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Micropropagation of *Schefflera amate* Plants

Ahmed A. Barakat and Nader A. El-Shanhorey

Botanical Gardens Research Department, Horticultural Research Institute, Agric. Res. Center, Alexandria, Egypt.

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ABSTRACT

Schefflera Amate an evergreen ornamental plant native of Australia and New Guinea which is one of the largest plant groups in the family Araliaceae. The aim of this study is to develop an efficient protocol for the rapid in vitro micropropagation of *Schefflera Amate* Plants via enhanced axillary bud proliferation from single nodal explants cultured on full strength MS basal medium, fortified with 30g/l sucrose, 4g/l gelrite augmented with diverse concentrations of phytohormones as BA, NAA, IBA and their combinations. In general, the prevailing observe concluded that the height effects for initiation level turned into recorded on MS medium fortified with NAA and BA at 1.00 and 0.50 mg/l, respectively. Meanwhile, supplemented medium with BA and NAA at 4.00 and 1.00 mg/l, respectively, gave upward push to the height effects for multiplication level. Regarding rooting level, the height effects have been recorded whilst the neoformed shoots of multiplication level have been divided singly and cultured on MS medium plus IBA and NAA at 2.00 and 0.250 mg/l, respectively which led to the maximum mean number of roots formed per propagule. Neoformed plantlets had been acclimatized *ex vitro* and *in vivo* vigorously in combination of perlite and peatmoss at (1:1) or (2: 1) and (2: 2) consecutively, further to fixed volume (1 portion) of sterile sand; which resulted in the highest mean value of survival percentage/plant (100%) and showed true-to-type plants *ex vitro*.

Keywords: *Schefflera amate*, Araliaceae, Micropropagation, *in vitro*, *in vivo,* Acclimatization.

1. Introduction

Schefflera J. R. Forst. & G. Forst (1775) is currently the biggest genus of family Araliaceae and represents about half of the family with an estimated 600–900 species, a standing that has been performed in large part via the broadening of its circumscription to encompass all araliads that lack prickles and feature palmately compound leaves, ligulate stipules and unarticulated pedicels (Frodin, 1975 and Fiaschi P. *et al.,* 2020). This broadening tendency culminated withinside the reputation of 582 regular species (Frodin & Govaerts, 2003) and a bunch of extra species looking forward to formal description. This trend, however, has been reversed with the invention that the genus is polyphyletic, representing 5 awesome clades, none of that are sister groups (Plunkett *et al.,* 2005; Fiaschi and Plunkett 2011; Gostel *et al.,* 2017). The genus is known as in honor of Johann Peter Ernst von Scheffler (born in 1739), health practitioner and botanist of Gdańsk, and later of Warsaw, who contributed flora to Gottfried Reyger for Reygers book, 'Tentamen Florae Gedanensis' (Schumann 1893).

Schefflera amate an evergreen ornamental plant native of Australia and New Guinea, it is a leafy green plant found in the flourishing rainforests of Australia. Propagation asexually rather than from seed. It is mostly used indoors as a foliage pot plant because of the attractiveness of the umbrella-like palmately compound leaves (Gilman and Watson, 1994; Chen *et al*., 2002). *Schefflera's* cap potential to clean the air and its tolerance to harsh indoors environments has in addition improved its global popularity (Yang *et al*., 2009; Dela Cruz *et al*., 2014). Propagation of *Schefflera* plant is mainly by seeds which results with the segregation of the progeny traits and limited to its native plantation in the tropics (Griffith, 1998; Chen *at al.*, 2002). Other propagation ways are leaf-bud cuttings (Hansen, 1986),

Corresponding Author: Ahmed A. Barakat, Botanical Gardens Res. Dept., Hort. Res. Inst., Agric. Res. Center, Alexandria, Egypt. E-mail: ahmed.barakat@arc.sci.eg

air layering (Gilman and Watson, 1994) and stem cuttings (Hansen, 1986). These practices are hindered by difficulties such as low number of propagules per plant, increased time of production and the risk of disease spread from several pathogens (Chase and Poole, 1986).

Tissue culture could be an alternative approach for economic production of *Schefflera amate* plants and to speed up the propagation rate and to reduce the need for mother plants (George and Sherrington, 1993, Debergh *et al.,* 1991). Moreover, *in vitro* culture of tropical ornamental plants has been recommended as a tool to eradicate the diseases that are frequently widespread in the mother plants (Hartmann and Kester, 2011). The current research developed, for the first time, a micropropagation protocol for *Schefflera amate* so as to succeed in a high propagation rate and acquire healthy plantlets, the consequences of plant growth regulators were studied on shoot proliferation and rhizogenesis of *Schefflera amate* under *in vitro* culture conditions. Subsequent acclimatization and survival rate of the micropropagated plantlets was also investigated using different potting media under *ex vitro* culture conditions.

2. Materials and Methods

2.1. Explant preparation

The explant materials of *Schefflera amate* were picked up from healthy mother plants grown in the greenhouse of Antoniades Botanical Garden, Horticultural Res. Inst., Agric. Res. Center, Alexandria, Egypt, during the period of 2021 and 2022.The collected material, were brought to the Plant Tissue Culture laboratory of The Faculty of Agriculture Saba Basha, Alexandria University to process, leaves had been eliminated and washed after that to be prepared for sterilization and tissue culture manipulation.

2.2. Explant sterilization

The shoot explants from cuttings had been washed very well with inside the water, the use of liquid cleaning soap for 30 min., after which the excised explants were placed under running tap water for 90 minutes then dipped in 70% ethanol for 15 sec. After pretreatment with ethanol, the explants have been washed with double distilled water two times to decrease the poisonous impact of ethanol. Nodal segments of only (2cm) long which contained a single node were then surface sterilized with concentration of mercuric chloride ($HgCl_2$) at 0.1% (v/v) with a few drops of wetting agent "Tween-20" (surfactant agent) for fifteen minutes (Barakat, 2021). The similar procedure was repeated; however the explants had been immersed in awareness of sodium hypocholrite solution (NaOCl) at 30%. After the surface sterilization of explants with mercuric chloride and sodium hypocholrite solution were decanted and the explants were washed with sterile double distilled water for four times, so as to lower the toxic effects of $HgCl_2$ and NaOCl and became ready for culturing (Barakat *et al.,* 2021).

2.3. In vitro experimental stages

1. Initiation stage, the explants during this stage had been cultured on solidified Murashige and Skoog medium (1962) solidified with gelrite (3g/l). The pH of the tested media was adjusted to 5.7 before adding gelrite, and then sterilized autoclaving at 121°C for 20 min., then explants were cultured into the given MS medium augmented with different concentrations of cytokinin (BA) at four concentrations: 0.00, 0.25, 0.50, and 1.00 mg/l, in combinations with auxin (NAA) at four concentrations 0.00, 0.50, 1.00, and 2.00mg/l.
2. Multiplication stage, the neoformed propagules of the initiation stage have been sectioned into equal leaflets nodal segment measured 1 cm. The excised nodal cuttings explants of the different positions were cultured, randomly, on multiplication media which supplemented with BA at four concentrations: 0.00, 1.00 2.00, and 4.00 mg/l, in combinations with NAA at four concentrations: 0.0, 0.25, 0.50 and 1.00 mg/l.
3. Rhizogenesis The obtained shoots from the multiplication stage were, individually, cultured on a rooting medium, contained two types of auxins were tested, Indole-3-Bytric acid (IBA) at four concentrations: 0.00, 0.50, 1.00 and 2.00 mg/l, in combinations with NAA at three concentrations: 0.0, 0. 125 and 0.250 mg/l.

Acclimatization of neoformed plantlets, the plantlets created from rooting stage have been washed lightly out of solidified medium under running tap water, accompanied with the aid of using immersing them into fungicide for 25 sec. They were, then, transplanted *ex vitro* in small plastic pots (6 cm) plastic pots contained a combination of an autoclaved mixture of the perlite (0, 1, 2 and 3 volume) and peatmoss at (0, 1 and 2 volume) each; and one constant volume of washed and autoclaved sand. Then, they were arranged in a factorial experiment and finally placed in transparent plastic bags (*ex vitro*), to maintain high relative humidity at (RH) 85% and 27±1°C, for hardening-off. However, the tested pots with different media were rearranged, randomly, weekly within same plot to avoid the experimental error. Ten days later, the plastic bags had been perforated for gaseous exchange, then transferred into plastic house (*in vivo*) persevered for in addition hardening. After four weeks, the plastic bags were removed and the acclimatized plantlets were watered, as needed, and fertilized weekly with mineral fertilization (20:20:20) at 0.5g/l.

Experimental layout and statistical analysis, all the experiments carried out during this study were designed as factorial experiments layout in completely randomized design (Gomez and Gomez, 1984). Recorded data were analyzed statistically using analysis of variance technique (ANOVA) and means were compared by L.S.D tests (Steel *et al*., 1997) and significance was determined at $p \leq 0.05$.

3. Results and Discussion

3.1. Initiation stage of *Schefflera amate*

Data presented in Table (1) and Fig. (1) Exhibit that both applied growth regulators (NAA and BA) and their combinations exerted significant effects on the initiation stage characters of *Schefflera amate* single node explants grown *in vitro*. Regarding the main effect of NAA tested levels on the studied characters, i.e. numbers of shoot, shoot length, numbers of leaflets and roots formed per propagule, commonly, the highest mean values were always recorded at 1.00 mg/l of NAA in the culture medium (2.00, 3.83, 9.44 and 4.03) respectively, and the lowest ones were noticed at its absence from the cultured medium (0.00 mg/l), except the mean number of roots formed per propagule. Concerning the main effect of BA on the above-mentioned traits, in general, the highest mean values were always recorded at (0.50 mg/l) BA in the culture medium of the above- mentioned traits (2.31, 4.06, 8.06 and 4.17), respectively, but the lowest ones were noticed at either the absence of BA for the above-mentioned traits.

Concerns the interaction between levels of both factors under the study, the NAA at 1.00 and BA at 0.50 mg/l, led to the highest mean values of the studied characters, in general. On the other hand, the lowest mean values of the above mentioned characters were recorded at the absence of both factors. This locating will be attributed to the mode of action of auxin (NAA) inside cultured tissues that's able to controlling various distinctive processes such as cell growth and elongation as stated by Leyser *et al.,* (1996) and George *et al.,* (2008).

On the other extreme, the high concentration of NAA used affected well the root formation of *Schefflera amate in vitro*. This might be due to the role and mode of action of auxin for their abilities to enhance root formation, as stated by George *et al.,* (2008) and Waseem *et al.,* (2011). Concerns to the interaction between both growth regulators at 0.50 BA and 1.00 mg/l NAA, expressed, significant effects on the various tested traits. The above-mentioned results, generally, indicated that increasing the mean values of the studied characters was concomitant with the high BA levels. BA has been the most efficient cytokinin for multiple shoot induction. Structural balance and brief metabolization of BA with the aid of using plant tissue in comparison to other synthetic cytokinins are the reasons for supremacy of BA for shoot

Induction. BA additionally reasons the manufacturing of natural hormones such as zeatin inside the plant tissue inflicting more advantageous shoot production (Ahmad *et al.* 2013; Malik *et al.* 2005). These results are in agreement with previously published reports suggest that higher concentrations of NAA in combination with BA play a crucial role in regeneration of plants, such as *Tupidanthus* (George *et al., 1987*), *Panax ginseng* (Choi *et al.,* 1990), 5-leaf aralia (Yang and Read 1997), *Cussonia paniculata* (Tetyana 2001), Aralia plants (Cheng *et al.,* 2011), *Aralia elata* (Karim *et al.,* 2007), *Fatsia japonica* (Choi *et al.,* 2005), *Polyscias fruticosa* (Sam 2005;Sakr *et al.,* 2014; Pandya *et al.,*2018), and *Schefflera arboricola* (Baghbidi, and Jowkar 2018).

Table 1: Effect of different levels of NAA and BA (mg/l) and their combinations on the initiation stage of *Schefflera amate* cultured *in vitro* for 35 days.

Characters	BA Levels (mg/l)	NAA levels (mg/l)				Mean (BA)	Significance		
		0.0	0.5	1.0	2.0		NAA	BA	NAA X BA
(a) Mean number of shoots formed/propagule:									
	0.0	0.89	1.22	1.33	1.33	**1.19**	**	**	*
	0.25	1.00	1.56	2.11	1.89	**1.64**			
	0.50	1.44	2.22	3.11	2.44	**2.31**			
	1.00	1.11	1.22	1.44	1.33	**1.28**			
Mean (NAA)		**1.11**	**1.56**	**2.00**	**1.75**				
L.S.D. (0.05)							**0.24**	**0.28**	**0.83**
(b) Mean shoot length (cm)/propagule:									
	0.0	1.36	2.52	3.01	2.30	**2.30**	**	**	**
	0.25	1.91	2.90	3.22	2.99	**2.76**			
	0.50	2.18	3.89	6.06	4.10	**4.06**			
	1.00	2.10	2.71	3.04	1.79	**2.41**			
Mean (NAA)		**1.89**	**3.01**	**3.83**	**2.79**				
L.S.D. (0.05)							**0.23**	**0.29**	**0.89**
(c) Mean number of leaflets formed/propagule:							**	**	*
	0.0	4.11	5.67	6.33	5.44	**5.39**			
	0.25	4.44	7.56	9.00	8.56	**7.39**			
	0.50	4.78	7.89	11.89	7.67	**8.06**			
	1.00	4.56	7.67	10.56	9.00	**7.94**			
Mean (NAA)		**4.47**	**7.19**	**9.44**	**7.67**				
L.S.D. (0.05)							**0.92**	**0.96**	**3.18**
(d) Mean number of roots formed/propagule:							**	**	**
	0.0	0.33	1.22	2.44	3.56	**1.89**			
	0.25	1.78	2.78	3.89	5.11	**3.39**			
	0.50	1.67	4.67	6.11	4.22	**4.17**			
	1.00	1.33	2.89	3.67	4.22	**3.03**			
Mean (NAA)		**1.28**	**2.89**	**4.03**	**4.28**				
L.S.D. (0.05)							**0.38**	**0.42**	**1.33**

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability. *, **: Significant or highly significant.

Fig. 1: Initiation stage of *Schefflera amate* single nodal grown *in vitro* on MS medium augmented with NAA at 1.00 and BA at 0.50 mg/l over 35 days

3.2. Multiplication stage of *Schefflera amate*

Results in Table (2) and Fig. (2) Describe the effect of various levels of both growth regulators (BA and NAA) and their combinations on the studied characters of *Schefflera amate.* Concerning the

mean number of shoot, the mean value of shoot length, the number of leaflets and roots formed per propagule, BA levels had a highly significant effect on this trait.

Table 2: Effect of different levels of BA and NAA (mg/l) and their combinations on the multiplications stage of *Schefflera amate* cultured *in vitro* for 35 days.

Characters	NAA Levels (mg/l)	BA levels (mg/l)				Mean (NAA)	Significance		
		0.0	1.00	2.0	4.0		BA	NAA	BA X NAA
(a) Mean number of shoots formed/propagule:									
	0.0	0.67	1.22	1.33	1.33	**1.14**	**	**	**
	0.25	1.00	1.67	2.33	2.78	**1.94**			
	0.50	1.56	3.56	4.33	4.67	**3.53**			
	1.00	1.56	2.67	4.33	6.11	**3.67**			
Mean (BA)		**1.19**	**2.28**	**3.\08**	**3.72**				
L.S.D. (0.05)							**0.27**	**0.27**	**0.96**
(b) Mean shoot length (cm)/propagule:									
	0.0	1.10	2.32	2.82	2.37	**2.15**	**	**	**
	0.25	2.02	3.18	3.60	3.14	**2.99**			
	0.50	2.74	2.96	4.13	2.44	**3.07**			
	1.00	1.88	3.19	5.00	4.58	**3.66**			
Mean (BA)		**1.94**	**2.91**	**3.89**	**3.13**				
L.S.D. (0.05)							**0.28**	**0.29**	**1.02**
(c) Mean number of leaflets formed/propagule:							**	**	*
	0.0	5.00	6.22	6.89	5.78	**5.97**			
	0.25	6.89	7.22	8.11	9.67	**7.97**			
	0.50	10.44	12.11	12.56	9.78	**11.22**			
	1.00	10.67	11.67	11.11	9.11	**10.64**			
Mean (BA)		**8.25**	**9.31**	**9.67**	**8.58**				
L.S.D. (0.05)							**0.68**	**0.69**	**1.17**
(d) Mean number of roots formed/propagule:							**	**	**
	0.0	0.00	0.33	0.33	0.33	**0.25**			
	0.25	2.33	2.22	2.89	2.56	**2.50**			
	0.50	3.78	3.89	4.89	3.67	**4.06**			
	1.00	4.22	3.11	2.11	0.33	**2.44**			
Mean (BA)		**2.58**	**2.39**	**2.56**	**1.72**				
L.S.D. (0.05)							**0.30**	**0.32**	**1.05**

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability. *, **: Significant or highly significant

Fig. 2: Multiplication stage of *Schefflera amate* explants grown *in vitro* on MS medium fortified with BA at 4.00 mg/l and NAA at 1.00 mg/l over 35 days.

The highest mean value was recorded at 2.00 and 4.00 mg/l BA as (3.72, 3.89, 9.67 and 2.56) respectively, but, the least response was observed with the absence of BA. On the other hand, NAA had a highly significant effect on the above mentioned traits. The highest mean value was recorded at 1.00 mg/l NAA as (367, 3.66, 10.64 and 4.06), respectively. Meanwhile, the interaction between both growth regulators exerted a highly significant effect. However, the combination of BA and NAA, at 2.00 and 1.00 mg/l respectively, resulted in the highest mean value, except the mean number of roots formed per propagule (4.89) at BA and NAA, at 2.00 and 0.50 mg/l respectively. The interaction between both BA and NAA at 2.00 and 1.00 mg/l, respectively, resulted in the maximum mean value. With respect the number of roots formed per propagule, both growth regulators had a highly significant effect on the given trait.

These results could be brought about to the mode of action of cytokinins (BA) on stimulation both cell division and promotion growth of axillary shoots in plant tissue culture as, also, found by George *et al*. (2008), and Trigiano and Gray (2000).

As well as, this is probably because of the mode of action of auxin (NAA) at the above-stated stage inside cultured tissues may also enhance, manipulate numerous special approaches which includes cell growth and elongation (George and Sherrington, 1984). Wilkins (1989), additionally, said that auxin caused range of responses which concerned cell division, cell enlargement, protein and nucleic acids synthesis which can be concomitants of auxin-caused growth and modifications in wall plasticity of plant cell and increase the apical dominance as there are critical and rapid processes involved in growth and elongation. With respect to the combinations between both growths regulators BA and NAA led to significant effects on the studied traits. These results are in agreement with previously published reports suggest that higher concentrations of BA in combination with NAA play a crucial role in regeneration greater number of shoot induction of plants, such as 5-leaf aralia (Yang and Read 1997), *Cussonia paniculata* (Tetyana, 2001), Aralia plants (Cheng *et al.,* 2011), *Aralia elata* (Karim *et al.,* 2007), *Fatsia japonica* (Choi *et al.,* 2005), *Polyscias fruticosa* (Sam 2005; Sakr *et al.,* 2014; Pandya *et al.,*2018), *Polyscias balfouriana* (Ilyas 2013 *et al.,*) and *Schefflera arboricola* (Baghbidi, and Jowkar 2018).

3. Rooting stage of *Schefflera amate*

Results in Table (3) and Fig. (3) Showed that the applied both auxin levels exerted a highly significant effects on the studied characters of *Schefflera amate* and the interaction between IBA at 2.00 mg/l and NAA at 0. 250 mg/l resulted in the highest mean value of number of roots formed /propagule (6.44). Concerning, the main effect of IBA tested levels on the mean value of shoot length and mean number of leaflets showed that IBA at 2.00 mg/l, led to the highest mean values of the above mentioned traits (5.57 and 10.67), respectively. On the other hand, NAA main effect disclosed that augmenting MS-basal medium with 0.250 mg/l of it, gave the highest mean values of the above mentioned traits (4.11 and 7.69), respectively . On the other side, the main effect of NAA indicated that supplying MS-basal medium with NAA at 0. 250 mg/l recorded the highest mean value of the number of roots (4.75).

The obtained results showed that the used auxins (NAA and IBA), in general, produced the best results in almost all studied traits. These results will be defined at the bases that auxin prompted quantity of responses which concerned cell division, cell enlargement, protein and nucleic acids synthesis which can be concomitants of auxin caused increase and modifications in wall plasticity of plant cell and increase the apical dominance as there are crusial and rapid processes worried in growth and elongation (Wilkins, 1989). The impact effect of NAA on rhizogenesis may be due to the reason that NAA is more motive than IBA, stays present with inside the tissue and may block further improvement of root meristemoids (De Klerk *et al*., 1997). These results are in agreement with previously published reports suggest that higher concentrations of IBA in combination with NAA play a crucial role in rhizogenesis of plants, such as *Panax quinquefolius* (Wang 1990)**,** 5-leaf aralia (Yang and Read 1997), *Cussonia paniculata* (Tetyana 2001), Aralia plants (Cheng *et al.,* 2011), *Aralia elata* (Karim *et al.,* 2007), *Fatsia japonica* (Choi *et al.,* 2005), *Aralia elata (Dai et al., 2011)*, *Panax notoginseng* (You *et al.,* 2012), *Polyscias balfouriana* (Ilyas 2013 *et al.,*), *Schefflera arboricola* (Baghbidi, and Jowkar 2018) and *Schefflera octophylla* (Luy *et al.,* 2021).

Table 3: Effect of different levels of IBA and NAA (mg/l) and their combinations on the rooting stage of *Schefflera amate* cultured *in vitro* for 35 days.

Characters	NAA Levels (mg/l)	IBA levels (mg/l)				Mean (NAA)	Significance		
		0.0	0.5	1.0	2.0		IBA	NAA	IBA X NAA
(a) Mean number of shoots formed/propagule:							**	**	**
	0.0	1.00	1.22	1.33	1.11	**1.17**			
	0.125	1.22	1.22	1.56	1.67	**1.42**			
	0.250	1.22	1.78	2.44	2.89	**2.08**			
Mean (IBA)		**1.15**	**1.41**	**1.78**	**1.89**				
L.S.D. (0.05)							**0.16**	**0.18**	**0.56**
(b) Mean shoot length (cm)/propagule:							**	**	**
	0.0	1.58	2.08	2.33	1.91	**1.98**			
	0.125	2.02	2.17	2.46	2.36	**2.25**			
	0.250	2.60	3.79	4.49	5.57	**4.11**			
Mean (IBA)		**2.07**	**2.68**	**3.09**	**3.28**				
L.S.D. (0.05)							**0.19**	**0.22**	**0.66**
(c) Mean number of leaflets formed/propagule:							**	**	**
	0.0	3.67	4.89	4.56	4.89	**4.50**			
	0.125	5.33	5.56	5.78	5.11	**5.44**			
	0.250	5.56	6.00	8.56	10.67	**7.69**			
Mean (IBA)		**4.85**	**5.48**	**6.30**	**6.89**				
L.S.D. (0.05)							**0.50**	**0.58**	**1.75**
(d) Mean number of roots formed/propagule:							**	**	*
	0.0	0.22	1.00	1.11	1.22	**0.89**			
	0.125	1.22	1.67	2.67	3.11	**2.17**			
	0.250	3.78	3.78	5.00	6.44	**4.75**			
Mean (IBA)		**1.74**	**2.15**	**2.93**	**3.59**				
L.S.D. (0.05)							**0.42**	**0.48**	**1.45**

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability. *, **: Significant or highly significant.

Fig. 3: Rooting stage of *Schefflera amate* shoots grown *in vitro* on MS medium augmented with IBA at 2.00 mg/land NAA at 0.250 mg/l over 35 days.

4. *In vivo* acclimatization of *Schefflera amate*

Data presented in Table (4) and Fig. (4) Exhibit that both applied mixtures of perlite and peatmoss (v/v) and their combinations, in addition to fixed volume (1 portion) of sand on acclimatization of neoformed plantlets of *Schefflera amate* grown *ex vitro* for four weeks.

Table 4: Effect of different levels of peat moss and perlite (v:v) and their combinations on the acclimatization stage of *Schefflera amate* cultured *in vitro* for 4 weeks.

Characters	Peat Levels (v:v)	Perlite levels (v:v)				Mean (Peat)	Significance		
		0.0	1.0	2.0	3.0		Perlite	Peat	Perlite X Peat
(a) Mean number of neoformed shoots:							**	**	**
	0.0	0.00	0.78	0.89	0.56	**0.56**			
	1.00	2.67	3.00	2.33	2.00	**2.50**			
	2.00	2.78	3.22	3.56	3.11	**3.17**			
Mean (Perlite)		**1.81**	**2.33**	**2.26**	**1.89**				
L.S.D. (0.05)							**0.25**	**0.22**	**0.76**
(b) Mean of plant height (cm):							**	**	**
	0.0	0.00	3.21	3.54	3.74	**2.63**			
	1.00	3.72	9.59	5.77	4.52	**5.90**			
	2.00	10.71	11.26	13.67	12.39	**12.01**			
Mean (Perlite)		**4.81**	**8.02**	**7.66**	**6.89**				
L.S.D. (0.05)							**0.90**	**0.92**	**2.67**
(c) Mean number of neoformed leaves:							**	**	**
	0.0	0.00	3.89	4.44	5.33	**3.42**			
	1.00	8.44	13.11	9.67	9.56	**10.19**			
	2.00	10.67	12.33	13.78	9.78	**11.64**			
Mean (Perlite)		**6.37**	**9.78**	**9.30**	**8.22**				
L.S.D. (0.05)							**0.78**	**0.94**	**2.72**
(d) Mean of survival percentage %:							*	**	**
	0.0	0%	11%	22%	22.22%	**14%**			
	1.00	78%	100%	56%	33.33%	**67%**			
	2.00	89%	100%	100%	88.89%	**94%**			
Mean (Perlite)		**56%**	**70%**	**59%**	**48%**				
L.S.D. (0.05)							**0.14**	**0.12**	**0.43**

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability. *, **: Significant or highly significant.

Fig. 4: Acclimatization stage of *Schefflera amate* shoots grown on potting medium of peat moss and perlite at (1:1, v/v) over 4 weeks (a) and over 3 months (b).

Concerning the average of survival percentage per plant, perlite had a highly significant effect on this trait. The highest mean value was recorded at levels (1v/v) (70%). Also, peatmoss had highly significant effect on the given traits was recorded at levels (2v/v) (94%). Meanwhile, the interaction between peatmoss and perlite exerted highly significant effect. However, the combination of peatmoss and perlite at either (1:1) or (2: 1) or (2:2), respectively, resulted in the highest mean value (100%) of survival percentage.

Respecting the number of new shoots and plant height, peatmoss and perlite combination and their interactions exerted significant effects on the given trait. In case of perlite main effect at (1 v/v), brought about the highest mean value (2.33 and 8.02), respectively. On the other side, peatmoss had highly significant effect on number of neoformed shoots and plant height per plant at (2 v/v) (3.17 and 12.01), respectively. However, the interaction between both added levels of perlite and peatmoss at (1:2), resulted in the highest mean value (3.56 and 13.67), respectively.

Concerning the number of newly formed leaves (true leaves) the highest mean value for the perlite was at (2 v/v) on the other hand, peatmoss recorded the highest mean value at (2 v/v). The mixture which contained both perlite and peatmoss recorded the highest mean value at either (1:1 v/v) or (1:2 v/v) and (2:2 v/ v) respectively, resulted in the highest mean value (13.78)

In this respect, substrates as peatmoss is one of the maximum essential ingredients of media because of its ability in affecting plant growth either indirectly or directly. Indirectly, improves the physical conditions of media by improving aggregation, aeration (8%) and water retention (77%), thereby growing an appropriate environment for root growth (Sensi and Loffredo, 1999). On the other hand, perlite is known to have a moderate capacity to retain water (38%) and provide' aeration (25%) and its neural pH and the fact that it is sterile and weed–free. Hence, it is good to be used in field developing substratum. Also, it is known that perlite decreases the bulk density of the soils and increases the porosity (Abido, 2016).These results are in agreement with previously published reports suggest that the combination of peatmoss and perlite resulted in the highest mean value of survival percentage of plants, such as *Cussonia paniculata* (Tetyana 2001), Aralia plants (Cheng *et al.,* 2011), *Fatsia japonica* (Choi *et al.,* 2005), *Aralia elata (Dai et al., 2011)*, *Panax notoginseng* (You *et al.,* 2012), *Schefflera arboricola* (Baghbidi, and Jowkar 2018) and *Schefflera octophylla* (Luy *et al.,* 2021).

5. Conclusion

In conclusion, an efficient protocol established for *in vitro* propagation and acclimatization for *Schefflera amate* from nodal explants. This is the first report on tissue culture of *Schefflera amate*. The highest number of shoots (6.11) obtained from the combination of BA and NAA at 4.00 and 1.00 mg/l augmented in cultured medium. The interaction between IBA at 2.00 mg/l and NAA at 0.250 mg/l resulted in the highest number of roots formed per propagule (6.44). The combination of peatmoss and perlite at either (1:1) or (2: 1) and (2: 2), respectively, resulted in the highest survival percentage (100%) per plant. Micropropagation would ensure a continuous supply of plants in exclusive time and space. This protocol enables large numbers of plantlets of this valuable plant to enrich and valorization the ornamental industry and increasing awareness for its conservation.

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