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THE EFFECT OF PLANT GROWTH REGULATORS ON SHOOT FORMATION FROM CORM EXPLANT OF *HEDYCHIUM CORONARIUM*

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ABSTRACT: This study was conducted for a micropropagation of *Hedychium coronarium*, an important medicinal herb, through sprouted rhizome buds. Rhizome buds were cultured on two different media; Murashige and Skoog medium (MS) and Gamborg medium (B_5) without any growth regulators during the establishment stage. B_5 medium gave a higher percentage of shoot formation 97.30% corresponding in shoot number and length comparing with MS medium. For shoot proliferation the micro shoots were placed on the B_5 medium with two different cytokinins, kinitein (kin) and benzyl adenine (BA) at the concentrations of 0.0, 0.5, 1.0, 2.0 and 4.0 mg/l. Four mg/l of BA led to obtain high value of shoot production. For root formation, the produced shoots were placed on B_5 medium plus different concentrations of two types of auxins IBA and IAA at the concentrations of 0.5, 1.0, 2.0 and 3.0 mg/l for each to study the best auxin and concentration which encouraged root formation (number and length). IBA proved a significantly efficient than IAA and encouraged root formation for both root number and root length. IBA at 3.0 mg/l gave the highest average of root number and length. All good rooted plants were cultured into pots containing vermiculite + peatmoss (1:1) in greenhouse, with 82% of survival percentage.

Key words: *Hedychium coronarium*, shoot formation, corm explant, plant growth regulators.

INTRODUCTION

Hedychium coronarium J. Koeniga 'white ginger lily' is an, aromatic rhizomatous plant, belongs to *Zingiberaceae* family, in tropical and sub-tropical Asia (Parida and Sanghanitra, 2019). Its extracts used for headache, sharp pain, inflammation and skin diseases due to rheumatism in traditional medicine (Kiem *et al*., 2011). It is also an analgesic, neuropharma-ecological, anti-inflammatory, antimicrobial and cytotoxic activities (Abdul Aziz *et al*., 2009; Priteshm *et al*., 2011; Sadhana *et al*., 2012). Essential oil extracted from leaves, flowers and rhizome has acaricidal properties, molluscicidal activity, antimicrobial activities, antifungal, anti-inflammatory, antibacterial, analgesic and potent inhibitory action. A reliable *in vitro* propagation study was required to obtain large number of shoots with a little cost efficient in short time. Most important factors for a successful micropropagation, is selection of explant source, genetic stability and the regeneration influence, for micropropagated plants (Larkin *et al*., 1989). Selection of media and plant growth regulator type and concentration is necessary for the success in the technology of tissue culture and choice of medium is conducted by the purpose from tissue culture, which

employed for plant species (Gamborg and Phillips, 1995).

The recent study aimed to establish a methodology for direct *in vitro* propagation of *Hedychium coronarium* to have a high-number of shoot initiation and regeneration of the plant directly.

MATERIALS AND METHODS

This work was carried out in Applied Research Center of Medicinal Plants and Natural Products (Tissue Culture Lab.), National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

Plant material:

The healthy rhizomes of *Hedycium coronarium* were collected from private farm in Cairo and maintained inside the greenhouse of the Applied Research Center of Medicinal Plants and Natural Products, the rhizomes were kept in a peatmoss and sand (1:1) for sprouting. Sprouted buds initiated within 4 weeks after that it transferred to the tissue culture laboratory of Applied Research Center of Medicinal Plants and Natural Products.

Explants preparation:

The rhizome with sprouted buds was cut to small pieces (0.5–1.0 cm) and all of the sheaths of the rhizomes were removed to be used as explants. The rhizome with sprouted was cleaned by tap water to remove all the residue of sand and peat after that it washed with tap water. Surface sterilization was done by using 0.1 % (w/v) aqueous solution of mercuric chloride ($HgCl_2$) for 10 min inside the laminar air flow, followed by washing with sterile distilled water for three times.

Treatments:

1. Shoot proliferation:

a. Effect of medium type:

Two nutrient media were tried, MS medium (Murashige and Skoog, 1962) and B_5 medium (Gamborg *et al.*, 1976) without any growth regulators. The pH of the media was adjusted at 5.7±0.1 before addition of the agar. The media distributed into jars each gar contained 50 ml after that it sterilized by autoclaving at 121 °C for 15 min. The design of the experiment was a complete randomize into three replicates each replicate consisted of 12 jars and one sprouted rhizome bud in each jar. The recorded data was taken after 30 days from culture to calculating the morphogenetic characters percentage.

b. Effect of cytokinins:

For shoot proliferation, the mini shoots were placed on the B_5 medium with two different types of cytokinins, kinitein (kin) and benzyl adenine (BA) at concentrations of 0.0, 0.5, 1.0, 2.0 and 4.0 mg/l were examined. Number and length of shoots were recorded after 30 days.

2. Rooting stage:

Uniform shoots (5 cm in length) which obtained from the previous experiments was transferred to jars containing 50 ml medium consisted of B_5 medium plus different concentrations of two types of auxins IBA and IAA at concentrations of 0.0, 0.5, 1.0, 2.0 and 3.0 mg/l for each to study the best auxin and concentration which encouraged root formation (number and length). Cultured jars were kept in constant temperature 25±2° C under fluorescent light of 1500 lux for 16 hours photoperiod.

3. Acclimatization:

The most important stage in tissue culture is transferring plantlets from the aseptic cultural environment to the free-living environment and ultimately to the field. The *in vitro* derived plantlets about 10 cm in height were washed with current tap water, and then disinfected by immersion in fungicide solution (Previcur N, 72.2%) and transferred to plastic pots (7.5 x 10.5 cm) containing peatmoss + vermiculite (1:1).

The pots were transferred to the greenhouse and covered with polyethylene sheets to maintain high relative humidity around the plants. In addition, spraying with water under the plastic sheets was carried out

daily, while the irrigation took place 3 times a week. Surviving plants were recorded after 4 weeks from transplanting.

Statistical analysis:

The experiments design was a completely randomized design. The recorded data subjected to statistical analysis of variance and all the means compared by using the L.S.D at 5% level of significance due to Snedicor and Cochran (1980).

RESULTS AND DISCUSSION

1. Shoot proliferation:

a. Effect of type medium:

A significant difference was found between the two different basal media for morphogenesis. B_5 medium was favorable for shoot formation (Table, 1 and Fig., 1). B_5 media was the best medium for shoot formation because of the high contains of thiamine concentration (vitamin B1), which gives a facilitated formation of shoots *in vitro* and it contained a low salt strength of nitrate (KNO_3) (Fatima *et al*., 2015).

b. Effect of cytokinins:

The addition of the two types of cytokinins from 0.5 to 4.0 mg/l to the media gave 100% of direct shoots formation. Four mg/l from both of two cytokinins led to obtain high value of shoots production and form callus on the base of shoots beside shoots production. The cytokinins has a high effect on the development of the plantlet, according to, the multiplication rate, the promotion of cell division expansion and the regulation of shoot formation as mentioned by Ashraf *et al*. (2013), similar results were obtained on *Chlorophytum borivilianum* and Verma and Bansal (2014) on *Hedychium coronarium*. George *et al*. (2008) who stated that presence of cytokinins into the media led to activated of axillary's buds, depressed of apical dominance which led to increase of the proliferation. Raising the concentration of cytokinins increased the proliferation of buds and the multi apexes plantlets.

Table 1. Effect of MS and B_5 media on shoot proliferation of *Hedychium coronarium*.

Medium	Shoot formation (%)	Number of shoots per explant	Shoot length (cm)
MS	88.6	3.3	2.1
B_5	97.3	5.1	3.6
L.S.D at 5%	2.11	0.87	0.91

B_5 medium **MS medium**

Fig. 1. Initiation of growth after 4 weeks from cultured the explants on B_5 medium and MS medium.

The data cleared that increasing of the concentration for both cytokinins led to increase the proliferated number of shoots while, it made an inhibitory effect on shoot elongation. The shortest shoots were recorded by 4.0 mg/l from both of the two cytokinins. The reduction in shoot elongation on the high level of two cytokinins may be due to that 4.0 mg/l produced callus on the base of shoots which was undesirable character because it is led to slow down the nutrient uptake from the medium which causing a less of shoot elongation (Barghchi and Alderson, 1993).

The data cleared that, BA has a significant effect on shoot multiplication but it is gives less effect on shoot elongation when it compared with Kin, so that BA increased the number of shoots but Kin promoted shoot elongation as shown in Fig. (2). Same results were obtained by Srivastava and Joshi (2009), they mentioned that BA alone is more effective than kin for shoot multiplication of *Portulaca grandiflora*. The effect of BA on the formation of shoots and the multiplication rate was high, but it gives a less effect on shoot elongation when it compared with kin for the micropropagation of *C. borivilianum*. Goba (2005) reported that the production of endogenous ethylene from the explants in the tissue culture containers led to less elongation. Ozden-Tokatli *et al*. (2005) and Saha *et al*. (2007) noted that high ethylene amount was released in medium containing BA in Bottle Gourd and Pistachio, respectively. Saha *et al*. (2007) noticed that the effect of Kin on shoot elongation in Bottle Gourd illustrated the critical inhibitory of the production of ethylene of *C. borivilianum*, the enhancing effect of Kin on shoot elongation due to its inhibitory effect on the released of ethylene in the medium.

2. Rooting stage:

The estimation of the percentage of root formation of shoot cultures were affected by IBA and IAA, the data cleared that the two studied levels of auxin gave rise of rooting percentage to 100% as shown in Fig. (3), referring the effect of IBA and IAA. IBA proved a significant efficient than IAA and encouraged root formation for both root number and root length. IBA at 3.0 mg/l gave the highest average of root number and length. Our result is supported by Scott and Ellen (1990), who suggested that IBA is more effective than any of other auxins because it is less quickly destroyed by autoclaving or light, it is escapes the attack of the endogenous degrading enzyme system and exhibits long tissue half-life such as IAA oxidase. Zaerr and Mapes (1982) mentioned that degradation of IBA facilitates, its localization and the slow movement was due

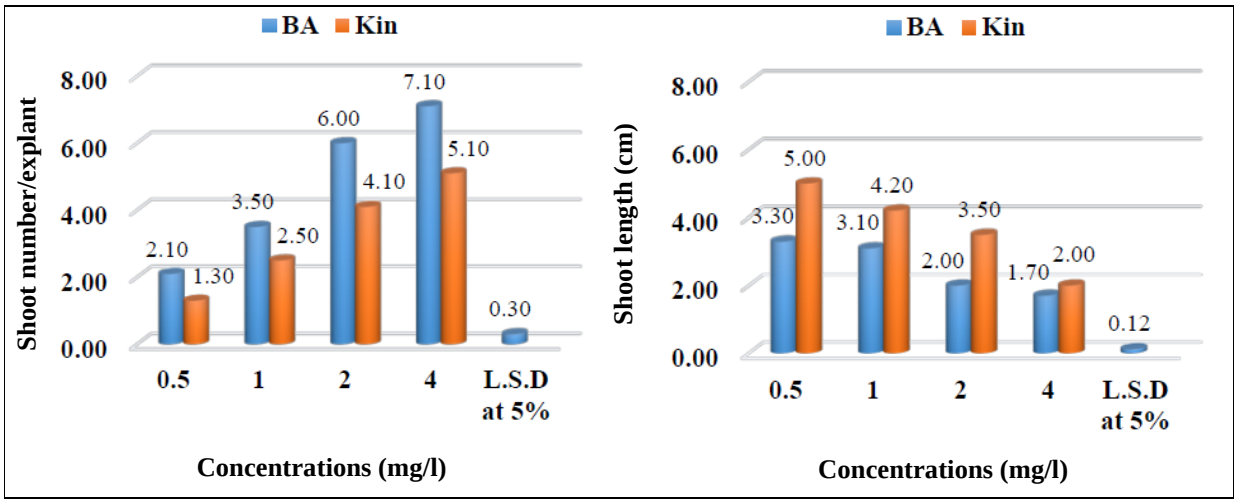

Fig. 2. Effect of both cytokinins (BA and Kin) on shoot number and length production of *Hedycium coronarium*.

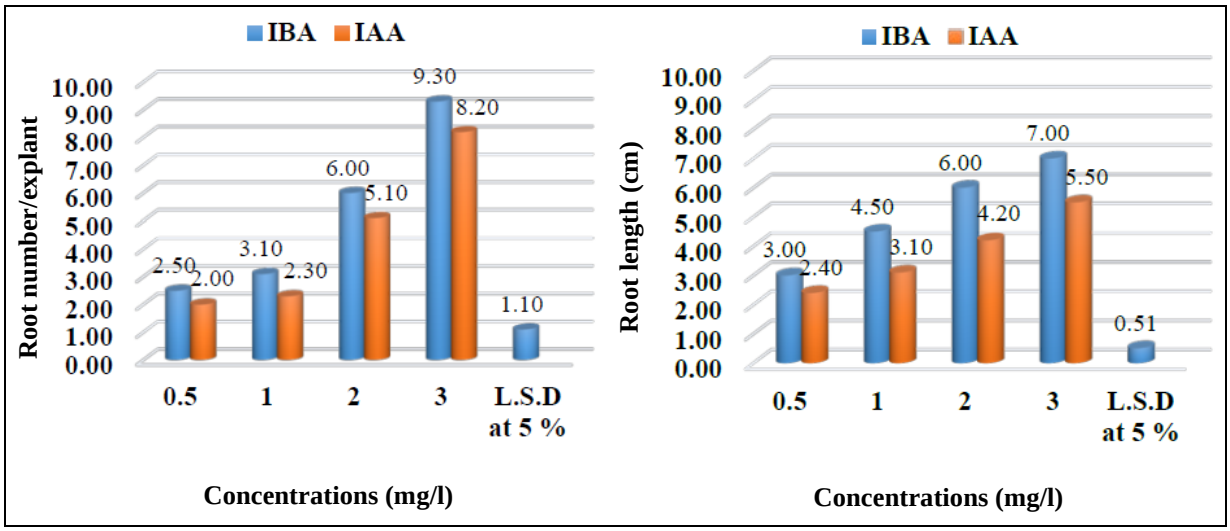

Fig. 3. Effect of IBA and IAA on root number and length during rooting stage *in vitro* shoots of *Hedycium coronarium*.

to the better function for inducing roots. Martin (2002) suggested that the promote effect of auxin for induction of roots is opposed by the inhibitory effect of auxin in inducing ethylene. Since, IAA is more effective than IBA in inducing ethylene production.

3. Acclimatization:

All good rooted plantlets were transferred to pots containing vermiculite + peatmoss (1:1) was placed into the greenhouse with 82% of survival percentage. All the micropropagated plants grew very well in the greenhouse. After acclimatization in the greenhouse all plants were placed into the field of the Applied Research Center of Medicinal Plants and Natural Products as shown in (Fig. 4).

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Fig. 4. The plantlet of *Hedycium coronarium* after transplanting in the greenhouse of the Applied Research Center of Medicinal Plants and Natural Products.

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تأثير منظمات النمو على إحداث تكوين بادرات من كورمات الهيديكيوم

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أجريت هذه الدراسة للإكثار الدقيق لنبات الهيديكيوم ذوالاهمية الطبية عبر إستخدام البراعم الموجودة على الريزومات. تمت زراعة هذه البراعم على بيئتين للنمو مختلفتين هما بيئة موراشيجى وسكوج وبيئة جامبورج بدون إضافة أى منظمات نمو خلال مرحلة التأسيس. أعطت بيئة جامبورج أعلى نسبة من تكوين البادرات وصلت الى٩٧,٣٪ ممثلة في عدد البادرات وطول البادرات المتكونة عند مقابلتها مع النسبة المتكونه على بيئة موراشيجى وسكوج. لتضاعف إكثار وإنتشار البادرات تمت زراعة البراعم الصغيرة المتكونه على بيئة جامبورج المضاف إليها نوعين مختلفين من السيتوكينين هما الكينيتين والبنزيل أدينين بتركيزات ٠,٠ ، ٠,٥ ، ١,٠ ، ٢,٠ و٤,٠ مليجرام في اللتر. أدى إستخدام تركيز٤,٠ مليجرام في اللتر من البنزيل أدينين إلى الحصول على أعلى معدل من البادرات المتحصل عليها (المنتجة). تكوين الجذور على البادرات المنتجة حدث عند زراعة البادرات على بيئة جامبورج المضاف إليها نوعين مختلفين من الأوكسينات هما إندول حامض البيوتريك وإندول حامض الخليك بتركيزات ٠,٥ ، ١,٠ ، ٢,٠ و٣,٠ مليجرام في اللتر لكل منهما لتحديد ودراسة أفضل أوكسين وأفضل تركيز لكلا من نوعي الأوكسين المستخدمين والذي يشجع على تكوين الجذور على البادرات المتكونه من ناحية عدد وطول الجذور. ثبت أن إندول حامض البيوتريك هو الاكثر معنويه وفاعليه من ناحية تشجيعه على تكوين جذور أكثر عددا وطولا. تركيز ٣,٠ مليجرام فى اللتر أعطى أعلى معدل من كل من عدد وطول الجذور. جميع النباتات التى تكونت جذور عليها تمت زراعتها داخل أصص تحتوى على خلطة من الفيرميكوليت والبيت موس بنسبة ١:١ حجماً داخل صوبة الاقلمة ووصلت نسبة النباتات الحية التى نجحت اقلمتها إلى ٨٢٪.