in line with those obtained on *Panax ginseng* for establishment stage by Zhao et al. [4].

The highest number of leaves was obtained at 1.0 mg/l BA and 2.0 mg/l kin.

The highest value of callus formation was recorded from culture medium supplemented with 3.0 mg/l BA or 3.0 mg/l BA plus 1.0 mg/l kin and so, 1.0 mg/l BA plus 0.5 mg/l kin and control (Fig. 1-a).

3.3 Multiplication Stage

3.3.1 Effect of MS medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of shoots, number of leaves and callus formation at multiplication stage

Data in Table 3 revealed that high level of BA (5.0 mg/l) at low of kin (0.5 mg/l) was the most effective combination.

The greatest number of shoots was obtained at 5.0 mg/l BA and 0.5 mg/l kin. Marcinek et al., [7] who found that most shoots formed in all BA concentrations for *Hedera helix* plant.

The highest number of leaves was produced when MS medium was supplemented with 5.0 mg/l BA and 0.5 mg/l kin.

The combination between 5.0 mg/l BA and 0.5 mg/l kin resulted in the highest value of callus formation.

3.3.2 Effect of WPM medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of shoots, number of leaves and callus formation at multiplication stage

Data in Table 4 demonstrated that the longest shoots for 5.0 mg/l BA combined with 2.0 mg/l kin.

The highest value was obtained as a result of supplementing the culture medium with 5.0 mg/l BA combined with 2.0 mg/l kin. These results are in harmony with that reported by Franco et al. [15] on *Didymopanax morototoni*; Chu and Sun [6] on *Acanthopanax senticosus* and Dai et al. [8] on *Aralia elata* that could be explained by the fact that cytokinins are well known to increase number of shoots.

Higher concentrations of BA (5.0 mg/l) combined with 2.0 mg/l kin were the most effective treatment on number of leaves.

BA at 5.0 mg/l and kin at 2.0 mg/l were giving the highest value for calls formation.

3.3.3 Effect of B5 medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of shoots, number of leaves and callus formation at multiplication stage

Data in Table 5 showed that the tallest shoots were found on BA at 5.0 mg/l and kin at 2.0 mg/l.

The greatest number of shoot was found at the combination of BA at 5.0 mg/l with kin at 2.0 mg/l.

The highest value of leaves for the treatment of combined BA at 5.0 mg/l with kin at 2.0 mg/l as compared with control treatment.

The greatest value of calls formation was recorded for BA at 5.0 mg/l and kin at 2.0 mg/l (Fig. 1-b). These results were similar to that obtained by, Marcinek et al. [7] on *Hedera helix*;

Karim et al. [16] and Dai et al. [8] on *Aralia elata*. The results were similar with scientists showed that cytokinins promoted cell division that stimulate shoot length, also induced axillary branching.

3.3.4 Effect of NAA in the presence of AC on number of roots, root length, number of leaves and shoot length on rooting stage

The data in Table 6 proved that the highest value for number of roots was obtained as the culture medium was supplemented with 1.0 mg/l NAA (Fig. 1-c).

NAA was raised to reach its maximum length for the concentration of 1.0 mg/l then decreased for 2.0 and 4.0 mg/l NAA.

NAA at 1.0 mg/l was the most effective on leaves formation.

Increasing concentrations of NAA enhanced the length of shoots from control to 0.1, 0.5 and 1.0 mg/l NAA.

3.4.5 Effect of IBA in the presence of AC on number of roots, root length, number of leaves and shoot length on rooting stage

Data in Table 7 showed that all the concentrations didn't affect root formation, except the rate of 0.5 mg/l NAA.

The only concentration of 0.5 mg/l IBA gave the single value.

Number of leaves was increased from control treatment to 0.1 and 0.5 mg/l IBA rates.

BA at 0.5 mg/l concentration was the most effective on shoot length. Similar results were reported by Lee et al. [17] on *Aconthopanax senticosus* cultured on medium supplemented with 0.5 mg/l IBA and 1.5 mg/l IAA. Activated charcoal is mode of action could be due to its adsorbation thus removal of inhibitory substances secreted by the cultured tissues.

3.5 Effect of Peatmoss and Sand on Survival Percentage during Acclimatization Stage

Data recorded in Table 8 demonstrated that the highest percentage of plant survival (60%) was achieved by transplanting of the plantlets to pots containing sand and peatmoss at the ratio of 1:1(v/v) (Fig. 1-d). Peatmoss and sand mixture were more effective than peatmoss alone or higher percentage of sand giving 0% for 1 Peatmoss plus 3 sand. Similar results were reported on *Aralia elata* by Dai et al. [8].

Table 1. Effect of clorox concentration (%) and soaking periods on survival (%), mortality (%) and contamination

Soaking periods (min) (B)	Survival (%)					Mortality (%)					Contamination (%)				
Clorox% (A)	10	15	20	25	Mean (A)	10	15	20	25	Mean (A)	10	15	20	25	Mean (A)
20	37.03	48.15	59.26	33.33	44.44	7.41	7.41	7.41	51.85	18.52	55.56	40.74	33.33	14.81	36.11
25	59.26	92.59	48.15	25.92	56.48	11.11	7.41	40.74	59.26	29.63	29.63	0.00	11.11	14.81	13.89
30	37.03	48.15	22.22	14.81	30.55	7.41	33.33	62.97	77.78	45.37	55.56	18.52	14.81	7.41	24.07
Mean (B)	44.44	62.96	43.21	24.69		8.64	16.05	37.04	62.97		46.92	19.75	19.75	12.34	
$LSD_{0.05}$ for															
Clorox (A)					4.94					4.68					4.13
Periods (B)					5.70					5.40					4.77
(AxB)					9.87					9.36					8.26

Table 2. Effect of MS medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of leaves and callus formation at establishment stage of *Polyscias fruticosa*

Kin (mg/l)	Shoot length (cm)						No. of leaves						Callus formation (as scores)					
BA (mg/l)	0.0	0.5	1.0	2.0	4.0	Mean (A)	0.0	0.5	1.0	2.0	4.0	Mean (A)	0.0	0.5	1.0	2.0	4.0	Mean (A)
0.00	0.83	1.00	1.17	1.33	1.33	1.13	1.00	1.67	2.00	2.33	2.33	1.87	0.33	0.00	0.00	0.00	0.00	0.07
1.00	1.17	1.33	1.33	1.33	1.33	1.30	1.33	1.67	2.67	3.33	2.67	2.33	0.00	0.33	0.00	0.00	0.00	0.07
3.00	1.17	1.17	1.33	1.5	1.33	1.30	1.33	1.67	1.67	2.00	2.33	1.80	1.00	0.00	1.33	0.00	0.00	0.47
5.00	1.00	1.17	1.17	1.17	1.17	1.14	1.00	1.67	2.00	2.00	1.67	1.67	0.00	0.00	0.00	0.00	0.00	0.00
Mean (B)	1.04	1.17	1.25	1.33	1.29		1.17	1.67	2.09	2.42	2.25		0.33	0.08	0.33	0.00	0.00	
$LSD_{0.05}$ for																		
BA (A)						0.19						0.36						0.24
Kin (B)						0.29						0.40						0.27
(A×B)						0.44						0.81						0.53

Footnote: Results for callus formation /explant were calculated visually as scores (according to Pottino, 1981[10])
Negative (-) =1, Below average (+) =2, average (++) =3, Good (+++)= 4

Table 3. Effect of MS medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of shoots, number of leaves and callus formation at multiplication stage of *Polyscias fruticosa*

Kin (mg/l)	Shoot length (cm)						No. of shoots						No. of leaves						Callus formation (as scores)					
BA(mg/l)	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**
0.00	1.83	1.67	1.83	2.17	2.17	1.93	1.00	1.00	1.00	2.00	2.33	1.47	2.00	2.33	2.67	4.00	4.33	3.07	1.33	1.33	1.00	1.33	1.33	1.26
1.00	1.67	1.50	1.50	2.17	2.00	1.77	1.00	1.00	1.00	2.00	2.00	1.40	2.33	3.00	3.67	6.00	5.33	4.07	1.67	1.67	1.67	1.33	1.67	1.60
3.00	2.17	2.50	2.67	2.00	2.00	2.27	2.33	3.00	3.00	2.33	2.33	2.59	4.33	5.67	6.33	4.33	4.67	5.07	1.00	1.33	1.67	1.33	1.33	1.33
5.00	2.17	5.00	2.67	2.17	2.00	2.80	2.33	4.67	3.33	2.00	2.00	2.87	5.67	11.0	7.67	4.33	3.67	6.47	1.33	2.00	1.67	1.33	1.33	1.53
Mean (B)	1.96	2.67	2.17	2.13	2.04		1.67	2.42	2.08	2.08	2.17		3.58	5.50	5.09	4.67	4.50		1.33	1.58	1.50	1.33	1.42	
$LSD_{0.05}$ for																								
BA (A)						0.16						0.25						0.44						0.39
Kin (B)						0.18						0.28						0.49						0.44
(A×B)						0.35						0.57						0.98						0.89

Footnote: Results for callus formation /explant were calculated visually as scores (according to Pottino, 1981[10])
Negative (-) =1, Below average (+) =2, average (++) =3, Good (+++)= 4

Table 4. Effect of WPM medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of shoots, number of leaves and callus formation at multiplication stage of *Polyscias fruticosa*

Kin(mg/l)	Shoot length (cm)						No. of shoots						No. of leaves						Callus formation (as scores)					
BA(mg/l)	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**
0.00	1.83	1.67	1.83	2.17	2.17	1.93	1.00	1.00	1.00	2.00	2.33	1.46	2.00	2.67	3.00	4.67	5.33	3.53	1.33	1.67	1.67	1.67	1.67	1.60
1.00	1.67	1.67	1.50	2.33	2.00	1.83	1.00	1.00	1.00	2.33	2.33	1.53	2.67	5.33	5.67	7.00	6.67	5.47	2.00	2.00	2.00	1.67	1.67	1.87
3.00	2.17	2.17	2.17	2.50	2.67	2.34	2.00	2.00	2.33	3.33	3.67	2.67	4.33	5.33	6.33	7.67	8.00	6.33	1.33	1.67	1.67	2.00	2.00	1.73
5.00	2.16	2.17	2.17	5.17	2.50	2.84	3.00	3.00	3.33	4.67	3.33	3.47	4.67	6.33	7.67	11.67	8.33	7.73	1.00	1.00	1.33	2.67	2.33	1.67
Mean (B)	1.96	1.92	1.92	3.04	2.34		1.75	1.75	1.92	3.08	2.92		3.42	4.92	5.67	7.75	7.08		1.42	1.59	1.67	1.95	1.92	
$LSD_{0.05}$ for																								
BA (A)						0.21						0.29						0.45						0.11
Kin (B)						0.23						0.33						0.49						0.39
(A×B)						0.46						0.65						0.99						0.78

Footnote: Results for callus formation /explant were calculated visually as scores (according to Pottino, 1981[10])
Negative (-) =1, Below average (+) =2, average (++) =3, Good (+++)= 4

Table 5. Effect of B5 medium supplemented with different concentrations of BA and Kin on shoot length (cm), number of shoots, number of leaves and callus formation at multiplication stage of *Polyscias fruticosa*

Kin (mg/l)	Shoot length (cm)						No. of shoots						No. of leaves						Callus formation (as scores)					
BA(mg/l)	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean (A)**
0.00	1.83	1.83	1.83	2.17	2.17	1.97	1.00	1.00	1.00	2.00	2.67	1.53	2.00	2.33	3.33	4.67	6.33	3.73	1.33	1.67	1.67	1.67	1.67	1.60
1.00	1.67	1.67	1.50	2.00	2.00	1.77	1.00	1.00	1.00	2.33	2.67	1.60	3.33	4.33	5.33	6.67	7.33	5.40	2.00	2.00	2.00	2.00	1.67	1.93
3.00	2.17	2.17	2.17	2.00	2.17	2.14	2.00	2.00	2.67	3.67	3.67	2.80	5.67	6.33	7.33	9.33	11.00	7.93	1.33	1.67	1.67	2.33	1.67	1.73
5.00	2.17	2.17	2.17	5.17	2.50	2.84	3.00	3.33	3.67	6.00	4.33	4.07	6.67	7.33	10.67	15.00	11.67	10.27	1.33	1.33	1.33	3.00	2.00	1.80
Mean (B)	1.96	1.96	1.92	2.84	2.21		1.75	1.83	2.09	3.50	3.34		4.42	5.08	6.67	8.92	9.08		1.50	1.67	1.67	2.25	1.75	
$LSD_{0.05}$ for																								
BA (A)						0.19						0.28						0.49						0.35
Kin (B)						0.21						0.30						0.54						0.39
(A×B)						0.42						0.61						1.09						0.79

Results for callus formation /explant were calculated visually as scores (according to Pottino, 1981[10]) Negative (-) =1, Below average (+) =2, average (++) =3, Good (+++)= 4

Table 6. Effect of NAA in the presence of AC on rooting characteristics, number of leaves and shoot length of *Polyscias fruticosa*

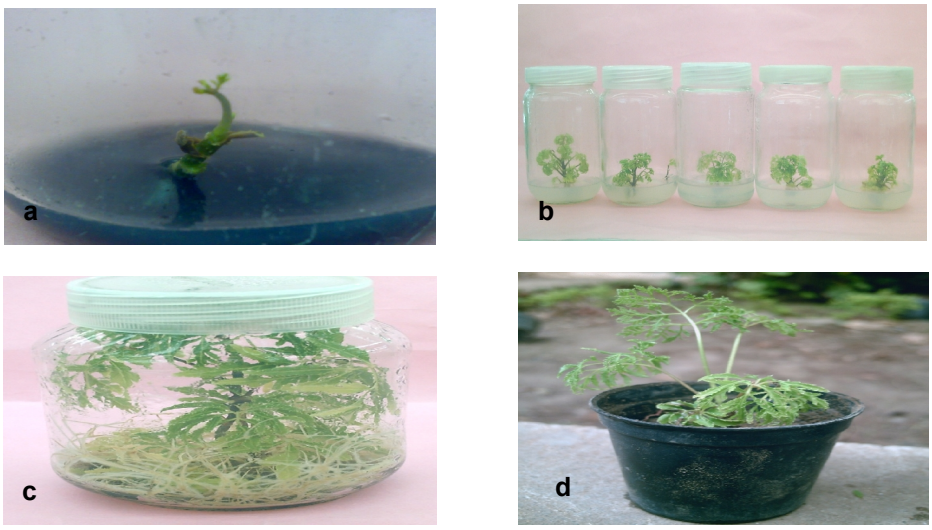
NAA (mg/l)	No. of roots	Root length (cm)	No. of leaves	Shoot length (cm)
0.0	2.33	1.67	5.33	1.33
0.1	6.67	2.67	6.33	1.83
0.5	9.33	4.33	8.33	2.50
1.0	14.33	9.67	13.3	4.83
2.0	7.67	5.67	7.33	2.33
4.0	6.33	4.67	6.67	1.83
LSD	1.16	1.03	1.14	0.74

Table 7. Effect of IBA in the presence of AC on rooting characteristics, number of leaves and shoot length of *Polyscias fruticosa*

IBA (mg/l)	No. of roots	Root length (cm)	No. of leaves	Shoot length (cm)
0.0	0.00	0.00	2.33	1.17
0.1	0.00	0.00	3.67	1.67
0.5	0.67	1.17	10.67	2.83
1.0	0.00	0.00	7.67	2.00
2.0	0.00	0.00	6.00	1.33
4.0	0.00	0.00	5.33	1.17
LSD	**0.43**	**0.77**	**0.92**	**0.46**

Table 8. Effect of peatmoss and sand on survival percentage during acclimatization stage of *polyscias fruticosa*

Peatmoss	Sand	Survival (%)
1	0	20.00
1	1	60.00
1	2	10.00
1	3	0.00
LSD		0.16



(Fig. 1) a. Effect of MS medium supplemented with 2mg/l kin and 3 mg/l BA on establishment stage of *Polyscias fruticosa*.
b. Effect of B5 medium supplemented with Kin (0.0, 0.5, 1.0, 2.0 and 4.0 mg/l) at 5.0 mg/l BA on multiplication stage of *Polyscias fruticosa* (from left to right).
c. Effect of MS medium supplemented with 1 mg/l NAA on rooting stage of *Polyscias fruticosa*.
d. Effect of peatmoss:sand at 1:1 (v/v) after one month on acclimatization stage of *Polyscias fruticosa*

4. CONCLUSION

Clorox at 25% for 15 minutes resulted the highest value for survival explants during surface sterilization. 3.0 mg/l BA and 2.0 mg/l kin showed the tallest shoots at establishment stage. The highest shoot length, number of shoots, number of leaves and callus formation was obtained at B5 medium supplemented with 5.0 mg/l BA and 2.0 mg/l kin at multiplication stage. The highest number of roots and root length was obtained on medium supplemented with 1.0 mg/l NAA at rooting stage. The highest percentage of plant survival was achieved by transplanting of the plantlets to pots containing sand and peatmoss at the ratio of 1:1(v/v).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ribas LLF, Zanette F, Kulchetscki L, Guerra MP. Micropropagation of *Aspidosperma polyneuron* from single node culture of juvenile material. Revista Arvore. 2005;29(4):517-524.
2. Granilshchikova M, Kopper E. Development of an efficient micropropagation method for *Gentiana lutea* L. Raumberg Gumpenstein, Austria. 2012;119-121.
3. Mantovani NC, Franco ETH, Guerra MP, Hoppe JM. Micropropagation of *caixeta Didymopanax* morototoni (Aubl.) Dcne. et Planch. Ciencia Florestal. 1999;9(1):47-61.
4. Zhao WJ, Wang Y, Jiang SC, Xu Y, Sun CY, Zhang MP. Establishment and optimization of *in vitro* regeneration system for *Panax ginseng*. Journal of Jilin Agricultural University. 2009;31(1):41-44.
5. Choi SK, Yun KW. *In vitro* propagation using shoot tip culture in gold tree [*Dendropanax morbifera* L EV]. Korean Journal of Crop Science. 2001;46(6):464-467.
6. Chu L, Sun Z. Tissue culture and rapid propagation of *Acanthopanax senticosus*. Bulletin of Botanical Research. 2009;29(4):505-508.
7. Marcinek B, Hetman J, Witek M. The influence of growth regulators upon the *in vitro* propagation of *Hedera helix* L. 'Dark Pittsburgh' and 'Kolibri'. Folia Universitatis Agriculturae Stetinensis, Agricultura. 2004;(94):119-124.
8. Dai JL, Tan X, Zhan YG, Zhang YQ, Xiao SA, Gao Y, Xu T Wang DW, Wang XC, You XL. Rapid and repetitive plant regeneration of *Aralia elata* Seem. via somatic embryogenesis. Plant Cell, Tissue and Organ Culture. 2011;104(1):125-130.
9. Murashige T, Skoog FS. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 1962;15:473-487.
10. Pottino G. In: Methods in Plant Tissue Culture. Dep. of Hort., Agric. College, Maryland Univ., College Park, Maryland, USA. 1981;8-29.
11. Lloyd GB, McCown. Use of microculture for production and improvement of *Rhododendron* spp. Hort Science. 1980;15:416.
12. Gamborg OL, Miller RA, Ojimak K. Nutrient requirements of suspension cultures of soybean roots cells. Exp. Cell Res. 1968;50:151-158.
13. Snedecor GW, Cochran WG. One-way Classification, analysis of variance. In: Statistical Methods (8th Ed.). Iowa State Univ. Press, Ames, Iowa, USA. 1989;12:217-236.
14. El-shamy MA, El-Gendy SA, Hosni AM, Hosni YA. Studies on Micropropagation of some woody ornamental plants. Doctor of Philosophy (Ph.D.) dissertation, Faculty of Agriculture. Ain Shams University. 2004;97.
15. Franco ETH, Gavioli LB, Ferreira AG. *In vitro* regeneration of *Didymopanax morototoni* Brazilian. Journal of Biology. 2006;66(2A):455-462.
16. Karim M Z, Yokota S, Rahman MM, Eizawa J, Saito Y, Azad MAK, Ishiguri F, Iizuka K, Yoshizawa N. Efficient adventitious shoot regeneration from root explants of *Aralia elata* Seem. International Journal of Botany. 2007;3(4):390-393.

17. Lee EJ, Kim, MK, Paek KY. Auxin and cytokinin affect biomass and bioactive compound production from adventitious roots of *Eleutherococcus koreanum*. Korean Journal of Horticultural Science & Technology. 2010;28(4):678-684.

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