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Secondary Metabolites Characterization of *in Vitro* Propagated *Antigonon leptopus* Cultures

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Abstract

This work was conducted on *Antigonon leptopus* to optimize the *in vitro* propagation conditions for shootlets multiplication and callus culture under effect of some factors such as types of cytokinins (BA and 2ip) at different concentrations (0.2, 0.4 and 0.6 mg/l) plus 0.1 mg/l of IBA that were examined for proliferated shoots with high rooting ability. Also, different concentrations of growth regulators (2,4-D, TDZ and NAA) under both light and darkness incubation were evaluated for callus tissues development. The secondary metabolites for both shootlets and callus tissues were estimated. For obtaining shootlet culture development with more proliferation and elongation of shootlets with the highest number of leaves, rooting percent and roots length, MS medium was added with 0.2 mg/l 2ip plus 0.1 mg/l IBA. While, MS supplemented with 0.4 2iP plus 0.1 IBA mg/l resulted in the highest values of all estimated pigments (Chl. a, b, carotenoid and anthocyanin) in shootlets. Using high concentration of 2ip (0.6 mg/1) enhanced the production of phenolic, flavonoids compounds and antioxidant activity in shootlets. Low concentration of both NAA (2.5 mg/l) and TDZ (1.0 mg/l) under darkness condition produced the highest callus fresh weight. However, the same treatment (NAA at 2.5 mg/l+ TDZ at1mg/l) under light condition caused significant increment of the above-mentioned active compounds in callus tissues. The successfully acclimatized plantlets showed variations in morphological characters due to transferring them to different growth media (peat moss, perlite, clay and sand) in the greenhouse.

Key words: Antigonon leptopus; micropropagation; callus; active compounds

Introduction

Antigonon leptopus (Polygonaceae family) or coral vine is native to Mexico and commonly found in tropical Asia, the Americas, Africa and the Caribbean [1]. It is usually grown in gardens and is often wild. It is a climbing vine; stems slender. The color of flowers is most generally pink, found in terminal panicles or axillary racemes. The use of *Antigonon leptopus* as an ornamental plant for its strong growth, and bountiful pink flowers and its ability to hide ugly landscapes [2].

Traditionally, *Antigonon leptopus* has been used to treat diabetes, cough, asthma, liver and spleen disorders and throat constriction [3]. Recent realization of traditional and alternate medicine created great awareness to investigate the medicinal plants on earth and aerial parts (leaves and flowers) of *Antigonon leptopus* that are utilized to make tea in numerous countries which are used as pain relief, throat constriction and remedy for cold in traditional medicine [4]. Leaves of *A. leptopus* are rich of alkaloids, tannins, saponins, unsaturated sterol and/or flavonoids, triterpenes, coumarins and glycosides [5]. Some other investigations found that the leaves of *A. leptopus* are used to decrease swelling and to treat hypertension, diabetes, and menstrual pains [6] while, vines are used in the treatment of throat constrictions and cough. It has been utilized in Chinese folk medicine to treat nephritis, hepatitis and colitis [7].

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The traditional propagation of this plant could not cover the increasing demand from it for use in medical purposes or decoration. Therefore, the tissue culture technique can be used as a substitutional to the continuous progress of plant supplies. Moreover, tissue culture technique is a way for rapid mass propagation for large scale re-vegetation, biodiversity conservation and enhancing the production and quantity of secondary metabolites that can be even bigger than in parent plants [8].

The choice of plant growth regulators (PGRs) is one of the main factors affecting the success of *in vitro* plant propagation. So, PGRs (especially cytokinins) are often supplied to culture media for the aim of controlling various physiological responses in vitro, leading to the production of organs as shoots and roots, tissues as callus or whole plants [9].

For induction of secondary metabolite production, plant growth regulators (PGRs) are required [10, 11], since they have significant effects on the metabolism of secondary metabolites. The quality and quantity of plant growth regulators plays a major role in the production capability of a given *in vitro* culture.

The current study was conducted for two objectives: First, to optimize an *in vitro* propagating protocol for this plant by testing different types and concentrations of cytokinin and testing two different types of auxins with cytokinin on callus formation. Second, evaluation of active compounds in the obtained shootlets and callus as affected by plant growth regulators.

Experimental

This study was carried out in Tissue Culture Technique Laboratory- Central Labs - Ornamental Plants and Woody Trees Department, National Research Centre (NRC), through two seasons 2019 / 2020 on *Antigonon leptopus* with the aim to examine the effect of some factors on consecutive propagation of this plant *in vitro* (shootlets multiplication, rooting, callus formation), *in vivo* acclimatization and evaluation of secondary metabolites for both shootlets and callus tissues.

Plant materials and disinfection

Stem nodel explants (2-5 cm) of *Antigonon leptopus* were selected from a healthy mother climbing grown in Zohrya Botanical Garden, Zamalek- Cairo, Egypt as explant materials for this work. Old leaves were removed and rinsed in liquid soap with shaking for 15 min then were placed under running tap water for one hour after that to be ready for sterilization. Under aseptic conditions in a laminar air-flow cabinet, the explants were dipped in 70% ethanol for 30 sec then, immersed in commercial Clorox (NaOCl, 5.25% free chlorine) at concentration of 15% (v/v) for 5 min and the explants were rinsed with sterile distilled water for three times, followed by the sterilizing with mercuric chloride ($HgCl_2$) at 0.1% (w/v) with a few drops of wetting agent "Tween-20" for two minutes and the explants were rinsed with sterile distilled water for four times. The explants became ready for culturing on full salt strength of MS medium.

Culture media and incubation conditions

The explants were cultured on Murashige and Skoog medium [12] which was solidified by using 0.8% agar prior to autoclaving at 1.2 kg/cm2 for 15 min. The pH of the tested medium was adjusted to 5.8 before addition agar and then sterilized in the autoclave at 121°C. The planting was done in glass jars (200 ml) filled with 25 ml of the medium. All culture jars were incubated in the growth chamber at 24 ± 2 °C, 3000 Lux for 16 hr. light /8 hr. darkness daily (controlled automatically) which were provided from cool white fluorescent lamps.

In vitro shooting and rooting

The experiment of this stage was designed to study the effect of different types of cytokinins such as BA (6-benzyl amino purine) and 2iP (6-γ, γ-dimethylallylamine purine riboside) at different concentrations (0.0, 0.2, 0.4, 0.6 mg/l), in combination with Indole Butyric Acid auxin (IBA) at 0.1 mg/l on shooting multiplication and rooting of shootlets. The experiment involved 7 treatments, each treatment contains 7 replicates and the explants were re-cultured two times, at 45-day intervals. The following characters of the plantlets such as survival %, shootlet number and length, leaves number, rooting %, number and length of roots were recorded as the mean of two subcultures of this stage.

Callus culture development

Symmetric size (about 1 cm length) of nodel explants was cultured in glass jars containing sterile MS medium supplemented with different types of auxin such as 2,4- dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA) at 2.5, 5.0 and 10.0 mg/l, combined with different concentrations (0.5, 1.0 and 2.0 mg/l) of cytokinin Thidiazuron (TDZ). For each treatment, 10 jars were prepared, each jar contained three explants. The jars of each treatment were divided into two parts, the first part was incubated in the light and another was incubated in darkness condition at 24 ± 2 °C for four months. For evaluating the development of callus tissues as respond to various plant growth regulators, callus fresh

weights (g) and characters were recorded for both light and darkness.

Acclimatization

The rooted plantlets were removed from the culture jars and then washed with tap water, followed by immersing them into fungicide for 20 sec. The plantlets were individually transplanted to small plastic pots (5 cm diameter) filled with different soil mixtures (v/v) involving peat moss: perlite (1: 1), peat moss: clay (1: 1), peat moss: sand (1: 1) and peat moss: perlite: clay: sand (1:1:1:1). White transparent bags were used to cover the culture pots to maintain relatively high humidity. One opening was made per polyethylene bag after two weeks from planting then the bags gradually removed at the end of this month. After two months, plant height (cm), branches number/plant, leaves number/ plant and root length (cm) were recorded.

Secondary metabolites

Photosynthetic pigment

Pigments concentration (chlorophyll a, b and carotenoids) were measured with spectrophotometer according to Yang et al., [13].

Total Anthocyanin

Anthocyanin was extracted overnight from fresh shootlets with ethanol and 1% HCl (85:15) at 4°C. The optical density of the extract solution was measured at 535 nm. according to Francis [14].

Determination of anti-oxidative activity using DPPH assay

The free radical scavenging activity using (DPPH) the 1.1-diphenyl-2-picryl- hydrazil reagent was determined according to Brand-Williams et al., [15]. 0.5 ml of the methanolic extracts of *Antigonon Leptopus* were added to 1.5 ml of freshly prepared methanol DPPH solution (20 µg ml) and stirred. The decolorizing processes were recorded after 5 min of reaction at 517 NM and compared with a blank control.

Antioxidant activity = [(control absorbance - sample absorbance)/ control absorbance] ×100%.

Total Phenolic content (mg GAE/g DW)

The determination of total phenolic content in the extracts was based on the method of **[16].** Methanolic solution of *Antigonon Leptopus* extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, 0.5 ml of methanolic solution of extract and 2.5 ml 7.5% $NaHCO_3$. The absorbance was determined using a spectrophotometer at λ max = 765 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed.

Total Flavonoid

Total flavonoid was estimated by the method [17]. 0.5 ml of 2% $AlCl_3$ solutions was added to 0.5 ml of methanol extracts. After one hour at room temperature filtered, and then the absorbance was measured at 415 nm. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

Statistical analysis

The average of recorded data for different parameters was statistically analysed using randomized complete block design except for callus culture development and active compounds (Tables 2 and 6) which were arranged as two factorials in a completely randomized design. LSD test at 5% for comparison among means was used according to methods of Steel &Torrie [18].

Results and discussion

***In vitro* culture development**

1. Shoots proliferation

As shown in Table (1), two types of cytokinins (BA and 2ip) at different concentrations (0.2, 0.4 and 0.6 mg/l) were examined in the presence of auxin IBA (0.1 mg/l) to standardize the most suitable culture medium for *in vitro* culture development of proliferated shoots with high rooting ability. The results showed that adding 0.4 mg/l of BA and any concentration (0.2, 0.4 or 0.6) of 2ip to MS culture medium relieved the response of explants to survive in highest present (100%). While, cultures were able to develop on MS medium supplemented with 0.2 mg/l 2ip plus 0.1 mg/l IBA and showed more proliferation and elongation of shootlets with highest number of leaves, rooting percent and roots length (3.0, 73.4 mm, 13.0, 93.13 % and 45.3 mm, respectively) as compared to control and other treatments (Fig.1; A, B). Also, increasing the concentration of 2ip to 0.6 mg/l resulted in development of long shootlets (72 mm) with highest number of roots (1.6). However, it could be noticed that no roots were formed when MS medium supplemented with BA at any concentration that was used. This declared that using MS culture medium supplemented with 2ip at 0.2 mg/l plus IBA at 0.1mg/l showed superior response for obtaining *in vitro* culture development with high proliferated and rooted shoots. Similar results were revealed by Zenna and Taha [19] on *Antigonon leptopus* who noticed the stimulation effect of 2ip at 1.0 mg/l and IBA at 0.2 mg/l when were added to MS medium on the shootlets formation

percent. Using 2ip produced strong grown shoots of Rhubarb plant with 100% roots [20].

Previous reports pointed out the important roles of growth regulators in various developmental processes from germination to shoots and roots formation [21]. Cytokinin is the most important plant hormone for enhancing plant growth and development, that known to promote cell division and differentiation [22]. Kulaeva [23] attributed the physiological role of cytokinin to the activation of RNA, protein synthesis and enzyme activity. Multiple shoots formation could be formed by different types of cytokinins through breaking the apical shoots dominance [24].

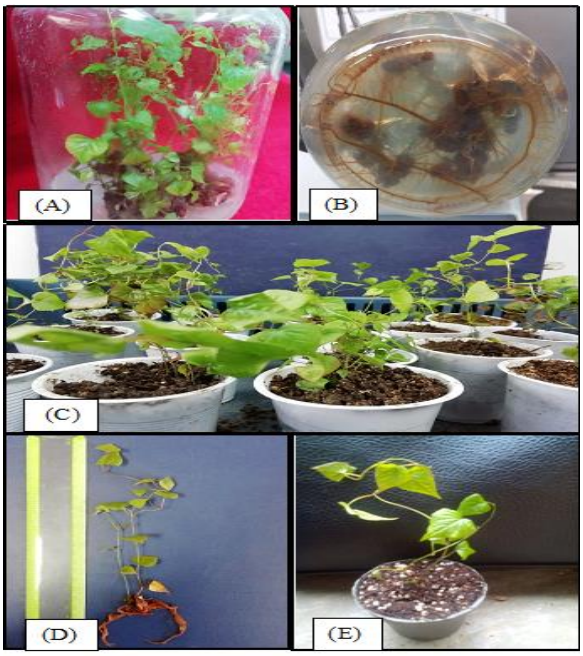

Fig. 1. Culture development of *Antigonon leptopus*: (A; B): *In vitro* shoots development and rooting of shootlets on MS medium supplemented with 0.2 mg/l 2ip plus 0.1 mg/l IBA; (C): Hardening off of plantlets in different combinations of growth media (peat moss, perlite, clay and sand); (D and E): Acclimatized plants in growth medium with combination of peat moss + perlite (1:1).

2. Callus culture development

Data in Table (2) revealed the variations in callus fresh weights and characters as response of calli derived from nodal explant to different concentrations of growth regulators (2,4-D, TDZ and NAA) under both light and darkness incubation. Interestingly, callus fresh weight was significantly highest (5.97 and 5.37 gm, respectively) when callus tissues were grown on MS medium supplemented with NAA at 2.5mg/l plus TDZ at 1.0 or 2.0.mg/l comparing with other treatments. The interaction effect of growth regulators concentration and light showed that low concentration of both NAA (2.5 mg/l) and TDZ (1.0 mg/l) under darkness incubation was superior for obtaining the highest callus fresh weight (7.03 g) as compared to other treatments. Moreover, using NAA at 2.5 mg/l plus any concentration of TDZ (0.5, 1.0 or 2.0 mg/l) had a promotion effect on callus growth especially in darkness. Hence, the development of callus tissues that was expressed by callus fresh weight was increased (2.64 g) under dark condition as compared to that was in light (2.25 g). Callus tissues that grown on MS medium supplemented with 2,4-D plus TDZ at any concentrations were appeared as compact tissues, characterized by creamy color and globular cells that were more indicated under darkness. While, callus tissues that were obtained from using NAA in MS medium plus TDZ at any concentrations seemed to be friable and dark brown tissues (Fig. 2).

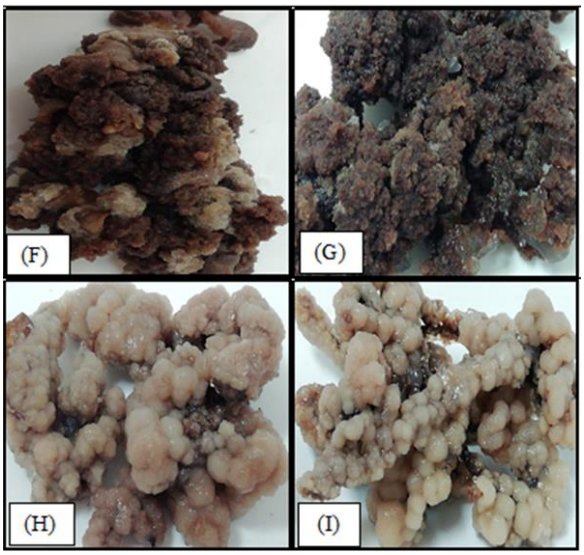

Fig. 2 Callus tissues development under effect of different plant growth regulators and light condition; (F, G): Callus tissues that were obtained from using NAA under light and dark conditions, respectively; (H, I): Callus tissues that were grown on MS medium supplemented with 2,4-D under light and dark conditions, respectively.

The variations of callus cultures growth as a result of using different plant growth regulators might be attributed to the sensitivity of explant that depends on the endogenous concentrations of plant growth regulators and the capacity of cells differentiation of these explants [25]. Earlier reports pointed out that the morphogenesis in culture system depends on the combination between auxins and cytokinins and their ratio that were used in plant tissue culture [26]. Also, light had a significant effect on callus growth and features such as color, appearance, compaction degree and morphogenetic potential [27].

Acclimatization

The presented data in Table (3) and Fig. (1; C, D and E) revealed the variation in morphological characters of successful acclimatized *Antigonon leptopus* plantlets in different combinations of growth media (peat moss, perlite, clay and sand). From these results, it could be noticed that the highest values of both plant height and leaves number (18.0 cm and 16.0 leaves) were obtained when the plantlets were transplanted in growth medium with combination of peat moss + perlite (1:1). Using peat moss combined with clay resulted in the highest number of branches per acclimatized plant (4.0) while, adding sand to peat moss (1:1) caused the longest roots (6.33 cm). These results pointed out the important role of peat moss as material constituent in growth media for plantlets acclimatization. Confirmed results by Sensi and Loffredo [28] attributed this effect to the ability of peat moss to improve plant growth indirectly through creating suitable physical conditions of media by enhancing aggregation, aeration, and water retention. However, the moderate capacity of perlite for use as growing media that was known to retain water, provide aeration and its neutral pH due to the fact that it is sterile and weed-free as well as its ability to decrease bulk density and increase the porosity of soils [29].

TABLE 1. *In vitro* shooting and rooting responses of *Antigonon leptopus* to different types and concentrations of cytokinins

Character / Treatments	Survival %	Shootlet number/ explant	Shootlet length (mm)	Leaves number /shootlet	Rooting %	Roots number	Roots length (mm)
Cont. (MS Free of hormones)	93.67	2.39	64.13	8.00	52.67	1.06	20.0
MS+0.2 BA+0.1 IBA	93.67	2.20	36.23	8.60	0.00	0.00	0.0
MS+0.4 BA+0.1 IBA	100.00	2.80	39.30	12.40	0.00	0.00	0.0
MS+0.6 BA+0.1 IBA	94.43	2.83	30.00	7.47	0.00	0.00	0.0
MS+0.2 2iP+0.1 IBA	100.00	3.00	73.40	13.00	93.13	1.20	45.3
MS+0.4 2iP+0.1 IBA	100 .00	2.19	70.77	9.17	46.43	1.06	42.0
MS+0.6 2iP+0.1 IBA	100.00	1.63	72.00	8.27	33.17	1.60	31.1
$LSD_{0.05}$	3.6	0.56	4.16	3.27	2.83	0.23	3.64

TABLE 2. Effect of different concentrations of growth regulators on callus culture development of *Antigonon leptopus* under light and darkness

Treatment (mg/l)	Light	Dark	Mean (A)
2,4-D 2.5 + TDZ 0.5	1.60	1.94	1.77
2,4-D 2.5 + TDZ 1.0	1.49	1.37	1.43
2,4-D 2.5 + TDZ 2.0	1.83	2.34	2.09
2,4-D 5.0 + TDZ 0.5	1.73	2.12	1.93
2,4-D 5.0 + TDZ 1.0	1.34	1.45	1.40
2,4-D 5.0 + TDZ 2.0	1.44	1.66	1.55
2,4-D 10.0 + TDZ 0.5	1.68	1.74	1.70
2,4-D 10.0 + TDZ 1.0	1.61	1.76	1.69
2,4-D 10.0 + TDZ 2.0	1.22	1.84	1.53
NAA 2.5 + TDZ 0.5	4.89	4. 91	4.89
NAA 2.5 + TDZ 1.0	4.90	7.03	5.97
NAA 2.5 + TDZ 2.0	5.10	5.64	5.37
NAA 5.0 + TDZ 0.5	2.32	2.38	2.35
NAA 5.0 + TDZ 1.0	2.28	1.91	2.09
NAA 5.0 + TDZ 2.0	1.96	3.63	2.79

NAA 10.0 + TDZ 0.5	1.69	2.00	1.85
NAA 10.0 + TDZ 1.0	1.77	2.10	1.94
NAA 10.0 + TDZ 2.0	1.63	1.76	1.70
Mean (B)	2.25	2.64	
LSD $_{0.05}$	A = 0.248	B = 0.083	
	A*B = 0.372		

TABLE 3. Acclimatization of *Antigonon leptopus* plantlets affecting by different growth media

Character / Treatment (v/v)	Plant height (cm)	Branches number /plantlet	Leaves number /plantlet	Root length (cm)
Peat moss+ Perlite (1:1)	18.00	2.00	16.00	5.00
Peat moss+ Clay (1:1)	16.67	4.00	13.00	5.33
Peat moss+ Sand (1: 1)	16.00	3.00	14.67	6.33
Peat moss+ Perlite+ Clay+ Sand (1:1:1:1)	17.00	2.33	13.00	4.67
LSD $_{0.05}$	1.73	0.99	2.83	1.10

TABLE 4. Effect of different types and concentrations of cytokinins on photosynthetic pigments contents of *Antigonon leptopus* shootlets

Pigment content (Mg/ 100g F.W.) / Treatment (mg/l)	Chl. a	Chl. b	Carotenoid	Anthocyanin
Control (MS Free of hormones)	51.20	19.94	97.40	15.00
MS+0.2 BA+0.1 IBA	38.53	13.70	78.06	14.13
MS+0.4 BA+0.1 IBA	50.23	20.13	88.33	10.27
MS+0.6 BA+0.1 IBA	33.10	13.50	63.00	10.73
MS+0.2 2iP+0.1 IBA	67.10	22.67	100.53	22.00
MS+0.4 2iP+0.1 IBA	82.97	33.20	161.53	25.47
MS+0.6 2iP+0.1 IBA	54.50	19.58	116.20	16.60
LSD $_{0.05}$	4.78	4.63	8.06	4.06

TABLE 5. Effect of plant growth regulators on active compounds (antioxidant activity, phenolics and flavonoids) contents of obtained *Antigonon leptopus* shootlets *in vitro*.

Treatments	Total phenols content (mg of GA/g DW)	Total flavonoids content (mg of Quercetin equivalent/g DW)	Antioxidant activity (%)
Control (MS Free of hormones)	15.93	2.82	55.2
MS+0.2 BA+0.1 IBA	19.97	3.17	67.5
MS+0.4 BA+0.1 IBA	21.13	3.78	72.4
MS+0.6 BA+0.1 IBA	22.63	3.67	56.7
MS+0.2 2iP+0.1 IBA	18.9	3.52	73.7
MS+0.4 2iP+0.1 IBA	20.14	4.26	74.4
MS+0.6 2iP+0.1 IBA	22.8	4.35	78.6
LSD $_{0.05}$	2.07	0.61	2.31

TABLE 6. Effect of plant growth regulators on active compounds (Phenolics, flavonoids contents and Antioxidant activity) of *Antigonon leptopus* callus tissues

Active compound	Total phenols content (mg of GA/g DW)			Total flavonoids content (mg of Quercetin equivalent/g DW)			Antioxidant activity (%)		
Treatments (mg/l)	Light	Dark	Mean (A)	Light	Dark	Mean (A)	Light	Dark	Mean (A)
2,4 D 2.5 + TDZ 2.0	21.00	21.17	21.08	4.07	3.52	3.79	66.00	63.31	64.65
NAA 2.5 + TDZ 1.0	29.72	24.78	27.25	5.01	4.40	4.83	82.33	79.00	80.66
NAA 10 + TDZ 2.0	25.02	24.51	24.76	4.52	4.65	4.46	67.12	65.00	66.06
Mean (B)	25.24	23.48		4.53	4.19		71.81	69.1	
L.S.D $_{0.05}$	A = 2.90 B = 1.67 A*B = 2.05			A = 0.66 B = 0.38 A*B = 0.46			A = 3.57 B = 2.06 A*B = 2.53		

Photosynthetic pigments contents

Concerning the effect of different types and concentration of cytokinins on photosynthetic pigments contents in shootlets, the tabulated data in Table (4) illustrated that the highest values of all estimated pigments (Chl. a, b, carotenoid and anthocyanin) were recorded (82.97, 33.20, 161.53 and 25.47 mg/100g F.W., respectively) in those shootlets obtained from MS supplemented with 0.4 of 2iP plus 0.1 of IBA mg/l with no significant difference between the mentioned value of anthocyanin and that recorded (22.0 mg/100 g F.W.) when MS medium contained 0.2 of 2iP plus 0.1 of IBA mg/l comparing with control and other treatments. These results highlighted the priority of the cytokinin 2ip at 0.4 mg/l in MS culture medium to obtain the best results of photosynthetic pigments. This stimulation effect of cytokinin attributed to stimulation and differentiation of chloroplast biogenesis [30]. The variation effects of cytokinins might be due to their enhancement effect especially, 2ip on pigments accumulation in shootlets tissues [31].

Antioxidant activity, phenolics and flavonoids contents in shootlets

Several studies showed that polyphenols are present in medicinal plants, especially flavonoids that have aroused great interest in research due to their medicinal properties [32]. These compounds are among the most studied class of secondary metabolites, making them desirable targets for *in vitro* culture [33]. In this context, Table (5) presents the total content of phenolic, flavonoids compounds and antioxidant activity in obtained shootlets of *Antigonon leptopus* on MS medium supplemented with two types of cytokinins (BA and 2ip) at three concentrations (0.2, 0.4 and 0.6 mg/l) combined with IBA at 0.1 mg/l. It can be observed that total phenolic content was increased with all used treatments more than control (MS-free Medium). The highest concentration of total phenolic compounds occurred with high concentration (0.6 mg/l) of 2ip or BA plus 0.1mg/l of IBA (with values of 22.80 and 22.63 mg GAE/100 g DW, respectively) with no significant differences of values due to these treatments. In addition, MS medium supplementation with cytokinins (BA or 2iP) and auxin IBA influenced on flavonoids content of *Antigonon leptopus* shootlets cultures in comparison to control (MS free medium), MS+ 0.4 or 0.6 mg/l of 2ip + 0.1 IBA caused the highest flavonoids content (with values of 4.26 and 4.35 mg Quercetin/g DW, respectively). Similar results were observed in previous studies on *Thymus vulgaris,* mentioned that addition of plant growth regulators (0.5 mg/L BA + 0.1 mg/L IBA) to the culture medium caused the elicitation of phenolic compounds that were recorded in plantlets [34]. Plant growth regulators were found to affect the accumulation of flavonoids in cell culture [35, 36].

In this work, different concentrations of 2iP, and BA combined with IBA produced significant effects on antioxidant activity as compared to MS-free medium (control). The highest antioxidant activity (78.6%) was recorded with MS+ 0.6 2ip + 0.1 IBA. It seemed that there was a strong relationship among total phenolic and antioxidant activity and this indicated that phenolic compounds were a major contributor of antioxidant activity in *Antigonon leptopus*. Our data are in agreement with [37] who reported the same relationship, as phenolics are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups.

Phenolics, flavonoids contents and Antioxidant activity of callus tissues

As shown in Table (6), the effect of different concentrations of growth regulators under light

conditions (light and darkness) on phenolics, flavonoids contents and Antioxidant activity of *Antigonon leptopus* callus tissues was revealed. The results illustrated that addition of NAA at 2.5 mg/l+ TDZ (1mg/l) in MS culture medium had a significant promotion effect on those active compounds that were in highest values (27.25 mg of GA/g DW, 4.83 mg of Quercetin equivalent/g D.W. and 80.66 %, respectively) comparing with other treatments. Incubating callus cultures under light condition was favoured for these compounds thus, the above-mentioned treatment (NAA at 2.5 mg/l+ TDZ at1mg/l) under light condition caused a significant increment of these compounds comparing with those were recorded under dark conditions.

Interestingly, there was significant increase in the phenolic content with increase in the weight of callus tissues when grown in presence of NAA at 2.5 mg/l+ TZD (1mg/l) on MS medium and browning of tissues that was observed. There is some evidence that phytohormone FAP (furfurilamino purine) causes browning of tissue due to excision in explant resulting in secretion of polyphenols in the medium [38].

Concerning the promotion effect of light and dark condition and NAA at 2.5 mg/l+ TZD at 1mg/l treatment on callus flavonoid content, confirmed reports mentioned that various combinations and concentrations of PGRs resulted in accumulations of phytochemicals like flavonoids and phenolics in callus [39,40]. The exogenous growth-regulating substances can alter the accumulation of secondary metabolites through regulating the expression of genes involved in the synthesis of secondary metabolites. Different types and concentrations of PGRs have been reported to regulate developmental processes and modify the concentration of secondary metabolites in plants differently [41]. Combination and concentration of the applied auxin and cytokinin were reported to strongly influence the biosynthesis and accumulation of flavonoids in the cultured plant cell [42]. The significant role of light on both stimulation and inhibition effects on the synthesis of secondary metabolism in many medical plants was also reported [43]. The present study was in line with these by Ali and Abbasi [44] on *Artemisia absinthium* suspension cultures who noticed the increased production of phenolic compound in response to light. Moreover, Joshi [45] showed that light significantly increased total flavonoid content in callus compared with dark conditions. Mir et al., [46] found that light enhanced the production with higher levels of total phenolic content than to dark conditions and the reason is that continuous light creates stress conditions within suspension cultures and hence stimulates signal transduction cascade for increment in production of phenolic compounds**.**

Conclusion

This study successfully established the optimal conditions for the *in vitro* shoots and callus tissue growth of *Antigonon leptopus* with a potential for secondary metabolite production. Dark and light conditions were used to enhance callus biomass and production of medicinally important metabolites. Since there are limited studies on tissue culture of this plant, these findings will be helpful for future scale-up productions of antioxidant compounds. Further investigations are necessary in order to increase the production of secondary metabolites by *in vitro* cultures.

Conflicts of interest

There are no conflicts to declare

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