Bull. Fac. Agric., Cairo Univ.,60 (2009):99-105.

PROPAGATION OF CALLA LILY (*Zantedeschia aethiopica* Spreng) PLANTS BY TISSUE CULTURE TECHNIQUE

(Received:8.9.2008)

By
M.A. El-Shamy, A. H.M. El-Feky and N. Y.L. Eliwa*

Botanical Gardens Research Department, Ornamental Plants Research Department*
Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

ABSTRACT

The following experiments were conducted using bud explants from tubers of *Zantedeschia aethiopica* Spreng, aiming to develop an *in vitro* propagation protocol for the production of multipurpose of *Z. aethiopica* plants.

Sterilization treatments were used for the production of contamination free explants. The results indicated that the highest value of survived plants resulted from the mixture of 2 % NaOCl plus 0.5 mg/l Hg_2Cl. The effect of NAA and Kinetin (Kin) at different rates on explant establishment was recorded . The recorded data indicated that using MS medium plus 3.0 mg/l NAA and 1.0 mg/l Kin resulted in a significant increase in shoot length and shoot number.

In the multiplication stage, shoots were cultured on MS media supplemented with either 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l 2ip or 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l BA. The data indicated that supplementation with 4.0 mg/l 2ip induced the highest number of shoots and shoot length, when compared to the other concentrations of 2ip or BA. During rooting stage: shoots were transferred to MS media at full or half strength supplemented with 0.0, 1.0, 3.0 or 5.0 mg/l IBA. MS medium at full strength supplemented with 3.0 mg/l IBA was more suitable to increase the number of roots and root length. The resulted plantlets were acclimatized in the greenhouse and they had healthy appearance and high survival percentage.

Key words: *Calla, in vitro, micropropagation, shoot tips, Zantedeschia.*

1.INTRODUCTION

Calla lily (*Zantedeschia aethiopica* Spreng.) blongs to family Araceae and is used as cut flowers and in landscape of gardens. Calla lily is a perennial plant grown in winter for its ornamental corolla-like spathes and as potted flowering plant (Bailey and Bailey, 1960).

The recommended method for sterilization of rhizome-bud explants of Calla lily (*Zantedeschia aethiopica* Spreng.) was by using 4% NaOCl solution for 5 minutes (Ebrahim, 2004).

In 4 Calla lily cultivars, Wang *et al.* (2001) found that the culture medium supplemented with 200 mg penicillin GK/litre efficiently inhibited bacterial growth without affecting normal growth and rapid propagation of cultured tissue.

Ebrahim (2004) found that the best medium for establishment of *Zantedeschia* was Murashige and Skoog (MS)- plus benzyladenine and agar.

Xiao *et al.* (1998), Chang *et al.* (2003) and Ebrahim. (2004) found that the multiplication of *Zantedeschia* can be induced by using MS medium supplemented with different plant growth regulators, such as, zeatin used by Xiao *et al.*, (1998). Meanwhile the number of shoots in Calla lily was increased with increasing benzyladenine (BAP) concentration (Purwito *et al.*, 2001). Also, they found that BA was more effective than thidiazuron (TDZ) for enhancing multiple shoot formation from explants of *Zantedeschia albomaculata* (Chang *et al.*, 2003).

Rooting *in vitro* was induced by zeatin (Xiao *et al.*, 1998). On the other hand, microshoots of *Zantedeschia* were rooted in half strength MS medium supplemented with IBA (Purwito *et al.*, 2001 and Chang *et al.*, 2003).

Adding NAA to a solid MS-medium at half salts strength produced the fastest growth and development of roots (Ebrahim, 2004).

The goal of this research was to set a protocol for *in vitro* propagation of *Zantedeschia* for

commercial production. This was done by studying the effect of sterilization treatments, growth regulators (NAA and Kin) on shoot establishment, BA or 2ip on multiplication stage and finally IBA and MS medium strength on rooting growth and development.

2.MATERIALS AND METHODS

This study was carried out in the Laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agricultural Research Center, Ministry of Agriculture. The experiments were done during the period from 2005 to 2006. The objective was to investigate the most suitable treatments for micropropagation of *Zantedeschia*. The mother plants were imported from Holland. The parts used as explants were buds from tubers.

Number of measurements were taken on *Zantedeschia aethiopica i.e.*, number of survival of explants, number of leaves, shoot length (cm), number of shoots, number of roots and root length.

Growth regulators Kin and NAA were used during establishment stage, Cytokinine, *i.e.* BA and 2ip were used for multiplication stage and IBA was used during rooting stage.

2.1.Experiment 1: Effect of some sterilization treatments on contamination of explants:

The aim of this experiment was to study the effect of some sterilization treatments, *i.e.* sodium hypochlorite (NaOCl) solution at 1.0, 2.0 and 3.0 % with mercuric chloride (Hg_2Cl) solution at concentrations of 0.1, 0.3, 0.5 and 0.7 g/l on contamination of *Zantedeschia* explants *in vitro*.

Buds of *Zantedeschia* were excised from the tubers, initially 1-2 cm in length then washed by soapy water for 5 minutes followed by 1 hour under running tap water. The explants were sterilized by immersion in mercuric chloride (Hg_2Cl) solution at the rate of 0.1, 0.3, 0.5 or 0.7 g/l containing 3-5 drops of Tween 20 for 5 minutes, followed by rinsing three times in sterile distilled water, then immersed in a sodium hypochlorite (NaOCl) solution at 1.0, 2.0 or 3.0 % (commercial Clorox) containing 3-5 drops of Tween 20 for 20 minutes. Finally the explants were washed 5 times with sterile distilled water. Each sterilized explant was cultured separately under sterile conditions in 100 ml jar.

For surface sterilization of explants, 12 treatments were initiated, each treatment consisted of 10 jars.

2.2. Experiment 2: Effect of NAA and Kin on explant establishment:

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used as culture medium.

For establishment stage, 16 treatments were studied, *i.e.* NAA (0.0, 1.0, 3.0 or 5.0 mg/l) and Kin (0.0, 0.5, 1.0 or 2.0 mg/l).

2.3. Experiment 3: Effect of BA and 2ip on multiplication stage:

For multiplication stage, 18 treatments were studied using 2ip (0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 mg/l) or BA (0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 mg/l) and three this was prolonged for subcultures.

2.4. Experiment 4: Effect of IBA and medium strength on rooting growth:

In rooting stage, 8 treatments including combinations of 4 IBA levels (0.0, 1.0, 3.0 or 5.0 mg/l) and two MS-strength (full or ½ MS) were used.

2.5.Experimental design and statistical analysis:

A complete randomized design was employed in all of the experiments. Analysis of variance was used to show the least statistical differences between treatments using the L.S.D at 5% probability level (Snedecor and Cochran, 1989).

3.RESULTS AND DISCUSSION

3.1.Effect of sodium hypochlorite and mercuric chloride on explant survival:

Results represented in Table (1) indicate that the effect of sodium hypochlorite (NaOCl) was positive in surface sterilization of *Zantedeschia* buds. The number of uncontaminated explants increased with the increase of NaOCl concentration, when mercuric chloride was present.

Table (1): Effect of different concentrations of sodium hypochlorite and mercuric chloride on explant survival.

Hg_2Cl(g/l) \ NaOCl(%)	0.1	0.3	0.5	0.7	**Mean (A)**
1	2.00	3.00	3.00	5.00	3.25
2	6.00	8.00	10.00	9.00	8.25
3	7.00	9.00	8.00	6.00	7.50
Mean (B)	5.00	6.67	7.00	6.67	

LSD at 0.05 NaOCl (A) = 0.4233
Hg_2Cl (B) = 0.4888 (AXB) = 0.8467

Table (2): Effect of different concentrations of Kin and NAA on growth *Zantedeschia* explants during establishment stage

NAA (mg/l)	No. of leaves					Shoot length (cm)					No. of shoots				
	Kin (mg/l)				Mean (A)	Kin (mg/l)				Mean (A)	Kin (mg/l)				Mean (A)
	0.0	0.5	1.0	2.0		0.0	0.5	1.0	2.0		0.0	0.5	1.0	2.0	
0.0	2.00	3.00	3.00	3.00	2.75	2.00	2.00	3.00	3.00	2.50	1.00	1.00	2.00	2.00	1.50
1.0	3.00	3.00	4.00	4.00	3.50	3.00	4.00	5.00	5.00	4.25	1.00	1.00	2.00	3.00	1.75
3.0	3.00	5.00	7.00	4.00	4.75	5.00	6.00	6.00	7.00	6.00	1.00	2.00	4.00	4.00	2.75
5.0	3.00	4.00	4.00	5.00	4.00	7.00	7.00	8.00	8.00	7.50	1.00	1.00	1.00	2.00	1.25
Mean (B)	2.75	3.75	4.50	4.00		4.25	4.75	5.50	5.75		1.00	1.25	2.25	2.75	

LSD at0 0 5
NAA(A)= 0 4597 = 0 4521 = 0 6064 Kin (B) = 0 4597 = 0 4521 = 0 6064 (AXB) = 0 9194 = 0 9042 1 2130

Table (3): Effect of different concentrations of 2ip on growth of *Zantedeschia* shoots prolonged for three subcultures during multiplication stage

2ip (mg/l)	No. of leaves				Shoot length (cm)				No. of shoots			
	Subculture			Mean (A)	Subculture			Mean (A)	Subculture			Mean (A)
	1	2	3		1	2	3		1	2	3	
0.0	3.00	5.00	7.00	5.00	7.00	9.00	11.00	9.00	2.00	3.00	4.00	3.00
1.0	3.00	4.00	5.00	4.00	7.00	7.00	8.00	7.33	4.00	8.00	15.00	9.00
2.0	4.00	5.00	6.00	5.00	7.00	8.00	9.00	8.00	6.00	14.00	23.00	14.33
3.0	5.00	7.00	8.00	6.67	6.00	7.00	8.00	7.33	8.00	20.00	35.00	21.00
4.0	7.00	9.00	11.00	9.00	6.00	7.00	8.00	7.00	9.00	22.00	38.00	32.00
5.0	8.00	9.00	10.00	9.00	6.00	7.00	7.00	6.67	10.00	26.00	43.00	26.33
Mean (B)	5.00	6.50	7.83		6.50	7.67	8.50		6.50	15.50	26.33	

LSD at 0.05 2ip (A) = 0.0315 =0.3859 =0.3482
Subculture (B)=0.0238 = 0.2917 =0.2632 (AXB) = 0.0629 =0.7719 =0.6964

Table (4): Effect of different concentrations of BA on growth of *Zantedeschia* shoots prolonged for three subcultures during multiplication stage

BA (mg/l)	No. of leaves				Shoot length (cm)				No. of shoots			
	Subculture			Mean (A)	Subculture			Mean (A)	Subculture			Mean (A)
	1	2	3		1	2	3		1	2	3	
0.0	3.00	5.00	7.00	5.00	7.00	9.00	11.00	9.00	2.00	3.00	4.00	3.00
1.0	3.00	4.00	5.00	4.00	7.00	8.00	8.00	7.67	3.00	7.00	18.00	9.33
2.0	4.00	5.00	5.00	4.67	6.00	6.00	5.00	5.67	5.00	12.00	24.00	13.67
3.0	3.00	3.00	2.00	2.67	5.00	4.00	4.00	4.33	7.00	18.00	32.00	19.00
4.0	3.00	2.00	2.00	2.33	4.00	3.00	3.00	3.33	7.00	15.00	21.00	14.33
5.0	2.00	1.00	1.00	1.33	3.00	2.00	1.00	2.00	6.00	11.00	18.00	10.67
Mean (B)	3.00	3.33	3.67		5.33	5.33	5.33		5.00	10.50	19.50	

LSD at 0 05 BA (A) = 0 6119 =0 7346 =1 6230
Subculture (B) = 0 4327 =0 5194 = 1 1470 (AXB) = 1.0600 =1 2720 =2 8110

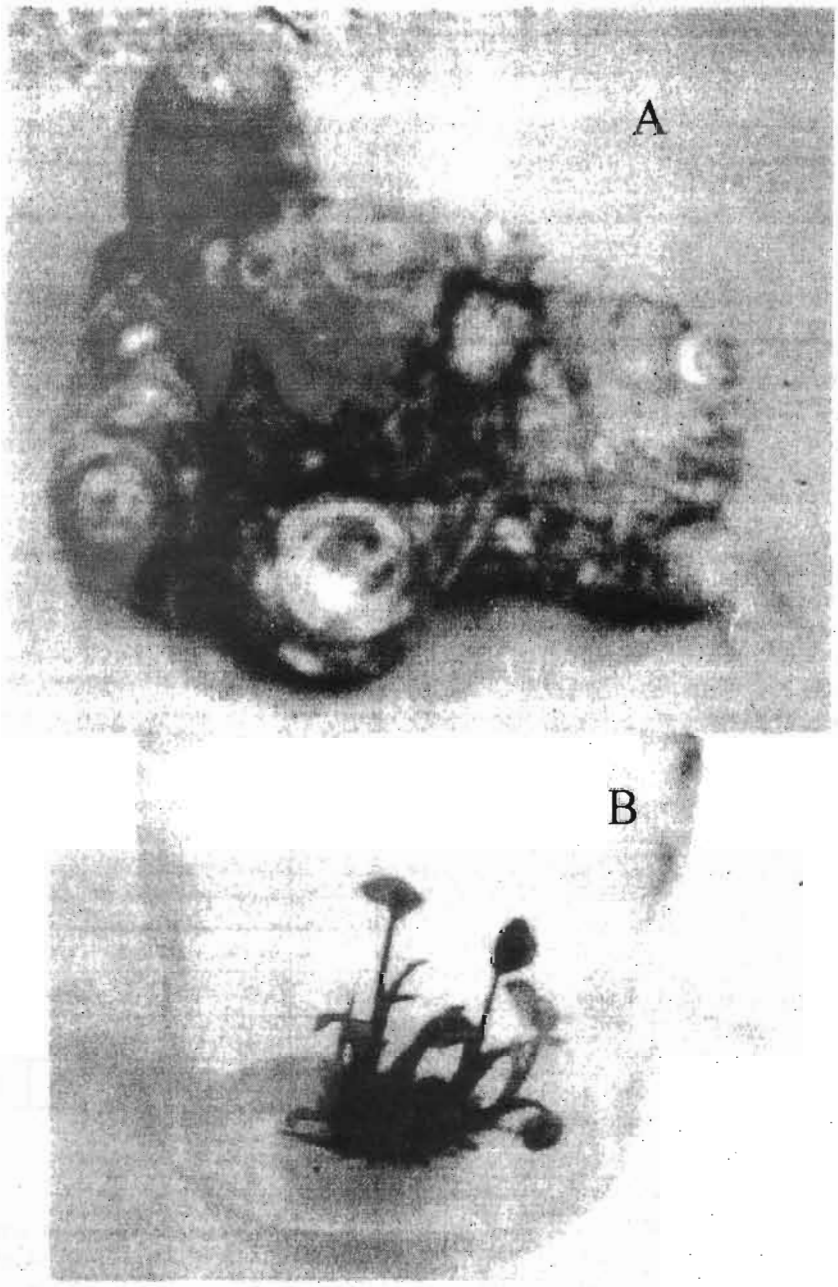

Fig (1): *In vitro* micropropagation of *Zantedeschia aethiopica* Spreng. a) Tubers b) Multiplication

Furthermore, the use of mercuric chloride (Hg_2Cl) augmented when sodium hypochlorite was supplemented to the solution of surface sterilization.

The interactions between NaOCl and Hg_2Cl were significant with the highest value of survived plants (10) when the mixture contained 2 % NaOCl and 0.5 mg/l Hg_2Cl.

As in the survival of the explants, the interaction between NaOCl and Hg_2Cl was decreased in survival of explants with the increase of concentration from NaOCl to 3% and higher than
0.5 g/l Hg_2Cl.

3.2. Effect of NAA and Kin on the stablishment of *Zantedeschia* bud

Results in Table (2) record that in the absence of Kin, NAA at a concentration of 5.0 mg/l increased shoot length (7 cm) and this was significant when compared with the other NAA concentrations. Also, in the absence of Kin, all media supplemented with NAA gave no shoots. However, the highest mean in the number of shoots (2.75) was found at 3.0 mg/l NAA, as well as at 2.0 mg/l Kin.

On the other hand, all buds formed 3 leaves at different concentrations of NAA comparing with 2 leaves at the zero level. While, the highest mean for the number of leaves was 4.75 at 3 mg/l NAA.

Regarding Kin addition, it was found that after four weeks the mean number of shoots was 2.75 at 2 mg/l Kin which was highly significant when compared with 0.0 or 0.5 mg/l Kin. The concentration of 1.0 mg/l Kin induced the higher number of leaves (4.50) while the highest shoot length was found when 2.0 mg/l was applied (5.75 cm).

The interaction between the different concentrations of NAA and Kin showed that the concentrations 3.0 mg/l NAA and 1.0 mg/l Kin was induced the highest number of leaves (7.00). For shoot length, the highest shoot length (8 cm) was induced from a medium supplemented with 5.0 mg/l NAA and 1.0 mg/l Kin while the best number of shoots (4) was produced from a medium supplemented with 3.0 mg/l NAA and 1.0 mg/l Kin.

3.3. Effect of 2ip on multiplication stage

Data recorded in Table (3) demonstrate that the number of shoots and number of leaves were increased by increasing the concentration of 2ip when compared with the control. However, all concentrations of 2ip resulted in significant shorter shoot length when compared to zero-level (control).

Regarding subculture effect, it was found that all studied parameters were positively increased with the subculture progress.

3.4. Effect of BA on multiplication stage

Results presented in Table (4) show that the highest value for shoot length and the number of leaves were recorded with zero BA level. The mean shoot length was fixed on 5.33 cm during the three subcultures of multiplication stage.

Purwito *et al.* (2001) indicated that the number of harvested shoots and leaves of calla lily increased with increasing BAP concentration.

In addition, the results clearly demonstrated that 2ip was more effective than BA in increasing different growth parameters of *Zantedeschia* shoots.

3.5. Effect of IBA and MS-strength on rooting stage

Data illustrated in Table (5) indicate that the medium-strength clearly affected the rooting stage of *Zantedeschia*. The full strength medium was superior than 1/2MS in the number of roots and root length.

Table (5): Effect of different concentrations of IBA (mg/l) and MS-Strength on *Zantedeschia* shootlets during rooting stage.

IBA (mg/l)	No. of Roots			Root length (cm)		
	Full-Strength	Half-Strength	Mean (A)	Full-Strength	Half-Strength	Mean (A)
0.0	1.00	1.00	1.00	2.00	1.50	1.75
1.0	3.00	2.00	2.50	3.00	2.00	2.50
3.0	7.00	5.00	6.00	8.00	5.00	6.50
5.0	7.00	6.00	6.50	9.00	5.00	7.00
Mean (B)	4.50	3.50		5.50	3.38	
LSD at 0.05						
IBA	(A)	0.9790			0.6564	
MS-Strength	(B)	0.6922			0.4642	
(AXB)		1.3840			0.9283	

This is in agreement with reports elsewhere with using Murashige and Skoog medium to different stages of *Zantedeschia*. (Xiao *et al.*, 1998).

Data exhibited in Table (5) also show that IBA at all tested concentrations caused an increase in the number of roots and root length of *Zantedeschia* plantlets as compared with the zero-level (control). The highest number of roots and root length was found when 5.0 mg/l IBA was used.

These results are in harmony with the results obtained on *Zantedeschia*, to favor IBA for the rooting stage (Purwito *et al.*, 2001). Finally, the high level of IBA utilized in this study, *i.e.* 3.0 mg/l might cause an enhancement for the number of roots and root length of *Zantedeschia*.

After acclimatization, about 90% of the obtained plantlets succeeded to be transplanted to the greenhouse conditions in pots containing sand and peatmoss in a ratio of 1:1.

4. REFERENCES

Bailey L.H. and Bailey E.Z. (1960). Hortus third A Concise Dictionary of Plants Cultivated in the United States and Canada, 3rd Ed, Macmillon, New York, P. 3534.

Chang H.S., Chakrabarty D., Hahn E.J. and Paek K.Y. (2003). Micropropagation of calla lily (*Zantedeschia albomaculata*) via *in vitro* shoot tip proliferation. *In Vitro* Cellular and Developmenta Biology Plant, 39(2): 129-134.

EbrahimM.K.H.(2004).Comparison,determination and optimizing the conditions required for rhizome and shoot formation, and flowering of *in vitro* cultured calla explants. Scienti Horticulturae, 101(3): 305-313.

Murashige T and Skoog F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum, 15: 473-497.

Purwito A., Aisyah S.I. and Tjandra A. (2001). Micropropagation of calla lily (*Zantedeschia* sp) by tissue culture. Tropenlandwirt Beiheft, 73: 307-312.

Snedecor, G.W. and Cochran, W.G. (1989): One-way classification, analysis of variance. In: Statistical Methods (8th Ed.). Iow a State Univ. Press, Ames, Iowa, U.S.A., ch. 12, 217- 236.

Wang Y.F., Lu R.J., Zhou R.M., Sun Y.F. and Huang J.H. (2001). Inhibition of endophytic bacteria during tissue culture of calla (*Zantedeschia aethiopica*). Acta Agriculturae Shanghai, 17(2): 82-83.

Xiao T.J., Li H., Long C.L. and Xia L.F. (1998). Studies on the micropropagation of four *Zantedeschia* cultivars. Acta Botanica Yunnanica,101-103.

إكثار نبات الكلا (*Zantedeschia aethiopica* **Spreng.**) بواسطة تقنية زراعة الأنسجة

ممدوح أحمد إبراهيم الشامى - علاء الدين حسن محمد الفقى - نجلاء يوسف لبيب عليوة*

قسم بحوث الحدائق النباتية،* قسم بحوث نباتات الزينة -معهد بحوث البساتين -مركز البحوث الزراعية -الجيزة-مصر.

ملخص

أجريت هذه الدراسة خلال الفترة من سنة ٢٠٠٥ - ٢٠٠٦ فى معمل زراعة الأنسجه بحديقة الزهرية التابع لمعهد بحوث البساتين-مركز البحوث الزراعية-وزارة الزراعة-جمهورية مصر العربية.

يهدف هذا البحث إلى معرفة أنسب المعاملات لإكثار نبات الكلا عن طريق زراعة الأنسجة وذلك لوضع بروتوكول للإكثار الدقيق لهذا النبات.

ويمكن تلخيص أهم النتائج فى الاتى:

أمكن إكثار نبات الكلا بواسطة البراعم الموجودة فى الدرنات كمنفصلات نباتية ثم تعقيمها بواسطة هيبو كلوريت الصوديوم بتركيز ١، ٢، ٣ ٪ و كلويد الزئبقيق بتركيز ٠,١، ٠,٣، ٠,٥، ٠,٧ جم/لتر بالإضافة إلى التداخلات بينهما .تبين أن أحسن تركيز هو ٢٪ هيبو كلوريت الصوديوم بالإضافة إلى ٠,٥ جم/لتر كلويد الزئبقيق وذلك للحصول على أعلى نسبة بقاء للنباتات وأقل نسبة تلوث.

أستخدمت فى مرحلة التأسيس بيئة موراشيجى وسكوج المضاف إليها نفثالين حمض الخليك بتركيز صفر ،١، ٣، ٥ مجم/لتر بالإضافة إلى الكينتين بتركيز صفر ،٠,٥، ١,٠، ٢,٠ مجم/لتر بالإضافة إلى التداخلات بينهما .فكان أفضل تركيز هو ٣,٠ مجم/لتر نفثالين حمض الخليك و ١,٠ مجم/لتر الكينتين لإستطالة النباتات و زيادة عدد الخلف.

أستخدمت فى مرحلة التضاعف بيئة موراشيجى وسكوج المضاف إليها البنزيل أمينو بيورين بتركيز صفر ،١، ٢، ٣، ٤، ٥ مجم/لترأو بيئة موراشيجى وسكوج المضاف إليها الأيزو بنتيل أدينين بتركيز صفر ،١، ٢، ٣، ٤، ٥ مجم/لتر. كانت أفضل معاملة هى٥ مجم/لتر الأيزو بنتيل أدينين من حيث عدد الخلف الناتجة.

أستخدمت فى مرحلة التجذير بيئة موراشيجى وسكوج ذات القوة الكاملة ونصف قوة الأملاح المضاف إليهما إندول حمض البيوتريك بتركيز صفر ، 1، 3، 5مجم/لتر.فتبين أن أفضل معاملة هى بيئة موراشيجى وسكوج ذات القوة الكاملة للأملاح المضاف إليها 5مجم/لتر إندول حمض البيوتريك.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٦٠) العدد الأول (يناير ٢٠٠٩):٩٩-١٠٥.