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UTILIZATION OF *ALOE VERA* GEL AS GROWTH ENHANCER ON MICROPROPAGATION OF *EUCALYPTUS CITRIODORA* HOOK PLANT

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ABSTRACT: This study aimed to examine the influence of *Aloe vera* gel at different concentrations as growth enhancer on *Eucalyptus citriodora*, Hook micropropagation. The experiments were conducted from April 1st 2019 until January 15th, 2020 at Plant Tissue Culture Laboratory of Prof. Dr. Abd El-Fatah H. Belal, Fac. Environ. Agric. Sci., Arish Univ. The obtained results presented that, soaking *Eucalyptus citriodora* explants on (Clorox)® solution at 20% (v/v) for 20 minutes recorded the highest survival percentage of explants (87.33%) on establishment stage. Furthermore, addition of BA at 1 mg l^{-1} in combination with NAA at 0.1 mg l^{-1} recorded the highest shoot number/explant, shoot length (cm) and leaf number/shoot (7.33, 4.66 and 9.50, respectively) on multiplication. Also, the maximum shoot number/explant, shoot length (cm) and leaf number/shoot (9.33, 7.00 and 11.50, respectively) were achieved with addition of *Aloe vera* gel at 20 m l^{-1}. Moreover, Murashige Skoog (MS) medium with IBA addition at 1.0 mg l^{-1} recorded the maximum growth of *Eucalyptus citriodora* during rooting stage. i.e., shoot number/explant, shoot length (cm), leaf number/shoot, rooting percentage, root number/explant and root length (cm) (3.44, 8.13, 12.86, 99, 3.27 and 5.07, respectively). Finally, rooted shoots were highly successful (above 80%) on soil mixture contains peatmoss and sand at a rate of (1:1, v:v).

Key words: *Eucalyptus citriodora,* BA*, Aloe vera* gel*,* IBA.

INTRODUCTION

Eucalyptus citriodora Hook, family, Myrtaceae considered one of the most eucalyptus famous genus, commonly known as lemon-scented gum or lemon-scented eucalyptus is an retains green leaves throughout the year with 24-40 m height (max. 50 m); leaves are strongly have aromatic scent like lemon and fruits like urn (Chen and Craven, 2007). *Eucalyptus citriodora* Hook wood used in producing pulp which used for paper production, house building and fixed oil which widely used in manufacture of perfumes, toiletries and disinfectants. Oil of *Eucalyptus citriodora* Hook contained citronellal which used for the production of menthol and citronellol. The leaves have own antiseptic properties and treatment various skin diseases, antibacterial, antifungal, acaricidal and insect expeller activities (Low *et al*., 1974; Warrag *et al*., 1990; Ramezani *et al*., 2002; Luqman *et al*., 2008 and Verbel *et al*., 2009).

For large scale, the *Eucalyptus* genus can propagate by seeds with about 50% successful with high variations in seedlings characteristics (Zobel, 1993). Moreover, it is very difficult to propagate by traditional methods such as air-layering, cuttings and grafting which face a problem of incompatibility between scion and rootstocks

(Verma *et al.,* 2013 and Bryant and Trueman, 2015).

Synthetic growth regulators have been used in tissue culture and agriculture for a long time. Synthetic growth hormones have many disadvantages like, so expensive, toxicity risk in plants, humans and animals so, finding alternative compounds as a replacement of traditional growth regulators is becoming an important demand (Cutler and Schneider, 1990).

Natural extracts from leaves of *Aloe vera* plants considered alternative compounds rich with natural plant hormones which can be used as growth enhancer. *Aloe vera* plants are a succulent herb which grows in many countries (Pandey and Singh, 2016). Parenchyma tissue of *Aloe vera* leaves contains liquid of yellow latex and gel which contains water at rate 99.5%, other 0.5 – 1% performed solid material which contains many other compounds like, water-soluble and fat-soluble, auxins, amino acids, vitamins, minerals, polysaccharides, enzymes, phenolic compounds and organic acids (Ni *et al.,* 2004; Boudreau and Beland, 2006; Surjushe *et al.,* 2008; Chatterjee *et al.,* 2013 and Raman *et al.,* 2013).

This study aimed to examine the effect of *Aloe vera* gel at different concentrations as growth enhancer on *Eucalyptus citriodora*, Hook micropropagation.

MATERIALS AND METHODS

Establishment stage:

Plant material:

Plant materials were obtained from cultivated *Eucalyptus citriodora* trees (2 years old) cultured on the Experimental Farm of Fac. Environ. Agric. Sci., Arish Univ., North Sinai, Egypt. The experiments were conducted from April 1st, 2019 until January 15th, 2020 at Plant Tissue Culture Laboratory of Prof. Dr. Abd El-Fatah H. Belal, Fac. Environ. Agric. Sci., Arish Univ.

Explants sterilization:

Eucalyptus citriodora shoot tips about 0.5-1.0 cm were washed by liquid soap for 10 minutes then washed with running tap water for 1 hour. After that explants were rinsed for 30 sec in ethyl alcohol (70%). Explants surface sterilized with different concentrations (5, 10, 15 and 20%) of sodium hypochlorite (Clorox)® for different durations (10, 15, 20, 25 min). Finally, explants were washed 3-5 times with sterilized distilled water.

Sterilized explants were cultured on MS-free medium (without growth regulators) with addition of 100 mg l^{-1} myo-inositol and 30 g l^{-1} sucrose (Murashige and Skoog, 1962). Medium pH was adjusted for 5.7-5.8 then adding agar at 8.0 g l^{-1}. Jars (60×120 mm) were used every jar filled with 50 ml of culture medium then jars were sterilized in autoclave at 121 °C and 1.1 kg cm^{-2} for 20 min.

Incubation of culture:

Culture Jars at all stages were incubated in growth room at temperature 25±2 ºC under 16 hrs artificial light with cool florescent light intensity of 2500 lux and 8 hrs dark/day.

Data recorded:

Survival percentage (%) of culture explants after 6 weeks was calculated as follows:

$$\frac{\text{Survival explants}}{\text{Total cultured explants}} \times 100$$

Stage of multiplication:

Effect of cytokinin types:

Nodal explants about (1-2 cm) from the previous experiment were cultured on MS-medium fortified with three cytokinins types; 6-benzyladenine (BA), kinetin (kin) and 2-isopentenyl adenine (2ip) at the rate of 1.0 mg l^{-1} compared with MS medium (control) with addition of naphthalene acetic acid (NAA) at 0.1 mg l^{-1} to examine the more effective cytokinin type.

Data recorded:

Six weeks later from culture date the following data were recorded: shoot

number/explant, main shoot length (cm) and leaf number/shoot.

Concentrations of *Aloe vera* gel:

From obtained results of pervious experiment MS-medium supplemented with 1mg l^{-1} 6-benzyladenine (BA) combined with 0.1 mg l^{-1} naphthalene acetic acid (NAA) were used with addition of *Aloe vera* gel concentrations at 0, 5, 10 and 20 ml l^{-1} to examine the best *Aloe vera* gel concentration which could record the highest multiplication rate.

The minerals in *Aloe vera* were analyzed at laboratories of Agriculture Research Center, Ministry of Agriculture, Giza, Egypt according to the standard procedures of Association of Official Analytical Chemists (1975) and Jackson (1973).

Aloe vera gel contained (mg/100 g F.W.) 75.14 N, 4.68 P, 54.32 K, 0.233 Fe, 32.00 Ca, 11.47 Mg, 0.021 Zn, 0.025 Mn and 46.14 Na.

Data recorded:

Six weeks later from culture date the following data were recorded: shoot number/explant, main shoot length (cm) and leaf number/shoot.

Rooting stage:

Auxins type and concentrations:

Before rooting stage shoots about (3-4 cm) were transferred to MS-free medium (without growth regulators) for 1 month to get rid of growth regulators effect from the previous experiment. Then shoots about (3-4 cm) were transferred to MS media fortified with naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA) or indole acetic acid (IAA) at 0, 0.5, 1.0 or 2.0 mg l^{-1} to determine the best auxin type which will increase root growth and formation.

Data recorded:

Shoot number/explant, shoot length (cm), leaf number/shoot, rooting percentage, main root number/ explant and root length (cm) were recorded after six weeks later from culture date.

Acclimatization stage:

Plantlets of *Eucalyptus citriodora* with high root formation and about 6-8 cm were transferred to plastic pots (9 × 7 cm) containing seven different soil mixtures as follows:

1. Peatmoss, vermiculite, perlite and sand at rate of (1: 1: 1: 1, v/v).
2. Peatmoss, vermiculite and sand at rate of 1: 1: 1, v/v.
3. Peatmoss and sand at a rate of 1:1, v/v.
4. Perlite, vermiculite and sand at rate of 1: 1: 1, v/v.
5. Perlite and sand at rate of 1:1, v/v.
6. Perlite and peatmoss at rate of 1:1, v/v.
7. Perlite and vermiculite at rate of 1:1, v/v.

Experimental design and statistical Analysis:

A completely randomized design (CRD) was used in this study with three replicates. Collected data were analyzed using MSTAT-C Statistical Software Package (Michigan State University, 1983). Duncan's multiple range test was used to compare variance between means (Duncan, 1955).

RESULTS AND DISCUSSION

Establishment stage:

Effect of different concentrations and durations of sodium hypochlorite (Clorox) solution on survival percentage of *Eucalyptus citriodora* explants:

Data presented in Fig. (1) clear that increasing both Clorox concentrations and duration significantly decrease the contamination percentage which enhancing the survival percentage. Moreover, data show that soaking *Eucalyptus citriodora* explants in (Clorox) solution at 20% (v/v) for 20 minutes recorded the highest survival percentage (87.33%) with null microbial contamination. On the other side low

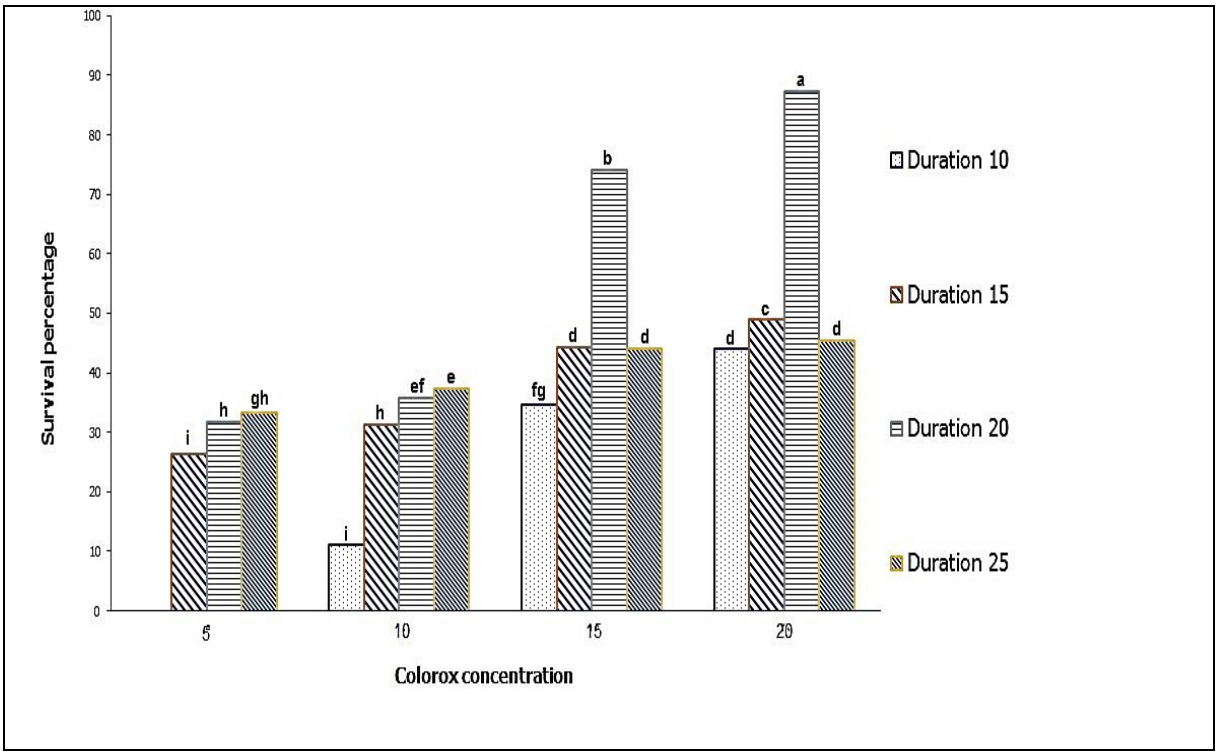

Fig. 1. Effect of different sodium hypochlorite (Clorox)® concentrations and duration on survival percentage of *Eucalyptus citriodora* explants.

concentration of Clorox® and low exposer time led to reduction on survival percentage due to the severity of microbial contamination. Our results were in agreement with Guanih *et al.* (2004) on *Dryobalanops lanceolata* Burck. The author's mentioned that, the lowest contamination percentage were observed when seeds cultured in 1/2 strength (MS) without seed coat after sterilization with 70% alcohol and 30% commercial Clorox for 5 minutes. Also, Hassan (2014) on *Cleome droserifolia* (Forssk.) Del. studied the effect of different concentrations of sodium hypochlorite (Clorox) 10, 15, 20 and 25 (v/v) for different durations (15, 20, 25and 30 min) on seeds sterilization. The obtained results showed the highest survival percentage (80%) achieved by using 20% (v/v) for 30 min. Moreover, Premjet et *al.* (2020) on *Melientha suavis* Pierre. reported that, sodium hypochlorite at 15% significantly recorded the highest survival rate of *M. suavis* leaf, young stem and shoot tip.

Multiplication stage:

Effect of BA, kin and 2ip at 1mgl^{-1}in combination with NAA at 0.1 mgl^{-1} on *Eucalyptus citriodora* growth during multiplication stage:

Results in Table (1) reveal that, addition of BA significantly increased the most growth parameters of *Eucalyptus citriodora* during multiplication stage. MS medium supplemented with BA at 1 mg l^{-1} combined with NAA at 0.1 mg l^{-1} recorded the maximum shoot number/explant, shoot length (cm) and leaf number/shoot (7.33, 4.66 and 9.50, respectively). These results are in the same way with those obtained by Saetiew *et al.* (2011) on *Pinguicula gigantean* who observed that, medium fortified with 2.0 mg/l BA and 0.1 mg/l NAA recorded the highest number of shoots after 8 weeks. Also, Abd El-Azeem *et al.* (2019) on *Eucalyptus citriodora* reported that the maximum shoots were observed by using nodal explants planted on MS medium fortified with 1.00 mg l^{-1} BA combined with 0.1 mg l^{-1} NAA.

Table 1. Effect of BA, kin and 2ip at 1 mg l^{-1} in combination with NAA at 0.1 mg l^{-1} on *Eucalyptus citriodora* growth during multiplication stage.

Cytokinin types	Shoot number /explant	Shoot length (cm)	Leaf number /shoot
Control	2.50 d	2.50 d	5.33 d
BA	7.33 a	4.66 a	9.50 a
Kin	4.50 b	3.00 b	7.66 b
2ip	3.66 c	2.75 c	6.50 c

Effect of different *Aloe vera* gel concentrations on *Eucalyptus citriodora* growth during multiplication stage:

The presented results in Table (2) & Fig. (2) show the effect of BA at 1 mg l^{-1}, NAA at 0.1 mg l^{-1} with different *Aloe vera* gel concentrations on *Eucalyptus citriodora* growth during multiplication stage. It is clear that increasing of *Aloe vera* gel concentrations significantly increase all *Eucalyptus citriodora* growth parameters comparing with control (*Aloe vera* gel-free). The maximum shoot number/explant shoot length (cm) and leaf number/shoot (9.33, 7.00 and 11.50, respectively) were achieved with addition of *Aloe vera* gel at 20 ml l^{-1}. The same trend was noticed by El Sherif (2017) on *Populus* trees, they reported that addition of *Aloe vera* leaves extract at 10 ml/l recorded the maximum values of *Populus* height and weight, shoot, leaf and root numbers and the root length, as well as mineral concentrations. The increase of *Eucalyptus citriodora* growth may be due to the nutrients and organic compounds on *Aloe vera* gel such as triglicerides, triterpenoid, gibberellin, potassium sorbate and salicylic acid (Hamman, 2008).

Rooting stage:

Effect of different auxins type and concentrations on *Eucalyptus citriodora* growth during rooting stage:

As shown in Table (3) & Fig. (2) that, IBA at the different concentrations under this study significantly increased growth of *Eucalyptus citriodora* during rooting stage. MS medium with IBA addition at 1.0 mg l^{-1} recorded the maximum growth of *Eucalyptus citriodora* during rooting stage. i.e., shoot number/explant, shoot length (cm), leaf number/shoot, rooting percentage, root number/explant and root length (3.44, 8.13, 12.86, 99, 3.27 and 5.07, respectively).

The previous results were in the same side with those suggested by Koriesh *et al.* (2003) they noticed that medium fortified with IBA compared with non-treated medium (control) enhanced root number and root length. Moreover, Karpov (2004) show that, the best rooting of microshoots of *Yucca aloifolia* was found on MS medium with addition indole-3-butyric acid at 1 mg l^{-1}.

Acclimatization stage:

From the illustrated results in Fig. (2, D) it is clear that, acclimatization of rooted shoots was highly successful (above 80%) on media culture mixture contains peatmoss and sand at a rate of (1:1v/v).

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Table 2. Effect of BA at 1mgl^{-1}, NAA at 0.1 mg l^{-1} with different *Aloe vera* gel concentrations on *Eucalyptus citriodora* growth during multiplication stage.

Aloe vera gel concentrations (ml l^{-1})	Shoot number /explant	Shoot length (cm)	Leaf number /shoot
Control	7.65 d	5.00 b	9.66 d
5	8.33 c	5.66 ab	10.22 c
10	8.66 b	6.33 ab	11.33 b
20	9.33 a	7.00 a	11.50 a

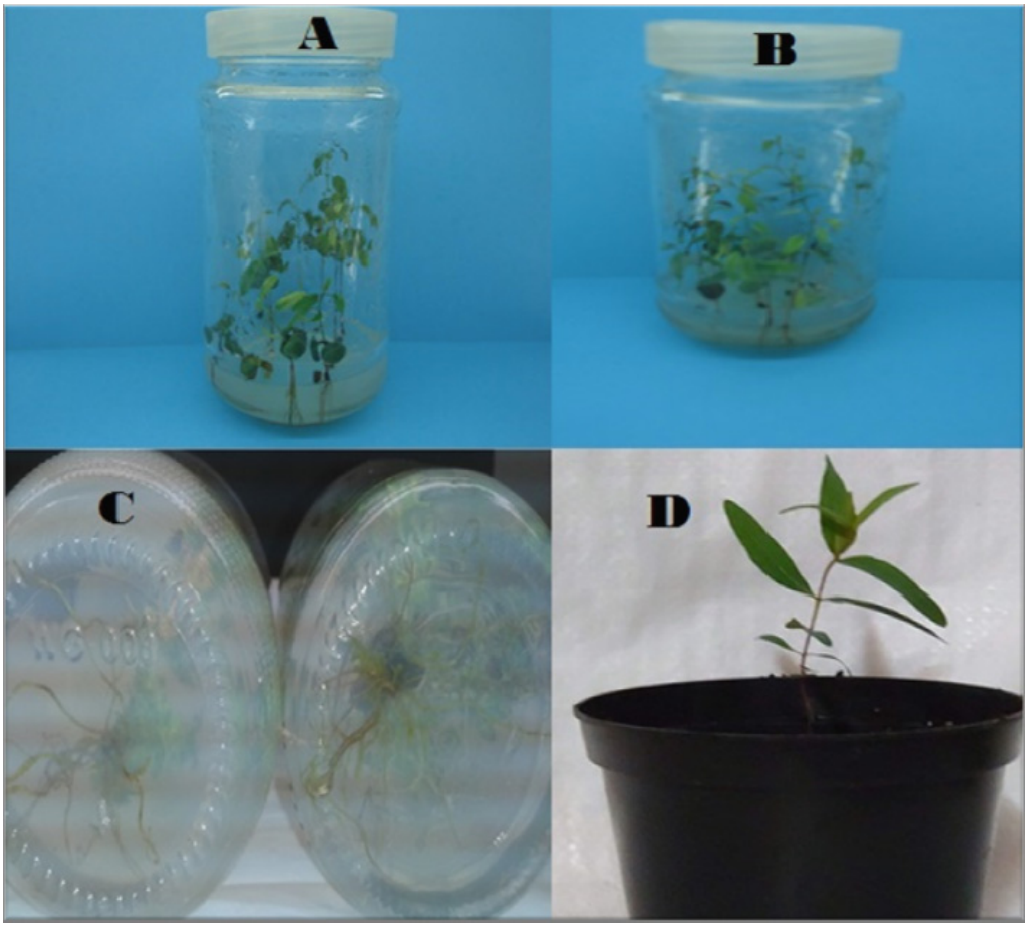

Fig.2. *Eucalyptus citriodora* micropropagation.

(A) BA at 1 mg l^{-1} in combination with NAA at 0.1 mg l^{-1} on *Eucalyptus citriodora* growth during multiplication stage, (B) BA at 1 mgl^{-1}, NAA at 0.1 mg l^{-1} and *Aloe vera* gel at 20 ml l^{-1} on *Eucalyptus citriodora* growth during multiplication stage, (C) IBA at 1mg l^{-1} on *Eucalyptus citriodora* growth during rooting stage, (D) Acclimatization of *Eucalyptus citriodora on* peatmoss and sand at a rate of (1v:1v).

Table 3. Effect of different auxins type and concentrations on *Eucalyptus citriodora* growth during rooting stage.

Auxin type	Auxin concentration (mgl^{-1})	Shoot number /explant	Shoot length (cm)	Leaf number /shoot	Rooting percentage (%)	Root number /explant	Root length (cm)
Control		1.77 fg	3.06 g	9.16 d	48.33 f	1.25 f	1.37 f
	0.5	1.86 ef	3.66 f	9.30 d	64.00 e	1.46 ef	1.76 e
NAA	**1.0**	2.29 d	4.44 d	10.33 c	69.33 d	1.83 cd	3.11 d
	2.0	2.68 c	4.73 d	10.90 bc	81.00 c	1.96 bc	3.36 cd
	0.5	2.38 d	5.90 c	11.33 b	84.66 b	2.13 b	4.23 b
IBA	**1.0**	3.44 a	8.13 a	12.86 a	99.00 a	3.27 a	5.07 a
	2.0	2.95 b	7.41 b	12.55 a	98.66 a	3.06 a	4.86 a
	0.5	1.65 g	3.06 g	8.33 e	62.33 e	1.33 f	1.90 e
IAA	**1.0**	2.00 e	3.86 ef	9.50 d	68.00 d	1.70 de	3.27 cd
	2.0	2.33 d	4.10 e	10.27 c	79.66 c	1.83 cd	3.46 c

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استخدام جل الصبار كمشجع نمو في الإكثار الدقيق لنبات الكافور الليموني

هاني محمد سامي حسن* و دينا محمد جلال هندي**
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تهدف الدراسة الحالية إلى دراسة تأثير تركيزات مختلفة من جل الصبار كمشجع نمو في إكثار نباتات الكافور الليموني. اجريت التجارب بمعمل أ.د/ عبد الفتاح حلمي بلال لزراعة الأنسجة النباتية بكلية العلوم الزراعية البيئية، جامعة العريش، مصر. وقد أوضحت النتائج المتحصل عليها أن نقع المنفصلات النباتية في محلول الكلوروكس بتركيز ٢٠٪ لمدة ٢٠ دقيقة أدى إلى الحصول على أعلى نسبة بقاء للمنفصلات وصلت إلى (٨٧,٣٣٪) وذلك أثناء مرحلة التأسيس. بالإضافة إلى ذلك وجد أن إضافة بنزيل أدينين بتركيز ١ مجم/لتر مع ٠,١ مجم/لتر نيفثالين حمض الخليك أدى إلى الحصول على أعلى عدد للأفرع/منفصل نباتي، طول الفرع (سم) وعدد الأوراق لكل فرع (٧,٣٣، ٤,٦٦ و ٩,٥، على التوالي) وذلك أثناء مرحلة التضاعف. كما تم أيضا الحصول على أعلى عدد للأفرع/منفصل نباتي، طول الفرع (سم) وعدد الأوراق لكل فرع (٩,٣٣، ٧,٠ و ١١,٥، على التوالي) عند إضافة جل الصبار بمعدل ٢٠ ملى/لتر، علاوة على ذلك أدى استخدام بيئة موراشيجى وسكوج مضافا إليها إندول حمض البيوتريك بمعدل ١ مجم/لتر أثناء مرحلة التجذير الحصول على أعلى معدل نمو للكافور الليموني (عدد للأفرع/منفصل نباتي، طول الفرع (سم) وعدد الأوراق لكل فرع، نسبة التجذير، عدد الجذور/منفصل نباتي وكذلك طول الجذر(سم) (٣,٤٤، ٨,١٣، ١٢,٨٦، ٩٩، ٣,٢٧ و ٥,٠٧ على التوالى). في النهاية تم أقلمة النباتات المستولدة بنسبة نجاح عالية وصلت إلى (٨٠٪) باستخدام مخلوط التربة يحتوى على (بيتموس:رمل) بنسبة ١:١ حجماً.

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