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# Micropropagation of dwarf schefflera [*Schefflera arboricola* (Hayata) Merr.] via direct shoot regeneration

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Key words: *In vitro* propagation, NAA, *Schefflera arboricola*, shoot induction, TDZ, variegated leaves.

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**Data Availability Statement:**
All relevant data are within the paper and its Supporting Information files.

**Competing Interests:**
The authors declare no competing interests.

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**Abstract: Schefflera [*Schefflera arboricola* (Hayata) Merr.] is one of the most popular ornamental house plants conventionally propagated by seeds. Rapid multiplication of elite clones is an important driving force for the pot plant's market. In this regard, *In vitro* clonal propagation of three schefflera cultivars, 'Luseane', 'Charlotte' and 'Gold Capella', was examined. Sterilization was done by 70% ethanol for 2 min and 1% sodium hypochlorite solution for 15 min. Shoot proliferation of the nodal segments was dependent on cytokinin supply. The greatest number of shoots was obtained when nodal explants were cultured on the MS medium with 0.5 mg $l^{-1}$ TDZ for 'Luseane', or 8 mg $l^{-1}$ BA for 'Charlotte' and 'Gold Capella'. Subculture of nodal segments harvested from the *in vitro* derived axillary shoots on the multiplication medium enabled continuous production of healthy shoots with similar frequency. Plantlets of 'Luseane' and 'Gold Capella' demonstrated 100% rooting using 2 $mgl^{-1}$ NAA, while 'Charlotte' showed 93.75% root induction by 1 $mgl^{-1}$ NAA. Plantlets were acclimatized successfully using peat moss and sand mixture ('Luseane'), loam soil, sand and leaf compost ('Charlotte') or peat moss and perlite mixture ('Gold Capella').**

## 1. Introduction

Dwarf Schefflera [*Schefflera arboricola* (Hayata) Merr.] is an evergreen ornamental plant of the Araliaceae family, native to China and Taiwan (Ohashi, 1993). It is mostly used indoors as a foliage pot plant because of the attractiveness of the umbrella-like palmately compound leaves and variegated cultivars (Gilman and Watson, 1994; Chen *et al*., 2002). Schefflera's ability to clean the air and its tolerance to harsh interior environments has further increased its worldwide popularity (Yang *et al*., 2009; Dela Cruz *et al*., 2014). To satisfy grower's calls for potted plants of schefflera, methods for rapid propagation of selected cultivars are crucial. Multiplication of this house 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) and only to cultivars of

schefflera which have non-variegated green leaves (Marcotrigiano, 1997). Other propagation means are leaf-bud cuttings (Hansen, 1986), 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 such as *Pseudomonas cichorii*, *Xanthomonas campestris*, *Phytophthora parasitica*, *Pythium splendens* and *Alternaria panax* (Chase and Poole, 1986).

Micropropagation is a reasonably more efficient way for schefflera production as a greater number of plants can be produced faster compared to the traditional cuttings. 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). Tissue culture has furthermore resulted in enhanced features compared to common propagations. It was revealed that micropropagated foliage pot plants such as syngonium, spathiphyllum, dieffenbachia (Conover, 1992) and philodendron (Chen *et al*., 2012) had a packed and denser plant forms in comparison to the conventional stem cuttings. Consequently, small tissue culture-grown scheffleras may be readily suitable for the limited space available in the terrariums or bonsai pots.

Despite the fact that micropropagation is extensively exploited by the floriculture industry, their formulas are not released to the general public. To our knowledge, there is no tissue culture protocol available in the literature for *S. arboricola*, though a few number of articles have been published so far for other Araliaceae species with horticultural importance such as *Cussonia paniculata* (Tetyana and van Staden, 2001), *Fatsia japonica* (Choi *et al*., 2005), *Eleutherococcus senticosus* (Amin *et al*., 2003; You *et al*., 2005), *Panax quinquefolius* (Uchendu *et al*., 2011) *Hedera helix* (Sivanesan *et al*., 2011) *Polyscias balfouriana* (Ilyas *et al*., 2013) and *Polyscias fruticosa* (Sakr *et al*., 2014).

The current research developed, for the first time, a micropropagation procedure for three cultivars of shefflera with green, white and yellow variegated leaves. In order to reach a high propagation rate and get well plantlets, the effects of various sterilization treatments, plant growth regulators' type and concentration were studied on the survival rate, shoot proliferation and rooting of scheffleras. Subsequent acclimatization of the micropropagated plantlets was also investigated using different potting media.

## 2. Materials and Methods

*Plant material and culture medium*

Three marketable cultivars with green, white and yellow variegated leaves (i.e. 'Luseane', 'Charlotte' and 'Gold Capella', respectively) were used for micropropapagation. The explants were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) of sucrose, and 0.65% (w/v) of agar. The pH of the medium was set to 5.7 before autoclave sterilization (i.e., 20 min, at 121°C). The plant growth regulators were added to the medium later by filter sterilization. The sterile nodal segments were established in culture medium containing 2 mg $l^{-1}$ benzyl adenine (BA). In order to proliferate the shoots, the emerging buds were removed after 30 days and subcultured to media containing different cytokinin treatments.

*Sterilization of three Schefflera cultivars*

The stock plants were sprayed with Ridomil fungicide (0.3%) one week before explant excision. Stem cuttings were soaked in 0.1% detergent solution for 10 min, then washed under running tap water for 30 min. The explants were moved to laminar flow cabinet and 1.5-cm long nodal segments were excised from the stem. For surface sterilization of the explants, 70% EtOH was tested for 0, 30, 60, 90, 120, 150 and 180 seconds. Explants were then disinfected using 0, 0.5, 0.75, 1 or 1.25% (v/v) sodium hypochlorite containing 0.01% Tween-20 for 5, 10 or 15 min on a shaker. After four rinses with sterile water and excision of the damaged edges, the explants were cultured on PDA medium (300 g potato extract, 30 g $l^{-1}$ of dextrose and 8 g $l^{-1}$ of agar) for the evaluation of the contamination. Five replicates were examined for each treatment with three explants in each flask. Contaminations and survival rates were recorded after 1 month and those having noticeable infection signs were instantly discarded.

*Effect of cytokinin type and concentration on shoot proliferation*

Inducing new shoots was carried out using BA and Kinetin (Kin) at 0, 1, 2, 4 and 8 mg $l^{-1}$ concentrations while thidiazuron (TDZ) was tested at 0, 0.125, 0.25, 0.5 and 1 mg $l^{-1}$ concentrations. Five replicate flasks were used for each treatment with three explants in each flask. The number and length of the proliferated shoots, and number of leaves produced per explant were recorded after 30 days. The regenerated shoots were later subcultured to the best cytokinin-containing MS medium.

*Effect of auxin type and concentration on root induction*

Shoots from the finest proliferation treatment were put in the glass flasks of MS medium containing 0, 0.1, 0.5, 1, 2 or 4 mg $l^{-1}$ of indole-3-butyric acid (IBA) or naphthaleneacedic acid (NAA). After 15 days the shoots were transferred to half-strength MS medium devoid of auxins. Five replicate flasks were tested in each treatment with three shoots in each flask. The number and length of the induced roots and rooting percentage of the shoots were recorded 15 days later.

*Effect of potting media on plant acclimatization*

Rooted shoots were carefully taken out from the glass flasks and washed under distilled water to get rid of attaching agar from the roots. The plantlets were then transferred to 10-cm plastic pots filled with different potting media: peat moss, peat moss and perlite (1:1), peat moss and sand (1:1), loam soil, sand and leaf compost (1:1:1). Ten replications were tested for each of the treatments. Potted plants were grown in a greenhouse with temperatures ranging from 24 to 28°C, relative humidity of between 70 and 90%, and light intensity of 35 µmol $m^{-2}$ $s^{-1}$ under a 12 h photoperiod. After 45 days the survival rate and plant height were analyzed.

*Culture conditions, experimental design and data analysis*

The *in vitro* researches were carried out with an environment temperature of 25±2°C and a 16/8 h light/dark photoperiod delivered by cool fluorescent lamps at 35 µmol $m^{-2}$ $s^{-1}$. All the tests were arranged in a completely randomized design. Analysis of the variances was done by SAS software (SAS Institute Inc., 2002) and means were compared by LSD test at 5% probability level.

## 3. Results

*Sterilization of three Schefflera cultivars*

For the determination of the most effective surface sterilization, explants were soaked in 70% EtOH solution for 0-180 sec. As the soaking time was increased to 180 sec, surface sterilization was increased gradually and eventually reached 100% in all three cultivars, however viability was rapidly decreased after 120 sec (Table 1). Thus, 120 sec, providing the highest disinfection (72.8%, 79.6% and 72.8% for 'Luseane', 'Charlotte' and 'Gold Capella', respectively) and viability (72.8%, 74.6% and 72.8% for Luseane', 'Charlotte' and 'Gold Capella', respectively) altogether, was selected. The results were not significant between the cultivars.

The explants of 'Luseane' were effectively disinfected when 1.25% sodium hypochlorite was applied for 15 min, however this application resulted with a significant decrease of the viability to 13% (Table 2). The most effective combination of disinfection (93.2%) and viability (86.4%) was obtained using 1% sodium hypochlorite for 15 min. 'Charlotte' demonstrated a similar pattern of sterilization. The utmost control of contamination (93.2%) and viability (86.4%) was observed using 1% sodium hypochlorite for 15 min or 1.25% sodium hypochlorite for 10 min. Similarly, also for 'Gold Capella', the best percentages of sterilization (93.2%) and viability (86.4%) were obtained by using 1% sodium hypochlorite for 15 min.

*Effects of cytokinin type and concentration on shoot proliferation*

*Effects of benzyl adenine and kinetin treatments on each schefflera cultivars.* The increase of BA concentration to 8 mg $l^{-1}$ significantly increased the num-

Table 1 - Effects of ethanol treatments on surface sterilization and viability of three schefflera cultivars

| 70% Ethanol (sec) | 'Luseane' | | 'Charlotte' | | 'Gold Capella' | |
|---|---|---|---|---|---|---|
| | Bacterial sterilization (%) | Viability (%) | Bacterial sterilization (%) | Viability (%) | Bacterial sterilization (%) | Viability (%) |
| 0 | 0±0 e | 0±0 e | 0±0 d | 0±0 d | 0±0 e | 0±0 d |
| 30 | 19.8±8.08 d | 19.8±0 d | 13.2±8.08 d | 13.2±0 d | 13.2±8.08 de | 13.2±0 cd |
| 60 | 33±0 d | 33±0 cd | 46.2±8.08 c | 46.2±0 b | 26.4±6.60 d | 26.4±0 c |
| 90 | 52.8±8.08 c | 52.8±0 b | 53.2±8.25 c | 53.2±0 b | 59.6±6.65 c | 59.6±0 a |
| 120 | 72.8±6.80 b | 72.8± 0 a | 79.6±8.33 b | 74.6±6.6 a | 72.8±6.80 bc | 72.8±0 a |
| 150 | 93.2±6.80 a | 39.6±6.60 c | 100±0 a | 33±0 c | 86.4±8.33 ab | 39.6±6.6 b |
| 180 | 100±0 a | 19.8±8.08 d | 100±0 a | 13.2±8.08 d | 100±0 a | 19.8±8.08 c |

Data (±SE) are the mean values of five replicates with three explants in each.
For each cultivar, different lowercase letters indicate significant differences among treatments (LSD multiple range test, P≤0.05).

ber of shoots per explant of ‘Luseane’ to 3.75 (Table 3). This concentration of BA also produced 12.5 leaves per explant. However, the largest shoot length of 0.88 cm was obtained using 4 mg $l^{-1}$ BA. On the contrary, increasing Kin concentration to 4 mg $l^{-1}$ and above resulted with the decrease in the shoots number. Kin also produced significantly lesser leaves at all the concentrations tested, and did not significantly increase the shoot length.

Treatment of ‘Charlotte’ with cytokinins revealed a similar pattern as ‘Luseane’. Indeed, the greatest shoot proliferation of 3.75 shoots per explant was obtained using 8 mg $l^{-1}$ BA, which also produced 15.3 leaves per explant and maximum shoot length of 0.90 cm. Also Kin increased the shoots number at the same concentration, though it was significantly lower than what was obtained with BA. The maximum shoot length and number of leaves observed with 8 mg $l^{-1}$ Kin were 0.73 cm and 5.67, respectively, and both were significantly lower than what was observed with BA.

Proliferation of the ‘Gold Capella’ with 8 mg $l^{-1}$ BA resulted with maximum of 1.75 shoots per explant, the largest shoot length of 0.83 cm and 7.58 leaves per explant, which were all significantly higher than Kin treatments (Fig. 1). Adding 2 or 4 mg $l^{-1}$ Kin to the medium slightly increased the shoot length compared to the control.

Fig. 1 - Effect of different cytokinin treatments on shoot proliferation of schefflera cvs. Luseane (a), Charlotte (b) and Gold Capella (c). Shoots were produced using MS medium supplemented with 0.5 mg $l^{-1}$ TDZ for ‘Luseane’ and 8 mg $l^{-1}$ BA for the others.

Table 2 - Effects of sodium hypochlorite treatments on sterilization and viability of three schefflera cultivars

| Cultivar | Minutes | Sodium hypochlorite (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sterilization (%) | | | | | Viability (%) | | | | |
| | | 0 | 0.5 | 0.75 | 1 | 1.25 | 0 | 0.5 | 0.75 | 1 | 1.25 |
| ‘Luseane’ | 5 | 13.2±8.08 g | 24.75±6.39 fg | 33±0 e-g | 41.25±6.39 d-f | 49.5±7.38 de | 13.2±0 f | 24.75±0 ef | 33±0 d-f | 41.25±0 c-e | 49.5±0 cd |
| | 10 | 13.2±8.08 g | 33±0 e-g | 52.8±8.08 de | 74.5±6.58 bc | 86.4±8.33 ab | 13.2±0 f | 33±0 d-f | 52.8±0 cd | 74.5±0 ab | 79.6±6.80 ab |
| | 15 | 13.2±8.08 g | 49.5±7.38 de | 59.6±12.51 cd | 93.2±6.80 ab | 100±0 a | 13.2±0 f | 49.5±0 cd | 59.6±0 cd | 86.4±6.80 a | 13.2±8.08 f |
| ‘Charlotte’ | 5 | 19.8±8.08 f | 33±10.44 ef | 33±0 ef | 41.25±6.39 de | 49.5±7.38 de | 19.8±0 ef | 33±0 d-f | 33±0 d-f | 41.25±0 c-e | 49.5±0 cd |
| | 10 | 19.8±8.08 f | 41.25±6.39 de | 52.8±8.08 de | 74.5±6.58 bc | 93.2±6.80 ab | 19.8±0 ef | 41.25±0 c-e | 52.8±0 b-d | 74.5±0 ab | 86.4±6.80 a |
| | 15 | 19.8±8.08 f | 49.5±7.38 de | 59.6±12.51 cd | 93.2±6.80 ab | 100±0 a | 19.8±0 ef | 49.5±0 cd | 59.6±0 bc | 86.4±6.80 a | 13.2±8.08 f |
| ‘Gold Capella’ | 5 | 13.2±8.08 g | 19.8±8.08 fg | 26.4±6.60 e-g | 39.6±6.60 d-f | 52.8±8.08 cd | 13.2 0 f | 19.8±0 ef | 26.4±0 d-f | 39.6±0 c-e | 52.8±0 bc |
| | 10 | 13.2±8.08 g | 26.4±6.60 e-g | 52.8±8.08 cd | 66±0 bc | 86.4±8.33 ab | 13.2±0 f | 26.4±0 d-f | 52.8±0 bc | 66±0 ab | 86.4±6.80 a |
| | 15 | 13.2±8.08 g | 46.2±8.08 c-e | 59.6±12.51 cd | 93.2±6.8 0ab | 100±0 a | 13.2±0 f | 46.2±0 b-d | 59.6±0 bc | 86.4±6.80 a | 13.2±8.08 f |

Data (±SE) are the mean values of five replicates with three explants in each.
For each cultivar, different lowercase letters indicate significant differences among treatments (LSD multiple range test, P≤0.05).

Table 3 - Effects of Benzyl adenine and Kinetin on shoot proliferation of each schefflera cultivars

| Cultivar | Control | BA ($mgl^{-1}$) | | | | Kin ($mgl^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | 1 | 2 | 4 | 8 |
| ‘Luseane’ | | | | | | | | | |
| No. of shoots | 0.83±0.1 f | 1.25±0.05 de | 1.5±0.07 c | 2.75±0.08 b | 3.75±0.25 a | 1.42±0.03 cd | 1.42±0.05 cd | 1.17±0.06 e | 0.92±0.08 f |
| Shoot length (cm) | 0.24±0.03 f | 0.50±0.02 c | 0.39±0.01 d | 0.88±0.01 a | 0.73±0.02 b | 0.27±0.03 ef | 0.27±0.02 ef | 0.31±0.005 e | 0.31±0.01 e |
| No. of leaves | 0.66±0.36 f | 8±0.64 c | 8.5±0.4 c | 10.42±0.81 b | 12.5±2.06 a | 1.42±0.32 e | 2±0.49 e | 2.75±0.52 d | 1.67±0.27 e |
| ‘Charlotte’ | | | | | | | | | |
| No. of shoots | 0.45±0.21 g | 1.67±0.14 f | 1.92±0.36 e | 2.5±0.48 c | 3.75±0.55 a | 2.25±0.28 d | 1.67±0.41 f | 1.67±0.14 f | 3±0.36 b |
| Shoot length (cm) | 0.36±0.11 f | 0.36±0.12 f | 0.45±0.16 de | 0.72±0.11 b | 0.90±0.15 a | 0.47±0.05 d | 0.40±0.08 ef | 0.38±0.05 f | 0.73±0.13 b |
| No. of leaves | 1.17±0.4 g | 10.5±0.74 c | 10.83±0.1 c | 11.83±0.74 b | 15.3±1.6 a | 2.25±0.31 f | 3.67±1.03 e | 3.33±0.36 e | 5.67±0.62 d |
| ‘Gold Capella’ | | | | | | | | | |
| No. of shoots | 0.42±0 g | 0.75±0.04 de | 0.83±0.1 cd | 1.08±0.08 b | 1.75±0.08 a | 0.67±0.08 ef | 0.75±0.08 de | 0.92±0.08 c | 0.58±0.08 f |
| Shoot length (cm) | 0.19±0.05 e | 0.59±0.04 b | 0.47±0.04 c | 0.76±0.06 a | 0.83±0.03 a | 0.25±0.02 e | 0.39±0.02 cd | 0.33±0.01 d | 0.23±0.01 e |
| No. of leaves | 0.58±0.08 e | 4.41±0.64 c | 5.66±0.56 b | 5.83±0.52 b | 7.58±0.75 a | 0.75±0.21 e | 0.83±0.22 e | 2±0.38 d | 1.66±0.14 d |

Data (±SE) are the mean values of five replicates with three explants in each.
For each cultivar, different lowercase letters indicate significant differences among treatments (LSD multiple range test, P≤0.05).

*Comparison of thidiazuron treatments on shoot proliferation of schefflera cultivars.* The highest shoot proliferation (6.25 shoots per explant) was observed in 'Luseane', using 0.5 mg $l^{-1}$ TDZ (Table 4) (Fig. 1). The maximum shoot proliferation of 'Charlotte' and 'Gold Capella' significantly lower than 'Luseane', were 3.33 and 1.33 shoots per explant, using 0.5 and 1 mg $l^{-1}$ TDZ, respectively. The largest shoot length of 2.33 cm was observed in the 'Charlotte' when 0.5 mg $l^{-1}$ TDZ was added to the medium. The other cultivars' shoot length were significantly lower. Furthermore, 'Charlotte' produced the maximum leaves per explant (i.e. 13.5 leaves) using 0.5 mg $l^{-1}$ TDZ, while other cultivars yielded significantly fewer leaves per explant (8.75 for 'Luseane', 2.42 for 'Gold Capella') at 0.25 mg $l^{-1}$ TDZ and 1 mg $l^{-1}$ TDZ, respectively.

*Effect of auxin type and concentration on root induction*

Rooting of 'Luseane' was 100% when 2 or 4 mg $l^{-1}$ IBA as well as 0.5, 2 or 4 mg $l^{-1}$ NAA treatments were applied (Table 5). The maximum number of roots per explant (i.e. 18.67 roots) was observed using 2 mg $l^{-1}$ NAA (Fig. 2). Moreover, the largest root length of 2.74 cm was produced by this treatment. However, 0.5 or 2 mg $l^{-1}$ NAA appeared to have no significant differences with the former treatment. Also, the highest shoot length of 2.96 cm was recorded using 1 mg $l^{-1}$ NAA, albeit no significant differences were found with 0.5, 2 and 4 mg $l^{-1}$ NAA treatments.

The rooting of 'Charlotte' raised to the maximum 93.75% using 2 mg $l^{-1}$ IBA or 1 mg $l^{-1}$ NAA. Other con-

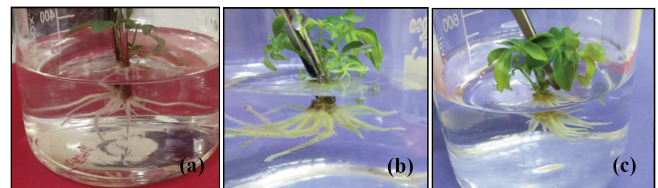


Fig. 2 - Root induction of schefflera cvs. Luseane (a), Charlotte (b) and Gold Capella (c) after using 2, 1 and 2 mg $l^{-1}$ of NAA, respectively. Note: the roots were submerged in distilled water for better visibility.

Table 4 - Effects of thidiazuron on shoot proliferation of schefflera cultivars

| TDZ (mgl$^{-1}$) | Luseane' | | | Charlotte' | | | 'Gold Capella' | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of shoots | Shoot length (cm) | No. of leaves | No. of shoots | Shoot length (cm) | No. of leaves | No. of shoots | Shoot length (cm) | No. of leaves |
| 0 | 0.83±0.1 gh | 0.24±0.03 I | 0.66±0 36 j | 0.45±0.21 I | 0.36±0.11 h | 1.17±0.4 I | 0.42±0 I | 0.19±0.05 I | 0.58±0.08 j |
| 0.125 | 1.65±0.02 e | 0.71±0.01 ef | 5.40±0.48 f | 1.75±0.08 e | 1.28±0.19 c | 5.75±0.67 f | 0.67±0h I | 1.02±0.01 d | 1.92±0.16 h |
| 0.25 | 5.58±0.08 b | 1.05±0.05 d | 8.75±0.76 c | 1.83±0.1 e | 1.34±0.13 c | 7.33±0.79 d | 0.75±0.08 h | 0.58±0.04 g | 1.75±0.21 h |
| 0.5 | 6.25±0.08 a | 0.66±0.04 fg | 6.25±0.82 e | 3.33±0.47 c | 2.33±0.25 a | 13.5±2.58 a | 1.08±0.08f g | 0.36±0.02 h | 1.75±0.16 h |
| 1 | 5.75±0.25 b | 0.80±0.02 e | 5.75±0.61 f | 2.33±0.24 d | 1.76±0.15 b | 9.5±1.31 b | 1.33±0.02 f | 0.41±0.02 h | 2.42±0.08 g |

Data (±SE) are the mean values of five replicates with three explants in each.
For each cultivar, different lowercase letters indicate significant differences among treatments (LSD multiple range test, P≤0.05).

Table 5 - Effects of auxins on rooting and young shoot growth of three schefflera cultivars

| Cultivar | Control | IBA (mgl$^{-1}$) | | | | | NAA (mgl$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.25 | 0.5 | 1 | 2 | 4 | 0.25 | 0.5 | 1 | 2 | 4 |
| 'Luseane' | | | | | | | | | | | |
| Rooting (%) | 8.25±6.39 d | 33±0 c | 66.25±10.59 b | 74.5±6.58 b | 100±0 a | 100±0 a | 33±10.44 c | 100±0 a | 91.75±6.39 a | 100±0 a | 100±0 a |
| No. of roots | 0.25±0.11 h | 2.58±0.26 g | 7.43±0.24 f | 11.24±0.35 d | 13.83±0.56 c | 10.38±0.37 de | 2.75±0.12 g | 9.75±0.54 e | 15.08±0.34 b | 18.67±0.33 a | 13.83±0.44 c |
| Root length (cm) | 0.31±0.24 c | 0.58±0.14 c | 1.78±0.16 b | 1.78±0.2 | 1.94±0.1 b | 1.49±0.08 b | 0.66±0.2 c | 2.46±0.17 a | 2.67±0.23 a | 2.74±0.08 a | 1.95±0.11 b |
| Shoot length (cm) | 1.85±0.1 c | 1.5±0.05 d | 1.95±0.08 c | 2.03±0.14 bc | 1.94±0.06 c | 1.89±0.06 c | 2.27±0.08 b | 2.87±0.12 a | 2.96±0.19 a | 2.77±0.12 a | 2.82±0.14 a |
| 'Charlotte' | | | | | | | | | | | |
| Rooting (%) | 58.25±12.44 c | 62.5±7.76 bc | 83.5±7.38 ab | 87.5±5.59 a | 93.75±4.84 a | 85.5±6.61 a | 62.5±7.76 bc | 91.75±6.39 a | 93.75±4.84 a | 91.75±6.39 a | 83.5±7.38 ab |
| No. of roots | 3.75±0.58 e | 5.02±0.33 d | 9.49±0.36 c | 12.25±0.45 b | 12.28±0.33 b | 10.18±0.56 c | 6.33±0.86 d | 15.06±0.46 a | 16.46±0.69 a | 13.33±1.21 b | 9±0.32 c |
| Root length (cm) | 1.13±0.12 c | 1.04±0.14 c | 1.88±0.04 b | 1.86±0.1 b | 1.88±0.24 b | 1.17±0.06 c | 1.32±0.19 c | 2.49±0.06 a | 2.47±0.15 a | 2.49±0.33 a | 1.49±0.09 b |
| Shoot length (cm) | 2.41±0.26 b | 1.53±0.07 c | 1.99±0.07 b | 2.28±0.11 b | 2.19±0.13 b | 1.52±0.1 c | 2.28±0.1 b | 2.92±0.1 a | 3.34±0.16 a | 3.21±0.18 a | 2.26±0.14 b |
| 'Gold Capella' | | | | | | | | | | | |
| Rooting (%) | 0±0 | 41.75±12.44 b | 85.5±6.61 a | 100±0 a | 100±0 a | 100±0 a | 50±16.70 b | 100±0 a | 100±0 a | 100±0 a | 100±0 a |
| No. of roots | 0±0 | 0.72±0.14 d | 6.99±0.3 c | 6.86±0.25 c | 9.39±0.46 b | 7.14±0.35 c | 1±0 d | 8.69±0.62 b | 8.88±0.26 b | 11.83±0.76 a | 9.13±0.32 b |
| Root length (cm) | 0±0 | 0.75±0.06 f | 1.63±0.03 e | 1.91±0.1 d | 2.09±0.07 cd | 1.92±0.02 c | 0.90±0.08 f | 2.15±0.04 c | 2.53±0.14 b | 2.78±0.09 a | 2.55±0.03 b |
| Shoot length (cm) | 1.29±0.01 g | 1.3±0.03 g | 1.66±0.13 d-f ** | 1.45±0.07 fg | 1.92±0.14 cd | 1.52±0.06 e-g | 1.77±0.11 de | 2.47±0.18 b | 2.17±0.09 bc | 2.83±0.19 a | 2.26±0.08 b |

Data (±SE) are the mean values of five replicates with three explants in each.
For each cultivar, different lowercase letters indicate significant differences among treatments (LSD multiple range test, P≤0.05).

centrations of IBA and NAA did not show significant discrepancies. Supplementing the medium with 1 mg $l^{-1}$ NAA produced the greatest number of roots per explant with an average of 16.46 roots (Fig. 2). On the other hand, 0.5 mg $l^{-1}$ NAA resulted with 15.06 roots which had no significant differences with the former treatment. Furthermore, using 0.5 and 2 mg $l^{-1}$ NAA produced the largest root length of 2.49 cm, though 1 mg $l^{-1}$ NAA produced similar root length of 2.47 cm with no significant differences. The maximum shoot length of 3.34 cm was measured using 1 mg $l^{-1}$ NAA in the medium, although 0.5, and 2 mg $l^{-1}$ NAA treatments did not yield significant differences with the former.

One hundred percent rooting was observed with 'Gold Capella' shoots when IBA was applied at 1, 2 or 4 mg $l^{-1}$, and NAA at 0.5, 1, 2 or 4 mg $l^{-1}$. The greatest number of roots per plantlet (i.e. 16.46 roots) was obtained using 2 mg $l^{-1}$ NAA treatment (Fig. 2). Moreover, the highest shoot and root length (2.78 and 2.83 cm respectively), were measured by addition of 2 mg $l^{-1}$ NAA, which showed significant differences compared to the other auxin treatments.

*Effect of potting media on plant acclimatization*

Schefflera's survival rate was not significantly affected by different potting media. The highest survival rate of 100% was obtained for 'Luseane' and 'Charlotte' using peat moss and sand (1:1) and loam soil, sand and leaf compost (1:1:1), respectively (Table 6). 'Gold Capella' showed a survival rate of 90% using peat moss or peat moss and perlite medium (1:1).

## 4. Discussion and Conclusions

Infection of tissue cultures of ornamental pot plants is a common phenomenon and an important barrier for their mass production. Contaminations in *Aglaonema* (Chen and Yeh, 2007), *Anthurium* (Kunisaki, 1980), *Dieffenbachia* (Brunner *et al*., 1995), *Spathyphyllum* and *Syngonium* (Kneifel and Leonhardt, 1992), *Zantesdeschia* (Kritzinger *et al*., 1998), as well as *Philodendron* (Fisse *et al*., 1987, Chen *et al*., 2012) are commonly reported. Thus, the current research was designed by setting up a disease-free shoot stock culture. Following surface sterilization with 70% EtOH for 120 sec and disinfection with 1% sodium hypochlorite for 15 min, more than 93% of the cultures were devoid of visible contaminations and they demonstrated 86.4% of viability for all tested cultivars. These results are consistent with the findings on *Fatsia japonica* Decne. (Choi *et al.*, 2005), *Polyscias balfouriana* (Ilyas *et al.*, 2013) and *Polyscias fruticosa* (Sakr *et al.*, 2014) from the Araliaceae family.

The green schefflera cultivar 'Luseane', showed a significantly greater number of shoot induction (6.25 shoots per explant) compared to the white- and yellow-variegated cultivars (Tables 3, 4). This phenomenon could be ascribed to the fact that variegated plants have a decreased propagation rate (Marcotrigiano, 1997). Vitrification problem and further necrosis of the new shoots was observed in 'Charlotte' and 'Gold Capella' when applying maximum concentration of 8 mg $l^{-1}$ BA (Fig. 3). Thus, a comparable 0.5 mg $l^{-1}$ TDZ was suggested for *in vitro* propagation of these cultivars instead of the former treatment. Sivanesan *et al*. (2011) reported increased number of shoots in *Hedera helix* 'Mini' by using a combination of 0.5 mg $l^{-1}$ TDZ and 0.1 mg $l^{-1}$ NAA. The mode of action of TDZ may be through alteration in energy levels, nutrient uptake, nutrient assimilation, or cell membranes of plants (Murthy *et al*., 1998). However, Tetyana and van Staden (2001) demonstrated that 2.5 mg $l^{-1}$ BA supplement to the media in

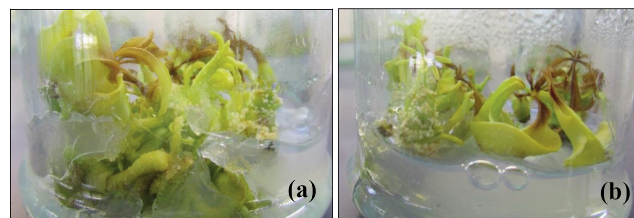


Fig. 3 - Vitrification and necrosis of schefflera plantlets using 8 mg $l^{-1}$ of BA in 'Charlotte' (a) and 'Gold Capella' (b).

Table 6 - Effect of potting media on acclimatization of three schefflera cultivars

| Mixture | Survival (%) | | | Shoots length (cm) | | |
|---|---|---|---|---|---|---|
| | 'Luseane' | 'Charlotte' | 'Gold Capella' | 'Luseane' | 'Charlotte' | 'Gold Capella' |
| Peat moss | 90±10 ab | 90±10 ab * | 90±10 ab | 3.86±0.07 b | 3.66±0.25 b | 3.48±0.18 bc |
| Peat moss and perlite | 90±10 ab | 90±10 ab * | 90±10 ab | 4.81±0.22 a | 4.49±0.2 a | 4.92±0.17 a |
| Peat moss and sand | 100±0 a | 90±10 ab * | 80±13.33 ab | 3.60±0.1 bc | 3.23±0.18 cd | 2.94±0.23 d |
| Loam soil, sand and leaf compost | 90±10 ab | 100±0 a * | 70±15.28 b | 3.65±0.08 bc | 3.72±0.24 bc | 3.79±0.17 b |

For each cultivar, different lowercase letters indicate significant differences among treatments (LSD multiple range test, P≤0.05).

*Cussonia paniculata* of the Araliaceae family, resulted with the highest shoot induction of 3.5 shoots per explant. On the other hand, the outcomings of You *et al*. (2005) in *Eleutherococcus senticosus*, the other member of the Araliaceae family, were different than our findings. It was revealed that 2 mg $l^{-1}$ BA proliferated shoots more effectively than TDZ, Kin and 2iP. Various cytokinin types and concentrations affect shoot proliferation of plant species, though all belong to the same family. Therefore, for each plant species, the shoot induction has to be newly investigated.

It was revealed that after the TDZ treatment, shoots' length of all cultivars were comparatively longer than those of the Kin and BA treatments. The white variegated schefflera 'Charlotte' showed the greatest length (2.33 cm) using 0.5 mg $l^{-1}$ TDZ. This effect could be related to further increase of auxin production by TDZ treatment (Murthy *et al*., 1998). It is assumed that TDZ has the ability to affect the amount of internal plant hormones (Murch and Saxena, 2001). Zaytseva *et al*. (2016) reported a similar high activity for low concentrations of TDZ in *Rhododendron*.

Although the highest number of leaves was recorded by using 8 mg $l^{-1}$ BA in all of the cultivars, other treatments are suggested due to the aforementioned vitrification problem. For 'Charlotte', 0.5 mg $l^{-1}$ TDZ treatment and for the two other cultivars 4 mg $l^{-1}$ BA produced maximum leaves without necrosis. Generally, cytokinins enhance the photosynthesis and help translocate the nutrients to the leaves (Taiz and Zeiger, 2006).

A sought-after plant tissue culture protocol essentially depends on sufficient rooting along with a successful acclimatization of the young plants. The results of the current study show that in all of the cultivars NAA is more effective in rooting than IBA. There are inconsistent reports on the rooting efficacy of different auxins. The research of You *et al*. (2005) indicated that 0.5 mg $l^{-1}$ NAA is more effective than IBA for rooting of *E. senticosus* shoots, while a concentration of 0.75 mg $l^{-1}$ IBA or 1 mg $l^{-1}$ NAA have proven useful for *C. paniculata* (Tetyana and van Staden, 2001). Auxin treatment of 'Charlotte', 'Luseane' and 'Gold Capella' increased the rooting percentage of the shoots, compared to the control by 35%, 92% and 100%, respectively. All shoots of 'Charlotte' and 'Luseane' were well acclimatized with a survival rate of 100% using media of loam soil, sand and leaf compost (1:1:1) and a mixture of peat moss and sand (1:1), respectively, while 'Gold Capella' demonstrated a none-significant lower survival frequency of 90% in the peat moss or peat moss and sand media (1:1).

To our knowledge, the current research is the first report on micropropagation of different cultivars of schefflera. Results demonstrated that green cultivar 'Luseane' had a greater shoot proliferation than the other two cultivars with average shoot number of 6.25 shoots per explant using 0.5 mg $l^{-1}$ TDZ. The multiplicated shoots could lengthen on PGR-free MS medium and later showed effective rooting on NAA-contained media. Although the yellow variegated cultivar 'Gold Capella' revealed a smaller proliferation rate of shoots compared to the control, its rooting was 100% improved. Subsequent relocation of the 'Luseane' and 'Charlotte' plantlets to the acclimatization greenhouse resulted with 100% survival rate of the rooted shoots. Generally, peat moss and perlite media (1:1) had a more positive effect on the young acclimatized plants of schefflera. The protocol developed in this research can be used for the aseptic production of schefflera.

## References


AMIN M.N., RAHMAN M.M., MANIK M.S., 2003 - In vitro *clonal propagation of* Paederia foetida L. *A medicinal plant of Bangladesh.* - Plant Tiss. Cult., 13: 117-123.

BRUNNER I., ECHEGARAY A., RUBLUO A., 1995 - *Isolation and characterization of bacterial contaminants from* Dieffenbachia amoena *Bull,* Anthurium andreanum *Linden and* Spathiphyllum sp. *shoot cultured* in vitro. - Sci. Hortic., 62: 103-111.

CHASE A.R., POOLE R.T., 1986 - *Effects of fertilizer rate on severity of Alternaria leaf spot of three plants in the Araliaceae*. - Plant Dis., 70(12): 1144-1145.

CHEN F.C., WANG C.Y., FANG J.Y., 2012 - *Micropropagation of self-heading* Philodendron *via direct shoot regeneration*. - Sci. Hortic., 141: 23-29.

CHEN J., HENNY R.J., McCONNELL D.B., 2002 - *Development of new foliage plant cultivars,* pp. 466-472. - In: JANICK J., and A. WHIPKEY (eds.) *Trends in new crops and new uses*. AHSH Press, Alexandria, USA.

CHEN W.L., YEH D.M., 2007 - *Elimination of* in vitro *contamination, shoot multiplication, and* ex vitro *rooting of* Aglaonema. - HortScience, 42: 629-632.

CHOI K.M., HWANG S.J., AHN J.C., LEE H.Y., KIM J.H., HWANG B. 2005 - In vitro *propagation from axillary bud explants of* Fatsia japonica *Deene. et Planeh*. - K. J. Med. Crop Sci., 13: 300-303.

CONOVER C.A., 1992 - *Foliage plants*, pp. 571-598 - In: LARSON R.A. (ed.) *Introduction to Floriculture*. Academic Press, London, UK, pp. 636

DELA CRUZ M., CHRISTENSEN J.H., DYRHAUGE THOMSEN J., MULLER R., 2014 - *Can ornamental potted plants*

*remove volatile organic compounds from indoor air? A review*. - Environ. Sci. Pollut. Res., 21(24): 13909-13928.

FISSE L., BATALLE A., PERA J., 1987 - *Endogenous bacteria elimination in ornamental plants*. - Acta Horticulturae, 212: 87-90.

GILMAN E.F., WATSON D.G., 1994 - Schefflera arboricola*, Fact Sheet ST-586*. - A series of the Environmental Horticulture Department, Florida Cooperative Extension Service, University of Florida, Florida, USA, pp. 1-3.

GRIFFITH L.P., 1998 - *Tropical foliage plants: A grower's guide*. - Ball Publishing, Batavia, USA, pp. 318.

HANSEN Y., 1986 - *Influence of cutting position and stem elongation on rooting of leaf-bud cuttings of* Schefflera arboricola. - Sci. Hortic., 28: 177-186.

HARTMANN H.T., KESTER D.E., 2011 - *plant propagation principles and practices: Aseptic methods of micro-propagation*. - Practice-Hall Inc., Englewood Cliffs, New Jersey, USA, pp. 509-532.

ILYAS S., NAZ S., JAVAD S., SHEHZADI K., TARIQ A., MUNIR N., ALI A., 2013 - *Influence of cytokinins, sucrose and pH on adventitious shoot regeneration of* Polyscias balfouriana *(Balfour aralia)*. - J. Med. Plants Res., 7(42): 3098-3104.

KNEIFEL W., LEONHARDT W., 1992 - *Testing of different antibiotics against gram positive and gram negative bacteria isolated from plant tissue cultures*. - Plant Cell Tiss. Org. Cult., 29: 139-144.

KRITZINGER E.M., VUUREN R.J.V., WOODWARD B., RONG I.H., SPREETH M.H., SLABBERT M.M., 1998 - *Elimination of external and internal contaminants in rhizomes of* Zantedeschia aethiopica *with commercial fungicides and antibiotics*. - Plant Cell Tiss. Org. Cult., 52: 61-65.

KUNISAKI J.T., 1980 - In vitro *propagation of* Anthurium andreanum Lind. - HortScience, 15: 508-509.

MARCOTRIGIANO M., 1997 - *Chimeras and variegations: Patterns of deceit*. - HortScience., 32(5): 773-784.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio assays with tobacco tissue cultures*. - Physiol. Plant., 15: 473-497.

MURCH S.J., SAXENA P.K., 2001 - *Molecular fate of thidiazuron and its effects on auxin transport in hypocotyls tissues of* Pelargonium x hortorum Bailey. - Plant Growth Regul., 35: 269-275.

MURTHY B.N.S., MURCH S.J., SAXENA P.K., 1998 - *Thidiazuron: A potent regulator of* in vitro *plant morphogenesis*. - In Vitro Cell Dev. Biol. - Plant, 34: 267-275.

OHASHI H., 1993 - *Araliaceae*, pp. 1002.- In: HUANG T. (ed.) *Flora of Taiwan 3*. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan.

SAKR S.S., MELAD S.S., EL-SHAMY M.A., ABD ELHAFEZ A.E., 2014 - In vitro *propagation of* Polyscias fruticosa *plant*. - Int. J. Plant Soil Sci., 3(10): 1254-1265.

SAS INSTITUTE, 2002 - *SAS/STAT® 9.2 User's Guide Introduction to Statistical Modeling with SAS/STAT Software*. - SAS Institute, Cary, NC, USA, pp. 60.

SIVANESAN I., SONG J.Y., JEONG B.R., 2011 - *Micropropagation of* Hedera helix L. *'Mini'*. - Prop. Orn. Plants, 11: 125-130.

TAIZ L., ZEIGER E., 2006 - *Plant physiology*. - Sundeland, Massachusetts, USA, pp. 643.

TETYANA P., VAN STADEN J., 2001 - *Micropropagation of* Cussonia paniculata*: a medicinal plant with horticultural potential*. - S. Afr. J. Bot., 67: 367-370.

UCHENDU E.E., PALIYATH G., BROWN D.C.W., SAXENA P.K., 2011 - In vitro *propagation of North American ginseng (*Panax quinquefolius *L.)*. - In Vitro Cell Dev. Biol. -Plant, 47: 710-718.

YANG D.S., PENNISI S.V., SON K.C., KAYS S.J., 2009 - *Screening indoor plants for volatile organic pollutant removal efficiency*. - HortScience, 44: 1377-1381.

YOU X.L., CHOI, Y.E., YI J.S., 2005 - *Micropropagation of* Eleutherococcus senticosus *through axillary bud culture*. - Forest Sci. Tech., 1: 38-44.

ZAYTSEVA Y.G., POLUBOYAROVA T.V., NOVIKOVA T.I., 2016 - *Effects of thidiazuron on* in vitro *morphogenic response of* Rhododendron sichotense Pojark. *and* Rhododendron catawbiense *cv. Grandiflorum leaf explants*. - In Vitro Cell Dev. Biol. - Plant, 52: 56-63.