OPEN ACCESS American Journal of Plant Physiology

ISSN 1557-4539
DOI: 10.3923/ajpp.2018.44.52

Research Article
In vitro Culture Development and Secondary Metabolites of *Dillenia indica* Tree

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Abstract

Background and Objective: There are some factors that affected the *in vitro* behavior of *Dillenia indica* and secondary metabolites such as different types of culture media, concentrations of BA and NAA as well as the physical state. The aim of this experiment was to study various factors on behaviors of *Dillenia indica* to attain suitable micropropagation protocol *in vitro* and *in vivo* stages. **Materials and Methods:** The experimental study was carried on *Dillenia indica* plant to evaluate the effect of three types of different media (MS, WPM and B5) with BA (0.5, 1.0 and 2.0 mg L^{-1}), three physical states (Solid, semi-Solid and liquid) of MS medium with NAA (0.0, 0.2, 0.4 and 0.6 mg L^{-1}) on various morphological and secondary metabolites characters. **Results:** The maximum numbers of the formed shootlets/explants and leaves/shootlet were recorded for MS medium or WPM+2 mg L^{-1} of BA while, B5 medium led to reduce those characters. Low concentration of BA (0.5 mg L^{-1}) supplemented to WPM or B5 medium had stimulation effect on shootlets elongation. The maximum phenol, tannin and pigments contents were found in shootlets that were cultured on WPM+1.0 mg L^{-1} BA. Using various culture media *in vitro* showed the highest antioxidant capacity as compared to control. Semi solid MS medium+NAA at 0.6 mg L^{-1} produced the maximum number of roots for the two culture periods and the longest roots in the first period. Culturing the shootlets on liquid MS+NAA at 0.4 mg L^{-1} resulted in strong and thick roots. The plants were highly survived and attained to highest height when the *in vitro* rooted plantlets were acclimatized to soil mixture of peat moss alone or peat moss+perlite+sand. Also, the maximum phenols content as well as antioxidant activity were detected in leaves of acclimatized plant in soil mixture of peat moss+sand+clay. **Conclusion:** The MS medium or WPM with 2 mg L^{-1} of BA was favored for *in vitro* propagation. Semi solid MS medium plus NAA at 0.6 mg L^{-1} produced the maximum number of roots. The maximum phenol, tannin and pigments contents were found in shootlets that were cultured *in vitro* on WPM medium plus 1.0 mg L^{-1} BA. The plants were successfully acclimatized in soil mixture of peat moss alone or peat moss+perlite+sand.

Key words: Dillenia, micropropagation, acclimatization, antioxidant activity and phenolic compounds

Citation: Lobna S. Taha, Eman A. Ibrahim, Nora M. Youssef and Iman M. El-Sayed, 2018. *In vitro* culture development and secondary metabolites of *Dillenia indica* tree. Am. J. Plant Physiol., 13: 44-52.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dillenia indica belongs to family Dilleniaceae and distributed in many Asian countries[1]. The genus Dillenia has 60 species. It is an evergreen tree, reaches 30 m high[2]. Colour of the bark is reddish brown. The leaves of the tree with corrugated surface and reticulate veins[3]. Fruits are aggregate and globose greenish yellow in colour and contain one to eight seeds[4]. In the conventional medicine, the juice of leaf, bark and fruit of the tree are used for cancer and diarrhea treatment[5,6]. The bark of the tree is also used for production of charcoal[7].

Various active compounds such as polyphenols, tannins, alkaloids, steroids, saponins and fixed oil and flavonoids were found in different solvent extracts of *Dillenia indica* which are responsible for the various pharmacological activities[8]. Betulin (pentacyclic triterpenoid) and betulinic are the main constituents present in the plant that exhibit spacious range of pharmacological activities including anti HIV, anti inflammatory, anti cancer, anti malarial, analgesic, anti diabetic, anti microbial, anti bacterial, anti diabetic, anti oxidant, anti diarrheal, cytotoxic and wound healing[1].

A large scale of plant production in the laboratory with quality integrated characteristics, cost effectiveness, maintain genetic fidelity, long term storage and virus free transplant could be obtained using *in vitro* propagation[9]. Besides, obtaining of medical metabolites using *in vitro* culture technique has been reviewed[10,11].

Tissue growth and the quality of morphogenetic responses are strongly influenced by various factors such as the composition of the culture medium[12]. The MS medium[13] formulation is the most widely used [14], since it contains all the nutrients essential for plant growth *in vitro*. Gamborg B5 medium is established by Gamborg[14] for callus and cell suspension culture as well as Woody plant Medium (WPM) that was originally formulated by Lloyd and McCown[15] and widely used for the propagation of many woody plants. Adding plant growth regulators to the culture medium could also effect on *in vitro* culture explants activity.

Therefore, the attempts were made to study various factors that affected the *in vitro* shooting and rooting behaviors of *Dillenia indica* to attain a suitable micro-propagation protocol and secondary metabolites in the plants after *in vitro* propagation.

MATERIALS AND METHODS

Area of study and sampling: This investigation were conducted during two years 2017 and 2018 at Tissue Culture Technique lab., Central laboratories, Department of Ornamental Plants and Woody Trees and Department of Plant Biochemistry, National Research Center (NRC), Egypt to obtain a suitable micropropagation protocol and investigate the changes in chemical constituents and antioxidant activity in both micropropagated and acclimatized shoots of *Dillenia indica* plants.

The shoots from of *Dillenia indica* tree were obtained from Zoo, Giza, Egypt and used as explant source (nodal explants) for *in vitro* propagation. The explants were washed under running tap water and a few drops of hand washing liquid for 20 min then surface sterilized in 70% (v/v) ethanol for 1 min, 15% commercial sodium hypochlorite solution for 10 min then rinsed three times with autoclaved distilled water followed with 7 min in 0.1 g L^{-1} $HgCl_2$ then rinsed finally three times with autoclaved distilled water.

Culture media: For shoots micropropagation, three types of different media [MS[13], WPM[15] and Gamborg B_5 medium[14] supplemented with various concentrations of BA (0.5, 1.0 and 2.0 mg L^{-1}) were investigated. The culture media were supplemented with 2.5% sucrose, pH 5.7±1 and solidified with 7 g L^{-1} agar. Data were recorded as: Number of shootlet/explant, length of shootlets (mm) and the number of leaves formed/shootlet.

For *in vitro* rooting, three physical states (Solid, semi-Solid and liquid) of MS medium supplemented with different concentrations of NAA (0.0, 0.2, 0.4 and 0.6 mg L^{-1}) were studied for two periods (1 and 2 months). Data were recorded as: Rooting%, number of roots/shootlet and length of formed roots (mm).

Culture conditions: The cultures were incubated on growth chamber at 25±2°C under 30 µmol m^{-2} sec^{-1} of light and 16 h photoperiod.

Acclimatization: The *in vitro* rooted plantlets were transferred to plastic pots containing different soil mixture of peat moss, peat moss+perlite (1:1), peat moss+clay (2:1), peat moss+perlite+sand and peat moss+clay+sand (1:1:1 v/v) and were covered with polythene bags. The survival percentage, plant height (mm), leaves number and root length (mm) were recorded after 8 weeks from acclimatization.

Secondary metabolites estimation

Preparation of *Dillenia indica* extraction: Fresh leaves of *Dillenia indica* (0.01 g) were extracted with 5 mL acetone (85%). The extracts were filtered and extracted twice. The final extract was used for the determination of phenolic compound, pigment and antioxidant capacity.

Total phenol and tannins: Total phenols were estimated according to Singleton *et al.*[16] and tannins assayed using methodology of Tambe and Bhambar[17].

Total antioxidant capacity: Total antioxidant activity assay was carried out according to Prieto *et al.*[18].

Photosynthetic pigment: Pigments level (chlorophyll a, b and carotenoids) were measured with spectrophotometer according to Yang *et al.*[19].

Statistical analysis: The data were analyzed using randomized complete block design with 3 replicates per treatment. The treatments' means were compared for significance by Duncan's New Multiple Range test at 0.05% level of probability[20] using COSTATV-63.

RESULTS

***In vitro* multiplication:** The effect of culture media types and BA concentrations on the number of shootlets formed/explants, shootlets elongation and leaves number/shootlet of *Dillenia indica* was shown in Fig. 1a-c. The data declared that using BA at 2 mg L^{-1} supplemented to MS medium produced the highest number of shootlets formed/explant (5.67 shootlet) (Fig. 1c, 2a). The same concentration of BA (2 mg L^{-1}) supplemented to WPM gave the maximum number of leaves/shootlet (47.33 leaves)

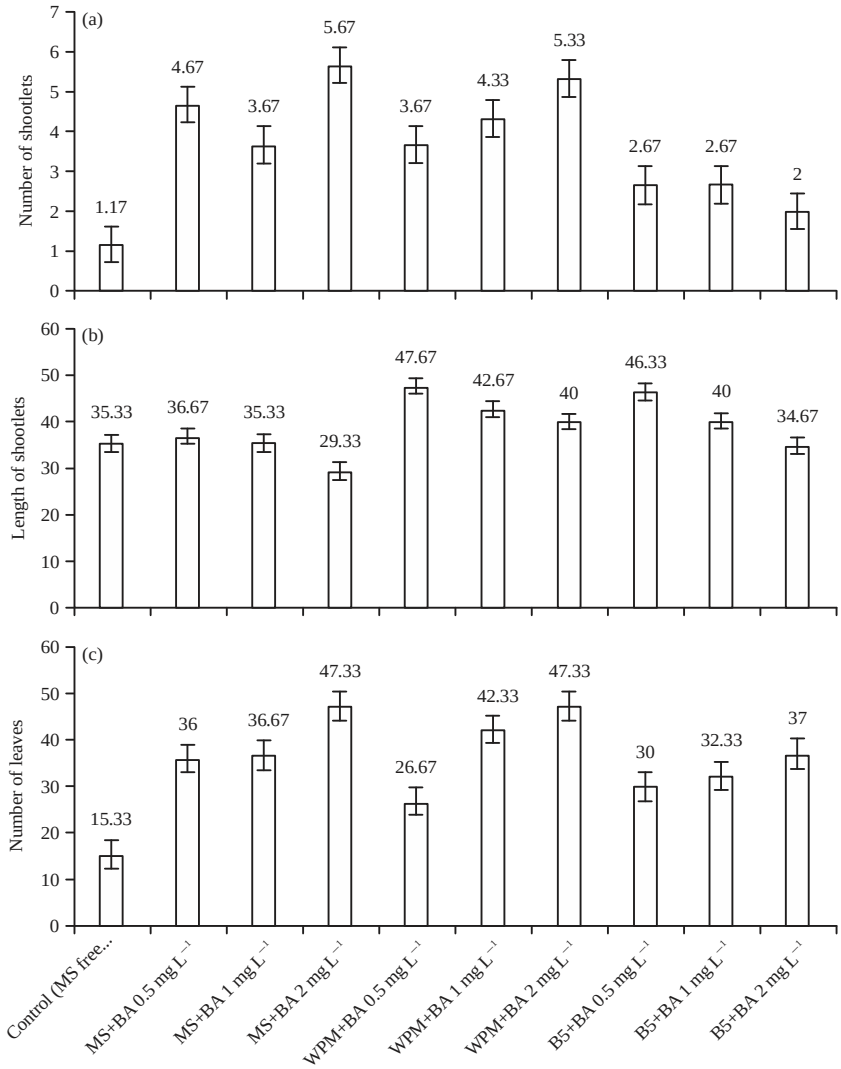

Fig. 1(a-c): *In vitro* multiplication affecting by culture media type and BA concentration according to Duncan's multiple range test (DMRT) at 5% level, (a) Number of shootlets/explant, (b) Length of shootlets (mm) and (c) Number of leaves/shootlet

Fig. 2(a-e): *In vitro* shooting and rooting ability of *Dillenia indica*, (a) Shootlets development that were produced from using MS+2 mg L^{-1} of BA, (b) Rooting of shootlets cultured on liquid MS+NAA at 0.4 mg L^{-1}, (c) Prepared rooted plantlets for the acclimatization stage and (d, e) Acclimatized plants to greenhouse

comparing with other treatments. Whereas, control treatment (MS free hormones) recorded the lowest values of shootlets and leaves numbers (1.17 and 15.33 leaves, respectively). Low concentration of BA (0.5 mg L^{-1}) that was supplemented to WPM or B5 media had stimulation effect on shootlets elongation (47.67 and 46.33 mm, respectively) while, adding BA at 2 mg L^{-1} to MS medium produced the shortest shootlets (29.33 mm) comparing with other treatments. It can be noticed that MS medium and WPM were more effective for inducing more shootlets and leaves as compared to B5 medium which reduced the multiplication rate and leaves number. High concentration of BA (2 mg L^{-1}) was favored for inducing new shootlets and leaves. These results are in agreement with those obtained by Abd El-Kadder and Hammad[21] on *Dillenia indica*, who observed that the proliferated shoots/explant were maximum when MS medium supplemented with BA (2mg L^{-1}) was used. Khan *et al.*[22] on *Salix tetrasperma* mentioned that the response of shoot induction was best on WPM supplemented with 6-benzyl adenine.

Table 1: *In vitro* rooting ability of *Dillenia indica* affecting by physical state of culture media (Solid, semisolid and liquid) and NAA concentration

Treatments	Characters					
	Rooting (%)		Number of roots/shootlet		Length of roots (mm)	
Period of rooting (month)	1st period	2nd period	1st period	2nd period	1st period	2nd period
MS free hormones	0.00	16.67^{c}	0.00	0.33^{g}	0.00	1.67^{d}
Solid MS+NAA 0.2 mg L^{-1}	27.67^{e}	41.67^{b}	1.40^{ef}	1.42^{fg}	8.33^{d}	10.00^{d}
Solid MS+NAA 0.4 mg L^{-1}	83.33^{b}	100.00^{a}	1.00	2.67^{ef}	85.00^{b}	105.00^{bc}
Solid MS+NAA 0.6 mg L^{-1}	100.00^{a}	100.00^{a}	8.00^{ab}	9.00^{ab}	65.00^{bc}	131.67^{a}
Semi solid MS+NAA 0.2 mg L^{-1}	44.33^{d}	50.00^{b}	3.33^{d}	7.33^{c}	59.33^{c}	91.67^{c}
Semisolid MS+NAA 0.4 mg L^{-1}	66.67^{c}	91.67^{a}	5.67^{c}	7.00^{c}	83.33^{b}	90.00^{c}
Semisolid MS+NAA 0.6 mg L^{-1}	100.00^{a}	100.00^{a}	9.67^{a}	10.00^{a}	120.00^{a}	130.00^{ab}
Liquid MS+NAA 0.2 mg L^{-1}	100.00^{a}	100.00^{a}	2.33^{de}	3.17^{de}	53.33^{c}	83.33^{c}
Liquid MS+NAA 0.4 mg L^{-1}	100.00^{a}	100.00^{a}	1.00^{ef}	4.33^{d}	115.00^{a}	130.00^{ab}
Liquid MS+NAA 0.6 mg L^{-1}	100.00^{a}	100.00^{a}	7.00^{bc}	8.00^{bc}	111.67^{a}	146.67^{a}

Means followed by different letters (a, b, c, d, e, f) are significantly different. Means having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level

The results in this work may attribute to that MS medium was most suitable for shoot proliferation which may be due to its high salt concentration and the presence[23] of NH_4N0_3. Nery *et al.*[24] pointed out that MS medium has been used successfully in propagating of many species but, Woody Plant Medium was favorable for some woody species. The varied results could impute to the different chemical and osmotic compositions of each media. Besides, Prakash and Pierik[23] reported that, BA had promotion effect shoots number formed/bud comparing with other cytokinins and with increasing BA concentration, the percentage of multiple shoots was increased. This attributed to the important role of cytokinin in cell division and differentiation and subsequently, the growth and development of plants[25].

***In vitro* rooting ability:** The results in Table 1 indicated that, using solid or semi solid MS medium plus 0.6 mg L^{-1} NAA or liquid MS plus any different concentrations of NAA (0.2, 0.4 or o.6 mg L^{-1}) led to the highest rooting percentage (100%) as compared to other treatments for the first period (one month). In the second period (two months), the same treatments as well as the concentration 0.4 mg L^{-1} of NAA added to solid MS medium or semi sold MS showed a significant increase (100 and 91.67%) in the rooting percentage of *Dillenia indica* plantlets as compared to other treatments. While, the lowest rooting percentage in both two periods (0 and 16.67%, respectively) was obtained with solid MS medium free of auxin. It can be observe that, the best rooting rate was happened on different media (solid, semi solid and liquid MS) supplemented with NAA at 0.6 mg L^{-1}. Based on that, auxin is essential to induce rooting in the *Dillenia indica* plantlets as no rooting was observed in the absence of auxin.

Concerning the effect of physical form of the culture medium and NAA auxin on number of roots per plantlet, the results in Table 1 showed that using semi solid MS medium plus NAA at 0.6 mg L^{-1} produced the maximum number of roots for two periods (9.67 and 10.0, respectively), followed by solid MS medium plus 0.6 mg L^{-1} of NAA treatment which recorded 8.0 and 9.0 roots in the first and second periods, respectively, comparing with other treatments. Whereas, the minimum values of roots number (0.0 and 0.33, respectively) in two periods were obtained with MS medium free of auxin.

Regarding the effect of different MS medium states combined with NAA on root length, the data presented in Table 1 indicated that, semi solid MS medium plus 0.6 mg L^{-1} NAA and application of 0.4 or 0.6 mg L^{-1} of NAA with liquid MS medium gave the longest roots (120, 115 and 111.67 mm, respectively) in the first period as compared to other treatments. In the second period, the similar treatments and using solid MS medium with NAA at 0.6 mg L^{-1} significantly increased the length of roots, compared to other treatments. Whereas, MS medium free of auxin produced the shortest roots of *Dillenia indica* plantlets (0.0 and 1.67 mm, respectively) for the first and second periods.

These finding were in agreement with those obtained by Seyyedyousefi *et al.*[26] reported that adding NAA at 1.0 mg L^{-1} to MS medium resulted in the highest root length and maximum root number. Also, Hamad *et al.*[27] mentioned that, solid MS culture medium supplemented with NAA (1.0 mg L^{-1}) led to the best rooting measurements of pineapple cultivars. The stimulation effect of auxins on root induction and growth was attributed to that auxins control main processes in division and elongation of the cells[28,29].

In this study, it had been noticed that culturing shootlets on liquid MS supplemented with NAA at 0.4 mg L^{-1} resulted plantlets with strong and thick roots which were prepared for acclimatization stage (Fig. 2b, c). This observation was confirmed by those found by Husen and Pal[30] mentioned that adding of auxin to the *in vitro* micro shoots strengthen the

Table 2: Acclimatization ability of *Dillenia indica* as effected by different soil mixtures

Treatments	Survival (%)	Plant height (mm)	Number of leaves	Root length (mm)
Peat moss	65.50[a]	96.33[a]	11.00	227.00[a]
Peat moss+clay	52.20[b]	79.00[b]	11.00	121.00[d]
Peat moss+sand+clay	35.67[b]	79.33[b]	10.00	92.17[e]
Peat moss+perlite	50.60[ab]	91.33[ab]	10.00	129.93[c]
Peat moss+perlite+sand	72.67[a]	103.33[a]	13.00	171.00[b]

[a,b,c]Means followed by different letters are significantly different. Means having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level

Table 3: Effect of culture media and BA concentration on phenolic compounds and pigments contents (mg g^{-1} F.W.) of *in vitro Dillenia indica* shoots

	Constituents					
Treatments	Phenols	Tannins	Chl. a	Chl. b	Total chlorophylls	Carotenoids
Control (MS free hormones)	101.33[d]	33.75[cd]	8.17[d]	3.77[c]	11.94[c]	2.70[b]
MS+BA 0.5 mg L^{-1}	99.78[d]	21.55[f]	6.63[f]	3.23[g]	9.87[h]	2.07[ef]
MS+BA 1 mg L^{-1}	121.51[c]	36.43[c]	5.86[g]	3.03[h]	8.89[i]	1.89[g]
MS+BA 2 mg L^{-1}	81.28[e]	22.36[e]	6.75[f]	3.25[g]	10.00[f]	2.03[f]
WPM+BA 0.5 mg L^{-1}	74.28[e]	14.50[f]	7.19[e]	3.62[e]	11.53[e]	2.22[d]
WPM+BA 1 mg L^{-1}	252.26[a]	47.83[a]	10.36[a]	4.86[a]	15.22[a]	3.82[a]
WPM+BA 2 mg L^{-1}	106.41[d]	20.90[e]	3.49[h]	2.40[j]	5.90[j]	0.86[h]
B5+BA 0.5 mg L^{-1}	125.33[c]	45.01[ab]	8.16[c]	3.68[d]	11.85[d]	2.46[c]
B5+BA 1 mg L^{-1}	122.91[c]	29.85[d]	8.95[b]	3.91[b]	12.86[b]	2.62[b]
B5+BA 2 mg L^{-1}	173.08[b]	42.60[b]	6.58[g]	3.33[f]	9.91[g]	2.19[de]

[a-j]Means followed by different letters are significantly different. Means having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level, Chl. a: Chlorophyll a, Chl. b: Chlorophyll b

adventitious roots by inducing the internal contents of enzymes that play an important role in regulation of various pathways of constituents such as protein, carbohydrates, nitrogen and phenolics, during roots initiation[31,32] and subsequently induce cell division, elongation and differentiation of tissues.

Acclimatization: As shown in Table 2 and Fig. 2d and e, when the *in vitro* rooted plantlets were acclimatized to different soil mixture [peat moss, peat moss+perlite (1:1), peat moss+clay (2:1), peat moss+perlite+sand or peat moss+clay+sand (1:1:1)], the plants were successful survived in highest percentage (65.5 and 72.67%) and attained to highest height (96.33 and 103.33 mm) with peat moss alone or mixed with perlite and sand, respectively. Also, the longest roots of acclimatized plants (227 mm) were obtained from using peat moss without any soil mixture followed by peat moss+perlite+sand which resulted roots length 171 mm. The shortest roots (92.17 mm) were resulted from peat moss+clay+sand. The number of leaves formed per acclimatized plant showed no significant variations when different soil mixture was used. In this share, Keng *et al.*[33] found that when acclimatized *Gynura procumbens* (Lour.) Merr. plants observed that the addition of sand had promotion effect on survival rate of the acclimatized plantlets and this might attributed to sands had reduced the retention of water commonly occurred in the organic soil which caused rotting of the roots and gradually the whole plantlets. Lobna *et al.*[34] found that the root length was in highest value when Paulownia plantlets were cultured in mixture of peat moss and sand (1:1).

Secondary metabolites

Phenolic compounds and pigments contents of *in vitro* shoots: Total phenols and tannins contents of *Dillenia indica* shootlets showed significant differences under effect of different culture media added with various concentrations of BA (Table 3). The total phenols and tannins in acetone extract were ranged from 74.28-252.26 mg g^{-1} F.W and 14.5-47.83 mg g^{-1} F.W., respectively. The maximum content of phenol (252.26 mg g^{-1}) and tannin (47.83 mg g^{-1}) were found with WPM supplemented with 1.0mg L^{-1} of BA treatment, followed by B5 medium plus 2.0 mg L^{-1} BA treatment which recorded 173.08 and 42.6 mg g^{-1} content as compared to other treatments. Also, the results illustrated that Chlorophyll a, b, total chlorophyll and carotenoids contents were significantly increased in micropropagation shoot on WPM medium supplemented with 1.0 mg L^{-1} BA (10.36, 4.86, 15.22 and 3.82 mg g^{-1} F.W., respectively) as compared to other treatments. Whereas, the lowest values of these constituents were obtained from using WPM plus BA at 2 mg L^{-1}. The biosynthesis of secondary metabolites in culture grown *in vitro* was clearly affected by BA[35]. Also, total phenolic content in *Gardenia jasminoides* were increased in all culture media (*in vitro*) more than control plant[36].

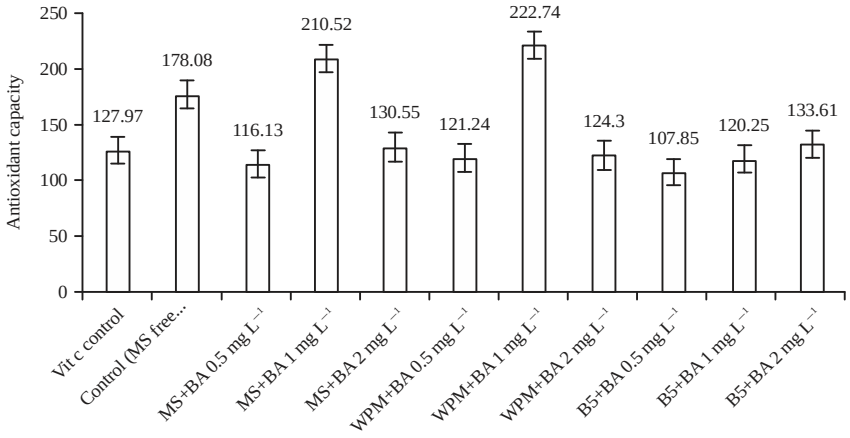

Fig. 3: Antioxidant capacity (µg/1 mL at concentration 100 µg) of *in vitro* propagated *Dillenia indica* shoots as effected by culture medium type and BA concentration

Table 4: Total phenols, tannin and pigments contents (mg g^{-1} F.W.) in leaves of acclimatized *Dillenia indica* plants

	Constituents					
Treatments	Phenols	Tannins	Chl. a	Chl. b	Total chlorophylls	Carotenoids
Peat moss	401.50^{b}	35.75^{c}	6.77^{d}	4.52^{b}	11.29^{c}	1.53^{b}
Peat moss+clay	374.90^{c}	197.50^{a}	11.77^{b}	5.50^{a}	17.28^{a}	2.60^{ab}
Peat moss+sand+clay	508.97^{a}	69.81^{b}	9.50^{c}	2.60^{c}	12.10^{b}	3.04^{a}
Peat moss+perlite	229.64^{d}	24.50^{d}	11.99^{a}	4.89^{ab}	16.89^{ab}	3.29^{a}
Peat moss+perlite+sand	134.82^{e}	12.37^{e}	4.86^{e}	2.61^{c}	7.48^{d}	1.27^{d}

$^{a-d}$Means followed by different letters are significantly different. Means having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level, Chl. a: Chlorophyll a, Chl. b: Chlorophyll b

Total antioxidant capacity of *in vitro* shoots: The extract of *Dillenia indica* shootlets showed variations for antioxidant capacity at concentration 100 µg mL^{-1} in all treatments (Fig. 3). Using WPM added with 1.0 mg L^{-1} of BA produced the highest antioxidant capacity (222.74 µg mL^{-1}) comparing with other treatments. While, B_5 medium plus 0.5 mg L^{-1} BA gave the lowest value (107.85 µg mL^{-1}) of antioxidant capacity. These results are similar to that obtained by Sayed *et al.*[36] found that all *Gardenia jasminoides* shootlets cultured on different concentrations of 2iP, kin and BAP had high antioxidant activity compared to MS free medium (control) and there phenolic compound in shootlet may be responsible for the antioxidant activity.

Phenolic compounds and pigments contents in acclimatized plants: In Table 4, the highest phenol content of plant leaves (508.98 mg g^{-1} F.W.) was detected when soil mixture of peat moss+sand+clay was used, followed by peat moss (401.5 mg g^{-1}). On the other hand, the minimum phenol content (134.82 mg g^{-1} F.W.) was observed with Peat moss+perlite+sand comparing with other soils.

The maximum tannin content (197.5 mg g^{-1}) was recorded with peat moss+clay, followed by peat moss+sand+clay (69.81mg g^{-1}) as compared to other soil types. Chlorophyll a, b and total chlorophyll content were significantly increased in leaves of *Dillenia indica* grown in mixture of peat moss+clay and peat moss+perlite. However, carotenoids content were significantly increased with peat moss+perlite and peat moss+sand+clay soils mixture (3.29 and 3.04 mg g^{-1}. F.W., respectively) comparing with other soils (Table 4).

Total antioxidant capacity of acclimatized plants: The results presented in Fig. 4, indicated that the leaves extract of *Dillenia indica* plant from all used soils showed higher antioxidant capacity than vitamin C (control). Soil mixture of peat moss+sand+clay or peat moss+clay led to the best results (415.3 and 430.2 µg) as compared to other treatments. High phenolic contents of plant are considered a good source for antioxidant activity because they have high reactivity as hydrogen or electron donors beside; they are capable of chelating metal ions owing to their hydroxyl group at various positions[37].

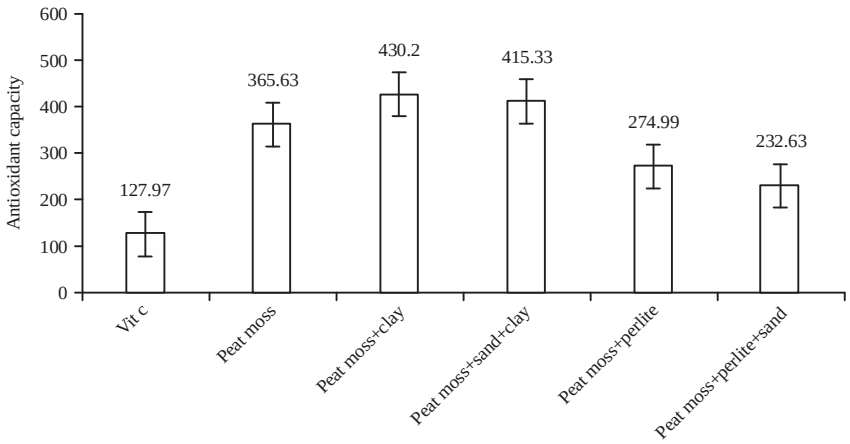

Fig. 4: Antioxidant capacity of acclimatized *Dillenia indica* plants under effect of different soil mixtures

CONCLUSION

Micropropagation protocol and secondary metabolites stimulation could be established for *Dillenia indica*. MS medium or WPM supplemented with 2 mg L^{-1} of BA was favored for *in vitro* propagation. Semi solid MS medium+NAA at 0.6 mg L^{-1} produced the maximum number of roots. The maximum phenol, tannin and pigments contents were found in shootlets that were cultured *in vitro* on WPM medium+1.0 mg L^{-1} BA. The plants were successfully acclimatized in soil mixture of peat moss alone or peat moss+perlite+sand. The maximum phenols content as well as antioxidant activity were observed in leaves of acclimatized plant in soil mixture of peat moss+sand+clay.

SIGNIFICANCE STATEMENT

Dillenia indica is considered an ornament tree and has various active compounds such as polyphenols, tannins, alkaloids, steroids, saponins and fixed oil and flavonoids were found in different solvent extracts of *Dillenia indica* which are responsible for the various pharmacological activities. The aim of this experiment was to study various factors on behaviors of *Dillenia indica* to attain suitable micropropagation protocol *in vitro* and *in vivo* stages. Micropropagation protocol and secondary metabolites stimulation could be established for *Dillenia indica*. Obtaining of the suitable protocol for *Dillenia indica* propagation lead up to a large scale of plant production in the laboratory with quality integrated characteristics, cost effectiveness, maintain genetic fidelity, long term storage and virus free, obtaining of medical metabolites using *in vitro* culture technique.

ACKNOWLEDGMENT

The authors of this work are greatly thankful to National Research Centre, 33 El Bohouth st. (formal El Tahrir st.), Dokki, P.O.12622, Giza, Egypt, for research facilities.

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