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IN VITRO PROPAGATION OF *PLECTRANTHUS BARBATUS* ANDREWS AS IMPORTANT MEDICINAL PLANT

[47]

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ABSTRACT

Plectranthus barbatus Andrew (*Coleus forskolii*) is one of the important species of the genus *Plectranthus* (*Coleus*) belonging to family Lamiaceae, with a many of traditional medicinal uses in India. *C. forskolii* is only known source of forskolin; a compound with a many uses in pharmaceutical industries. *C. forskolii* was lack in Egyptian flora. Moreover, there were no previously studies on this plant in Egypt. Therefore, the present study used tool of biotechnology to conserve the stocks of this plant by micropropagation. *C. forskolii* seedlings came from its native Thailand at June 2013 and were put in the greenhouse in Desert Research Center for creating an efficient micropropagation protocol. The study was carried out on the effect of growth regulators (cytokinins and auxins) on different micropropagation stages of the explants. In multiplication stage, initiated shoots were cultured on MS medium supplemented with various concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) of cytokinins (6-benzylaminopurine (BA), Kinetin (KIN) and Thidiazuron (TDZ). The mean number of axillary shoots per explant of *C. forskolii* reached the highest value 6.19 ±0.573 on MS medium containing 2.0mg/L TDZ. Where, the highest value of mean length was 6.44± 0.310 cm on MS medium containing 1.0 mg/L KIN. The mean number of roots / explant of *C. forskolii* reached the highest value and the mean length were 30.00 ± 0.577 and 11.8±0.860 cm respectively, on 1/2 MS medium containing 0.5mg/L indole-3-butyric acid(IBA). While, the highest value of shoot length was 11.8±0.860 cm on 1/2 MS medium containing 2.0mg/L naphthalene acetic acid (NAA). A percentage of 83% of rooted plantlets were successfully acclimatized after four weeks and grown normally in the greenhouse in sterile soil mixture of garden soil, vermiculate and sand (2:1:1/v/v/v). The protocol could be cost effective and useful in germplasm conservation and delivery of tissue cultured Coleus plants.

Key words: *Plectranthus barbatus; Coleus forskolii*; Micropropagation; Acclimatization; Egypt..

INTRODUCTION

Medicinal plants are very important source of drugs for the majority of the world's population. The biotechnology tools are important to conserve and select the important genotypes of medicinal plants **(Tripathi and Tripathi, 2003)** *In-vitro* Micropropagation is biotechnological tool for conserved medicinal plants and production of secondary metabolites. *Plectranthus barbatus* Andrew was one of the important species of the genus *Plectranthus* L′ Herit. (Lamiaceae), with a wide range of traditional medicinal uses in Indian and Ayurveda traditional medicine as well as in the folk medicine of tropical Africa, China and Brazil. The plant had therefore been a target for intensive chemical and pharmacological studies up to now **(Alasbahi and Melzig 2010)**. Forskolin is an important labdane diterpene because its mode of action as it activates the secondary messenger Cyclic Adino monophosphate (cAMP) between the cells. *Coleus forskolii* is the only known source of forskolin compound with a pharmaceutical activity such as heart

diseases, antiplatelet, bronchospasmolytic, cardiotonic, hypotensive, antiaging, antiallergic, smooth muscle, arterial relaxant, antiasthmatic, antiobesity, respiratory disorder, constipation, convulsion, asthma, bronchitis, intestinal disorders, burning sensation, insomnia , angina , epilepsy , antiglaucoma and cancer **(Verma et al 2012)**. This is the first study on *C. forskolii* in Egypt because the absence of this plant in the Egyptian flora so, this study aimed to introducing this plant to Egypt because of its medicinal importance including in its effective natural material (forskolin). Therefore, there is need to produce large scale from a limited source of plant by using micro propagation as biotechnology tool to conserve this plant.

MATERIALS AND METHODS

1. Explant collection and preparation

Coleus forskolii seedlings came from its native origin in Thailand at June 2013 and were put in the green house in the Desert Research Center, El-Matareya, Egypt.

2. Explant sterilization

Surface sterilization with mercuric chloride with concentrations (0.1 g/L) for15 minutes was carried out under complete aseptic condition in the laminar air flow hood to avoid the explant contamination with bacteria or fungi after culturing in the media. ***(Sreedevi et al; 2013)***

3. Basic nutrient medium and culture condition (MS)

The sterilized explants (stem node section from 2 to 3 cm long) were inoculated in sterilized solid basal medium **Murashige and Skoog's (MS, 1962).** The MS medium was supplemented with 30g/L (3%) sucrose, different concentrations from growth regulators were carried out.

Media were adjusted to pH 5.7, and then the solidified agent 7g/liter agar was added. Fifteen ml volumes of media were dispensed into 25x150 mm culture tubes or 30 ml volumes of large jars, then closed with polypropylene caps and autoclaved at 121°C at pressured of 1.1kg/cm² for 15 min.

The inoculation of sterilized explants is happened in aseptic condition in the laminar air flow hood, and then incubated in a growth room at a temperature of 25 ± 2°C under 8-16 hrs. photoperiod with light intensity of 2 Klux, provided by cool white light fluorescent tubes.

4. Effect of growth regulators on different growth stages of the explants

4. a. Establishment stage

The sterilized explants were inoculated in sterilized solid (MS) media containing 3% sucrose, various concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) of cytokinin Benzyle Adenine (BA) in 7g/liter agar each. Explants were cultured in fifteen ml volumes of media were dispensed into 25×150 mm culture tubes or 30ml into 350 ml jar and each treatment was repeated three times. The experiment was carried out in completely randomized design with three replicates.

4. b. Multiplication stage

Established shoots were subjected to be multiplied on MS medium supplemented with various concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) of cytokinin (BA), Kinetin (KIN) and Thidiazuron (TDZ). in 7g/liter agar each. The best medium was put in large jar to obtain stock materials to be used for the following experiments ***(*Dube et al 2011).**

4. c. Rooting stage

After *in vitro* regenerated shoots (5-6 cm long), shoots were transferred in sterilized solid 1/2 Murashige and Skoog's (MS) media supplemented with different concentrations (0.5, 1.0, 1.5 and 2.0mg/L) of auxins (indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) to initiate root formation. Mean number and length (cm) of root / explant were recorded after 60 days.

4. d. Acclimatization stage

Rooted shoots were washed from medium residue and soaked in 1.5g/l fungicide solution (Benlate) for three minutes. Mixture of garden soil, vermiculate and sand; (2:1:1v/v/v), respectively, was autoclaved at121°C at pressured of 1.1kg/cm² for 15 min, then was put in pots. Pots were covered with transparent polyethylene bags, and put in the green house. After 2 weeks, a small cut was made in the transparent bags for aeration and the data of survival was recorded. After 30 days, the transparent bags were removed and the survival % was recorded

5. Statistical analysis

Data of propagation parameters were statistical analyzed by using Model GLM of SAS software version (9.0) **(SAS, 1998).** The effects of growth regulators on the different growth stages of *Coleus forskolii* were tested. Duncan Multiple Range test was used to test the level of significance among the means **(Snedecor and Cochran, 1989).**

RESULTS AND DISCUSSION

Effect of growth regulators on the different growth stages of *Coleus forskolii*

In this study, the various concentrations from BA, TDZ or KIN as cytokinin were applied for shoot induction and multiplication of *Coleus forskolii*.

In vitro establishment of *C.forskolii* by using stem node sections is present in **Table (1).** Data showed the effect of various concentrations from BA, TDZ or KIN compared with the control medium without any growth regulators on the multiplication of *C. forskolii* .

From the data in **Table (1),** it was clear that the mean number of axillary shoots per explant of *C. forskolii* reached the highest value and highly significant (P< 0.01) (6.19) on MS medium containing 2mg/L TDZ followed by 1.5mg/L TDZ at the value (5.72), when compared with the control value (3.03). It was found that when used less concentrations of TDZ, the mean number of axillary shoots per explant decrease.

Multiplication was an increase of organs which can be whole plant; the increase was achieved by inducing axillary shoot initiation **(Murashige, 1974).** The stage was repeated at regular intervals to produce large-scale shoot multiplication to be commercially useful **(Smith and Murashige, 1970).**

TDZ is a substituted phenylurea (N-phenyl-N-1,2,3-thiadiazol-5-ylurea) it was used as a plant growth regulator to stimulate high rate of axillary shoot spread in many woody plant species and a synthetic herbicide **(Malik and Saxena, 1992)**. Thidiazuron released the lateral bud dormancy and stimulated shoot formation in a wide variety of plant species **(Fiola et al 1990; Malik and Saxena, 1992)**. It is considered more potent than most of the commonly used cytokinin **(Huetteman and Preece, 1993).** According to **Capelle et al (1983**), TDZ directly promoted growth because of its special biological activities that is similar to that of an N- substituted cytokinin or an endogenous cytokinin accumulation and synthesis may be induce. Thidiazuron (TDZ) has gained an attention for long time due to its important role in plant cell and tissue culture. Many of biological events in cells are enhanced by TDZ when morphogenic events were induced by application of TDZ. Other reports said that endogenous plant growth regulators may be modified by TDZ, either directly or indirectly and produces reactions in cell or tissue, necessary for its division or regeneration. Also, it can make modification in cell membrane, nutrient absorption, energy levels, transport and assimilation **(Guo et al 2011)**.

In vitro multiplication of *Achillea millefolium* with different concentration of TDZ, 1mg/L concentration gave the best number of shoots /explants **(Alvarenga et al 2015)**.

The highest value of mean length was highly significant (P< 0.01) 6.44± 0.310 cm on MS medium containing 1mg/L KIN followed by 0.5mg/L KIN 5.20±0.172 cm**,** when compared with the control value 4.81±0.470 cm**.** It was found that when different concentrations of KIN were used, the mean length of the shoots fluctuated. While, **(Sahai and Shahzad, 2010)** recorded that *In vitro* propagation of *C.forskohlii* gave the highest mean of shoot length at MS media supplement with 5.0µM/L KIN**. Dakah et al (2014)** found that 1 mg/L KIN concentration gave the best shoot length for the medicinal plant *Ziziphora tenuior* L. combined with 0.1 mg/L of NAA, but when the concentration of KIN increased up to 1.5 mg/L it led to the decrease in length 6.17± 0.072.

Table 1. The Effect of different concentrations of growth regulators (BA, Kin and TDZ) on the mean number and length of axillary shoots/ explant of *Coleus forskohlii*.

Growth regulator	Concentration (mg/L)	Mean no. of axillary shoots/explant			Mean length of axillary shoots (cm)/explant		
		Mean	± SE		Mean	± SE	
Control	**-**	3.03	**±**0.3604	F	4.81	**±**0.4703	B
BA	0.5	3.33	±0.1667	F	3.91	±0.2022	Bcd
	1	4.02	±0.1190	Def	3.09	±0.5448	De
	1.5	5.56	±1.0567	Abc	1.95	±0.0917	Ef
	2	4.38	±0.1986	cdef	0.71	±0.1100	F
KIN	0.5	4.95	±0.1507	abcd	5.20	±0.1723	Ab
	1	3.22	±0.2233	f	6.44	±0.3100	A
	1.5	4.69	±0.4719	bcde	4.20	±0.6185	Bcd
	2	3.42	±0.2205	ef	4.55	±0.6431	Bc
TDZ	0.5	3.64	±0.2421	ef	4.53	±0.1542	Bc
	1	3.56	±0.3521	ef	4.11	±0.2862	Bcd
	1.5	5.72	±0.4338	ab	4.61	±0.6343	Bc
	2	6.19	±0.5729	a	3.35	±0.3395	Cd
S.O.V		**			**		

Means with the same letter in the same column are not significantly different.
S.O.V: source of variance **: P> 0.01 is highly significant.

2. Rooting stage

The main aim of this stage was to encourage the root formation and elongation.

In the present study, the control did not respond to form roots. While the different concentrations of NAA and IBA as auxins induced root induction. The mean number of roots per explant of *C. forskolii* reached the highest value 30.00 ± 0.577 with significant (P< 0.01) on 1/2 MS medium containing 0.5mg/L IBA followed by 2mg/L IBA 25.67±2.728, While the lowest value was 11.80±3.023 on 1/2 MS medium containing 1.5mg/L NAA.

Thaniarasu et al (2015) were excised and placed shoots (4-6 cm) of *Plectranthus bourneae* on half strength MS supplemented with different concentrations of NAA, IBA and IAA for root induction± achieved well-developed roots within 20 days. It was found that IBA was the most effective for root induction. These results were confirmed by the previous findings of *Trichosanthes dioica* (**Awal et al 2005**) **and** (**Rajani and Patil, 2009**) who reported that IBA is the best auxin for root induction and development of ginger.

The highest value showed mean length of roots was 3.93±0.413cm on 1/2 MS medium containing 0.5mg/L IBA followed by 1.5mg/L IBA 2.88±0.861 cm, while the lowest value was significant (P< 0.01) 1.07±0.195 cm on1/2 MS medium containing 2mg/L IBA. It is noticed that the mean roots length will fluctuated in different concentrations as in **Table (2).** While, **(Sahai and Shahzad 2010)** found that the roots of *Coleus forskohlii* gave the highest mean length with ½ strength MS media supplied with 2.0µM/L IBA.

Mean length of shoots (cm) recorded the highly significant (P< 0.01) value at 11.8±0.860 cm on 1/2 MS medium containing 2mg/l NAA followed by 1.5mg/L NAA 11.4±0.872 cm ,while the lowest value was 7.50 ±0.645 on 1/2 MS medium containing 1mg/L IBA. It is noticed that when the concentration of NAA decreased, the mean length number of shoots will decrease as in **Table (2).**

The rooting percentage recorded the highest value 19.4% on 1/2 MS medium containing 0.5mg/L IBA followed by 2mg/L IBA 16.6%, while the lowest value was 7.6% on 1/2 MS medium containing 1.5mg/L NAA. The trend of increasing rooting % was correlated to the decrease in the concentration of IBA.

Table 2. Effects of two growth regulators (NAA and IBA) on the mean number of roots/ explant, length of roots, length shoots and rooting% of *Coleus forskohlii*.

Growth regulator	Concentration (mg/L)	Mean No. of roots/explant		Mean length of roots (cm) / explant		Mean length of shoots (cm) /explant			Rooting %
		Mean	± SE	Mean	± SE	Mean	± SE		
Control		0.0		0.0		0.0			**0.0**
NAA	**0.5**	12.00	±1.000 c	1.22	±0.130 b	8.67	±0.333	dc	**7.7**
	1	17.67	±2.333 bc	1.76	±0.891 b	9.67	±0.333	bc	**11.4**
	1.5	11.80	±3.023 c	2.86	±0.364 ab	11.4	±0.872	ab	**7.6**
	2	13.00	±3.146 c	2.58	±0.275 ab	**11.8**	**±0.860**	**a**	**8.4**
IBA	**0.5**	**30.00**	**±0.577 a**	**3.93**	**±0.413 a**	8.33	±0.333	dc	**19.4**
	1	22.25	±2.287abc	2.17	±0.281 ab	7.50	±0.645	d	**14.4**
	1.5	22.50	±4.628abc	2.88	±0.861 ab	10.0	±0.408	abc	**14.5**
	2	25.67	±2.728 ab	1.07	±0.195 b	8.67	±0.333	dc	**16.6**
S.O.V		**		*		**			

Means with the same letter in the same column are not significantly different S.O.V: Source of Variance
*: P< 0.05 is significant. **: P< 0.01 is highly significant. n: non-significant.

3. Acclimatization stage

The survived acclimatized plants were 93% after two weeks from transferring rooted plantlets of *Coleus forskolii* on a mixture of soil, vermiculite and sand (2:1:1: v/v/v). After a month from transferring the rooted plantlets, the percentage of surviving was 83%. In rooting stage, the plant was prepared to be transferred from the closed artificial environment to the open environment **(Hartmann and Kester, 1983). Thaniarasu et al. 2015** calculated the percentage of *Plectranthus bourneae* survival plants after two months, the rooted plantlets were successfully acclimatized with around 80% survival rate

CONCLUSION

The mean number of axillary shoots per explant of *Coleus forskolii* reached the highest value on MS medium containing 2mg/L TDZ. The highest value of mean length of axillary shoots was on MS medium containing 1mg/L KIN. The mean number of roots / explant of *Coleus forskolii* reached the highest value was on 1/2 MS medium containing 0.5mg/L IBA. While, the highest value of mean Length of shoots (cm) was on 1/2 MS medium containing 2mg/l NAA. The survived acclimatized plants of rooted plants of *Coleus forskolii* was 93% after two weeks and 83% after one month. Moreover, this is the first report of multiple shoots *in vitro* formation in nodal segments obtained from seedling of coleus .

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الموجـــــز

يعتبر نبات بلكترنثص باربـاتس اندروس (*كوليس فورسكولى*) هـو واحـد مـن أهـم الأنـواع التابعـة لجـنس *بلكترنثص* (*كوليس*) الذي ينتمي إلى العائلة الشفوية ، حيث لـه استخدامات طبية تقليدية واسعة في الهند. ويعتبر *كوليس فورسكولى* هو المصدر الوحيد لمادة الفورسكولين؛ وهو مركب واسع الاهمية فى االصناعات الدوائية، نظرا لعدم وجود نبات *كوليس فورسكولى* في الفلورا المصرية، بالإضافة لعدم وجود دراسات سابقة حول هذا النبات في مصر. لذلك، هناك حاجة الى الحفاظ على المصدر من هذا النبات من خلال استخدام التكنولوجية الحيوية. تم احضار شتلات *كوليس فورسكولى* من موطنها الأصلي بتايلاند في يونيو 2013 وتم وضعها في صوبة مركز بحوث الصحراءلإنشاء بروتكول للإكثار المعملى لنبات الكوليس. وقد أجريت الدراسة على تأثير منظمات النمو(سيتوكينين والاكسين) على مراحل الإكثار الدقيق المختلفة للمنفصلات النباتية. فى مرحلة التضاعف، نمت الافرع على وسط MS ذات تركيزات مختلفة (0.5، 1.0، 1.5 و 2.0 ملجم / لتر) من السيتوكينينات (BA، KIN و TDZ). حيث بلغ متوسط عدد الافرع الجانبية لكل منفصل نباتى من *الكوليس فورسكولى* أعلى قيمة 6.19 ±0.573 على وسط MS يحتوي على تركيز 2 مجم/لتر من TDZ. بينما كانت أعلى قيمة لمتوسط الطول 6.44 ± 0.310 سم على وسط MS يحتوي على تركيز 1 ملجم / لتر KIN. وبلغ أعلى متوسط فى عدد الجذور وطولها لكل منفصل نباتى من نبات *الكوليس فورسكولى* 30.00 ± 0.577 و الطول 11.8 ± 0.860 سم على التوالي ، على 2/1 MS التي تحتوي على تركيز 0.5 ملجم / لتر IBA. في حين أن أعلى قيمة لطول النبيته كانت 11.8 + 0.860 سم في وسط 2/1 MS تحتوي على تركيز 2 مجم/لتر NAA. تمت أقلمة النبيتات الناتجة بنسبة نجاح وصلت الى 83٪ فى الصوبه بعد أربعة أسابيع حيث نمت بشكل طبيعى فى خليط من التربة المعقمة ، من التربة و الفيرميكيوليت والرمل بنسبة (2: 1: 1 / v / v / v). ان هذا البروتوكول يعتبر وسيلة فعالة وقليلة التكلفة فى اكثار وحفظ وتبادل المصادر النباتية لنبات الكوليس.

الكلمـــات الدالـــة: بلكتـرنثص باربـاتس، كوليـوس فورسكولي، الإكثار الدقيق،التأقلم، مصر.

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