



ENHANCEMENT OF CALLI GROWTH IN THREE CULTIVARS OF JERUSALEM ARTICHOKE

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Neama A. Abdalla¹, Ragab^{2*} M.E., El-Miniawy² S.M. and Taha¹ H.S.

1- Plant Biotechnology Dept., National Research Center, Dokki, Giza, Egypt

2- Horticulture Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: mohamedragab99@hotmail.com

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ABSTRACT

Jerusalem artichoke is one of the most widely used plant groups in industrial and medicinal purposes and has been extensively used for inulin production. This plant is well known as a perennial plant and its tubers are rich in inulin. This study aimed to establish an applicable protocol for calli induction and production using different explants of Jerusalem artichoke cultivars (Balady, Fuza and Alba). Leaf, stem and root explants derived from *in vitro* growing plantlets were cultured on MS medium augmented with different combinations of naphthalene acetic acid (NAA) and benzyl adenine (BA). The highest percentage of calli induction (100%) was recorded with all tested media except MS free growth regulators medium and MS fortified with 1 mg L⁻¹ BA. The maximum value of calli fresh weight was obtained by culturing stem explants on MS supplemented with 2 mg L⁻¹ NAA and 1 mg L⁻¹ BA for all cultivars. Calli fresh weights increased weekly reaching the maximum value at the eighth week for all used explants cultured on the best medium for calli initiation. 'Balady' recorded the best results for calli induction and production as compared with 'Fuza' and 'Alba'. Stem explant was superior to root and leaf explants for all examined cultivars. The most suitable medium was MS medium fortified with 2 mg L⁻¹ NAA and 1 mg L⁻¹ BA compared to the other tested media for inducing calli and to enhance calli production from stem explant for all cultivars.

Keywords: *Helianthus tuberosus*, Asteraceae, calli, callus growth curve

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is considered one of the most widely used crops for the production of inulin. This inulin and other poly-fructosides are important for a wide range of scientific and industrial processes in particular the production of high-fructose syrups. Among alternative crops, inulin crops such as chicory (*Cichorium intybus*), globe artichoke (*Cynara cardunculus* var. *scolymus*) and wild yam (*Dioscorea* spp.) are being extensively investigated (Meijer et al 1993 and Smith et al 1997) for food and industrial purposes as well as for nutraceutical uses (Chubey and Dorrell, 1977; Spiegel et al 1994). The tubers of *Helianthus tuberosus* contain high levels of inulin (around 50% of dry weight; Kays and Nottingham 2007) which has been pursued as a bulking agent for artificial sweeteners (McLaurin et al 1999).

In light of recent events in plant cell and tissue culture, it is becoming extremely difficult to ignore its importance as an excellent alternative method could be used in the production of the plant secondary metabolites. It is well known that, callus is a relatively undifferentiated tissue consisting primarily of parenchymatous cells. Callus tissue can serve an experimental system to investigate and solve a broad range of basic research problems in organogenesis and embryogenesis related to the propagation of horticultural and agronomic plants (Caponetti, 2000). Furthermore, it used to study secondary metabolite production (Kondamudi et al. 2009), where callus has high potential for secondary metabolites enhancement compared with the original plant (Parsaeimehr et al. 2010; Molchan et al. 2012). There are a few literatures concerning Jerusalem artichoke *in vitro* (Gamburg et al 1999;

Volk and Richards 2006; Taha et al 2007; El-Mostafa et al 2008 and Abdalla et al 2014). A successful protocol for calli induction and production from leaf and nodal explants of Jerusalem artichoke was established. The best results of calli production from nodal and leaf explants were obtained using MS medium, which supplemented with 1 mg L⁻¹ of BAP and NAA (**Taha et al 2007**).

The establishment of an applicable protocol for calli induction and production from three Jerusalem artichoke cultivars was the main aim of this study.

MATERIALS AND METHODS

This study was carried out in the Plant Biotechnology Department, Genetic Engineering and Biotechnology Division, National Research Center, Cairo, Egypt, in cooperation with the Horticulture Department, Faculty of Agriculture, Ain Shams University, during the period of 2016/2017, in order to establish an applicable protocol for calli induction and production from different cultivars of Jerusalem artichoke.

Calli induction and growth development

Plant materials

Tubers of JA 'Balady' were obtained from Agricultural Research Center, Giza; whereas, tubers of 'Fuza' obtained from Ismailia and tubers of 'Alba' cultivar were obtained from Department of Agricultural Botany, Plant Physiology and Biotechnology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Hungary. Immature leaf, stem and root explants were taken from *in vitro* growing plantlets (five weeks old), which successfully obtained by culturing stem nodes explants of three cultivars of JA on ½ MS + 62.5 mg L⁻¹ Cefotax. These explants were cut into 3-4 mm segments followed by culturing them on solidified MS medium (Murashige and Skoog 1962), 3% (w/v) 80 sucrose, 0.7% agar fortified with different combinations of BA and NAA as follows:

C1= MS basal medium (free growth regulators)

C2= MS + 1 mg L⁻¹ BA + 0 mg L⁻¹ NAA

C3= MS + 0 mg L⁻¹ BA + 1 mg L⁻¹ NAA

C4= MS + 1 mg L⁻¹ BA + 1 mg L⁻¹ NAA

C5= MS + 1 mg L⁻¹ BA + 2 mg L⁻¹ NAA

C6= MS + 2 mg L⁻¹ BA + 1 mg L⁻¹ NAA

The pH of all media was adjusted to 5.8 with 1 N KOH or HCl. The media were distributed into 100 ml glass jars containing 25 ml and sterilized by autoclaving for 15 min at 121°C and 1.2 kg cm⁻².

Each treatment consisted of 5 replicates (jars) and each replicate contained 4 explants for leaf explants, 3 explants for stem and root explants. The cultures were incubated in dark condition at 26±1°C. After four weeks of culture, the frequency of calli formation (%) and calli fresh weights (g/jar) were recorded.

Frequency of calli formation

Frequencies of calli formation were calculated by the following equation:

$$\text{Frequency of calli formation (\%)} = \left(\frac{\text{Explants produced callus}}{\text{Total cultured explants}} \right) \times 100$$

Calli growth curve

One gram of callus, produced from leaf, stem and root explants of 'Balady', 'Fuza' and 'Alba' was cultured on MS medium supplemented 1 mg L⁻¹ BA + 2 mg L⁻¹ NAA, which was the best medium for calli induction. The pH of tested medium was adjusted to 5.8 with 1 N KOH or HCl. The tested medium was distributed into 100 ml glass jars containing 25 ml and sterilized by autoclaving for 15 min at 121°C and 1.2 Kg cm⁻². Each treatment consisted of 5 replicates (jars) and each replicate contained one gram of leaf, stem and root derived calli of the three cultivars. All cultures were incubated in dark condition at 26±1°C for growth estimation.

For estimate the growth curve of calli derived from the three explants of the three cultivars; fresh weights of calli were recorded weekly for eight weeks.

Statistical analysis

Data were statistically analyzed using Duncun's multiple range test at 5 % level (CoHort, 2004) according to Snedecor and Corchan (1982) to verify the differences between means of treatments. All experiments were designed in a completely randomized design.

RESULTS AND DISCUSSION

Calli induction and growth development

Table (1) and Fig. (A) present the effect of MS medium supplemented under different combinations of NAA and BA on calli induction from leaf, stem and root explants of 'Balady', 'Fuza' and 'Alba'.

Table 1. MS medium fortified with different combinations of BA and NAA on frequency of calli formation (%) from leaf, stem and root explants of 'Balady', 'Fuza' and 'Alba' after four weeks of the culturing and incubated under dark condition at 26±1°C

Treatment code	Balady			Fuza			Alba		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
C1	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d
C2	25 c	100 a	75 b	25 c	100 a	75 b	25 c	100 a	75 b
C3	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
C4	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
C5	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100a	100 a
C6	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

Each treatment was the average of 5 replicates (jars) and each jar contains three explants for stem and root; four explants for leaf. Values followed by the same letters were not significantly different by Duncan's multiple range test at 0.05 level.

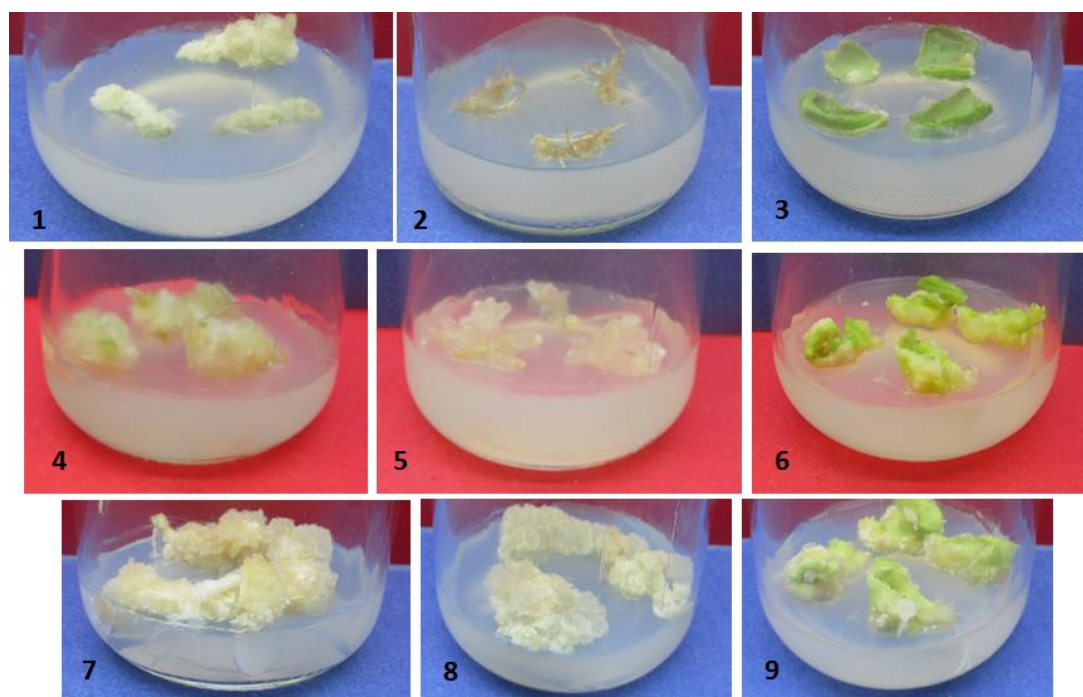


Fig. A. Calli formation after one week (photos 1, 2 and 3); after two weeks (photos 4,5 and 6) and after four weeks (photos 7, 8 and 9) from culturing explants of 'Balady' on C5 (MS + 1 mg L⁻¹ BA + 2 mg L⁻¹ NAA) and incubated under dark condition at 26±1°C. Where photos 1, 4 and 7 for stem explant; 2, 5 and 8 for root explant and 3, 6 and 9 for leaf explant.

Frequency of calli formation

Callus was successfully initiated on all tested media except C1 (MS free growth regulators medium), where the maximum callus induction (100%) was recorded on C3-C6 and 66.7% on C2 regardless of cultivars or explants used. Also, there were no differences between the cultivars in its ability to initiate callus, where they followed the same behavior regardless of media or explants, as they recorded the same value of frequency of callus formation (83.3%). The stem was the superior explant followed by root and leaf explants where they recorded (100%, 79.2% and 70.8% respectively) for all cultivars and irrespective of cultured media. Moreover, the same explant for different cultivars achieved the same percentage for callus formation under the examined media.

Abbasi et al (2010) reported that, callogenesis could be considered a significant tool in medicinal species for the production of biologically active compounds and the indirect organogenesis as well. The current investigation clearly verified that all examined explants for the three cultivars did not form calli on MS free growth regulators medium. Similar results were obtained by **Taha et al (2007)** on *Helianthus tuberosus* L, **Ahmad et al (2011)** on *Stevia rebaudiana*, **Al-Khateeb et al (2012)** on *Cichorium pumilum*, Asteraceae family and **Aghdasi et al (2018)** on *Ephedra major* Host, Ephedraceae family. Earlier report on callus induction of *Cichorium intybus* showed that NAA was the best auxin for callus induction when it used alone or in combination with BA. Moreover, the higher concentrations of NAA, which increased more 1 mg L⁻¹ was significantly increased callus induction rate (**Velayutham et al 2006**). These results agreed with ours on *Helianthus tuberosus* L. Conversely, the lowest frequency of callus induction achieved on MS medium augmented with 1 mg L⁻¹ BA alone. In this context, previous study reported that an auxin is generally required for callus induction from explants. Because its ability for altering the genetically programmed physiology of whole plant tissues. Where cells, revert to a dedifferentiated state and begin to divide forming callus when they subjected to auxin in the culturing medium (**Abdalla et al 2013**). In addition, supplementation of culture medium either Gamborg B5 (Gamborg et al. 1968) or MS medium with Kinetin in absence of auxin did not induce calli at all from stem explants of *Ephedra major* (**Aghdasi et al 2018**). In contrast to these results, there were no observed calli induced nei-

ther for all different auxin supplementations when each one used alone nor when BA used alone (**Muhamad et al 2018**) for bud explants of *Sargassum polycystum* cultured on solid medium.

Calli fresh weights

The best medium that achieved maximum values of calli fresh weight with all cultivars and all explants was C5 (MS + 2 mg L⁻¹ NAA + 1 mg L⁻¹ BA). While, the lowest calli fresh weight was observed with C2, Where calli fresh weight increased gradually from C2 to C5 then it decreased with C6 for all tested cultivars and all used explants. In general and concerning cultivars, 'Balady' was the best followed by 'Fuza' and 'Alba', respectively. Moreover, stem explant was the superior then root and leaf explant, respectively. The best results for calli fresh weights were noticed with stem explant of 'Balady' cultured on C5 medium followed by culturing stem explant of 'Fuza' and 'Alba' on the same medium, respectively as shown in **Tables (2, 3 and 4)**. It is noticed that adding NAA alone and by increasing it in combination with BA, the callus fresh weight increased significantly. This result was in agreement with that obtained by **Parsaeimehr et al (2010)** on *Ephedra procera*. On the other hand, supplementation of media with BA alone recorded minimum callus fresh weight for all examined explants and all cultivars and by increasing its concentration relative to NAA decreased callus fresh weight significantly.

Calli growth curve

About the growth of calli, fresh weights of stem, root and leaf derived calli of 'Balady' were measured weekly for eight weeks from culturing on the best medium for callus initiation and production (C5). There was a gradual increase in callus fresh weight week by week till it reached the maximum value at the eighth week for all explant. The growth curve of calli derived stem is superior to that of calli derived root followed by calli derived leaf. The optimum value of calli fresh weight (5.18 g) was recorded for calli derived stem after eight weeks from culturing on C5 medium. While it recorded (3.98 and 2.30 g for calli derived root and leaf, respectively). Also it is observed that the increasing in fresh weight values in the last three weeks is greater than that in the first five weeks for all examined explants. The same trend was recorded for 'Fuza' and 'Alba'; where they achieved gradually

Table 2. MS medium fortified with different combinations of NAA and BA on callus fresh weights (g/jar) from leaf, stem and root explants of Jerusalem artichoke 'Balady' after eight weeks of culturing and incubated under dark condition at 26±1°C

Treatments	Explants			Mean
	Leaf	Stem	Root	
C1= MS + 0 mg L ⁻¹ BA + 0 mg L ⁻¹ NAA	0.00 p	0.00 p	0.00 p	0.00 F
C2= MS + 1 mg L ⁻¹ BA + 0 mg L ⁻¹ NAA	0.56 o	2.00 m	1.00 n	1.18 E
C3= MS + 0 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	2.68 l	6.33 c	3.13 k	4.04 C
C4= MS + 1 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	3.89 i	7.43 b	4.76 e	5.36 B
C5= MS + 1 mg L ⁻¹ BA + 2 mg L ⁻¹ NAA	4.63 f	8.47 a	5.56 d	6.22 A
C6= MS + 2 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	3.35 j	4.36 g	3.99 h	3.90 D
Mean	2.51 C	4.76 A	3.07 B	

Each treatment was the average of 5 replicates and each Jar contains three explants for stem and root; four explants for leaf. Values followed by the same letters were not significantly different by Duncan's test at 0.05 level. The vertical means represent the effect of media on callus fresh weight regardless the effect of explants, whereas the horizontal ones indicate the explants.

Table 3. MS medium fortified with different combinations of NAA and BA on callus fresh weights (g/jar) from leaf, stem and root explants of Jerusalem artichoke 'Fuza' after eight weeks of culturing and incubated under dark condition at 26±1°C

Treatments	Explants			Mean
	Leaf	Stem	Root	
C1= MS + 0 mg L ⁻¹ BA + 0 mg L ⁻¹ NAA	0.00 o	0.00 o	0.00 o	0.00 F
C2= MS + 1 mg L ⁻¹ BA + 0 mg L ⁻¹ NAA	0.45 n	1.80 l	0.84 m	1.03 E
C3= MS + 0 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	1.84 l	3.87 f	3.23 h	2.98 C
C4= MS + 1 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	3.63 g	5.99 b	4.67 d	4.76 B
C5= MS + 1 mg L ⁻¹ BA + 2 mg L ⁻¹ NAA	4.51 e	8.20 a	5.45 c	6.05 A
C6= MS + 2 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	2.90 j	3.16 i	2.45 k	2.83 D
Mean	2.22 C	3.83 A	2.77 B	

Each treatment was the average of 5 replicates and each Jar contains four explants. Values followed by the same letters were not significantly different by Duncan's test at 0.05 level. The vertical means represent the effect of media on callus fresh weight regardless the effect of explants, whereas the horizontal ones indicate the explants.

Table 4. MS medium fortified with different combinations of NAA and BA on callus fresh weights (g/jar) from leaf, stem and root explants of Jerusalem artichoke 'Alba' after eight weeks of culturing and incubated under dark condition at $26\pm 1^\circ\text{C}$

Treatments	Explants			Mean
	Leaf	Stem	Root	
C1= MS + 0 mg L ⁻¹ BA + 0 mg L ⁻¹ NAA	0.00 o	0.00 o	0.00 o	0.00 F
C2= MS + 1 mg L ⁻¹ BA + 0 mg L ⁻¹ NAA	0.34 n	1.65 k	0.76 m	0.91 E
C3= MS + 0 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	1.55 l	3.62 f	3.00 h	2.72 C
C4= MS + 1 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	3.52 g	5.83 b	4.22 e	4.52 B
C5= MS + 1 mg L ⁻¹ BA + 2 mg L ⁻¹ NAA	4.39 d	7.93 a	5.12 c	5.81 A
C6= MS + 2 mg L ⁻¹ BA + 1 mg L ⁻¹ NAA	2.37 i	3.10 h	2.30 j	2.55 D
Mean	2.02 C	3.67 A	2.56 B	

Each treatment was the average of 5 replicates and each Jar contains three explants for stem and root; four explants for leaf. Values followed by the same letters were not significantly different by Duncan's test at 0.05 level. The vertical means represent the effect of media on callus fresh weight regardless the effect of explants, whereas the horizontal ones indicate the explants.

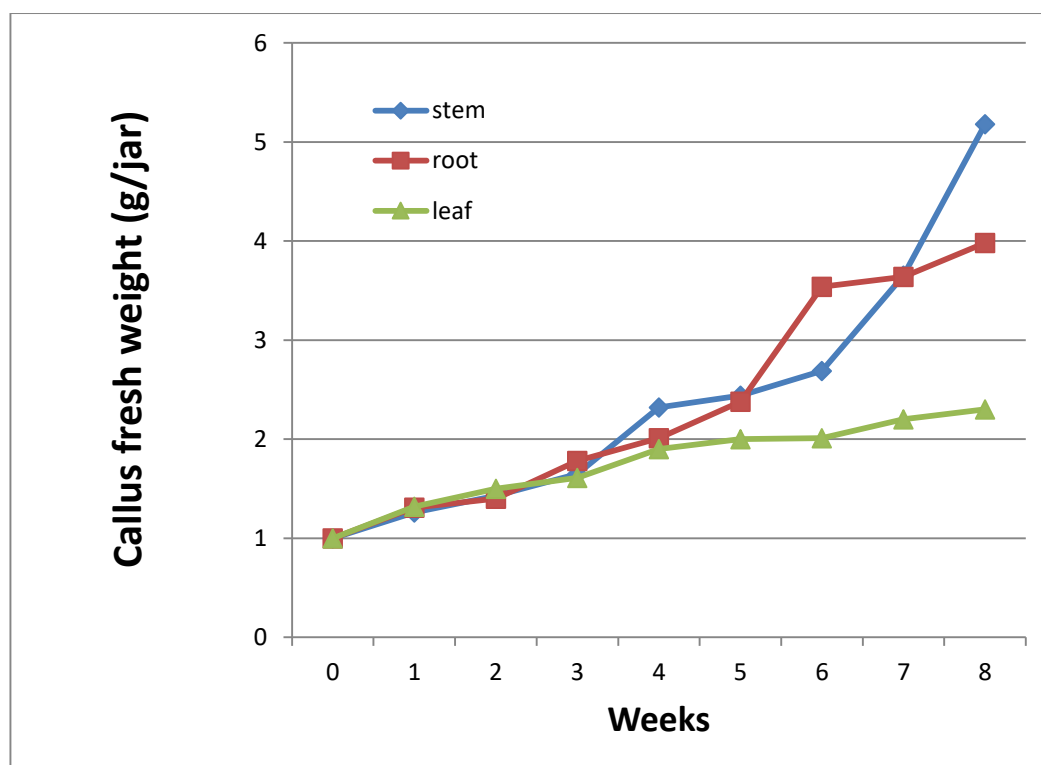


Fig. B. Calli growth curve of 'Balady' after eight weeks from the reculturing stem, root and leaf derived calli on callus induction and production medium (C5) and incubated under dark condition at $26\pm 1^\circ\text{C}$

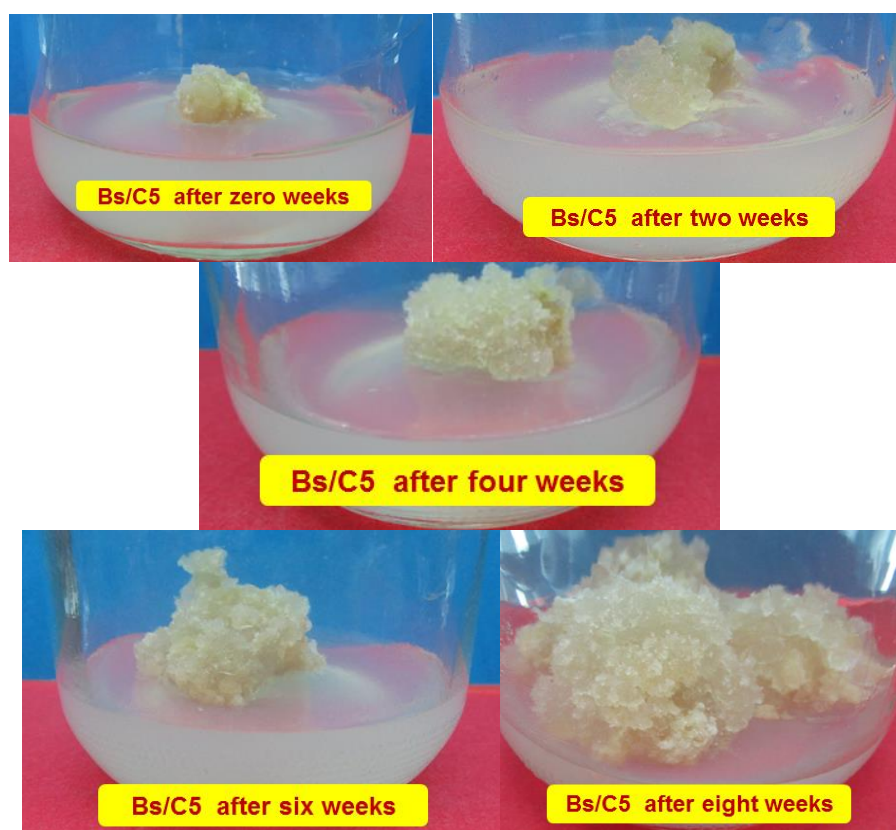


Fig. C. Calli growth curve of stem derived callus of 'Balady' weekly for eight weeks from the culturing on callus induction and production medium (C5) and incubated under dark condition at $26\pm 1^{\circ}\text{C}$. **Bs/C5** represents stem derived callus of 'Balady' cultured on C5

increase in calli fresh weight weekly till they reached the maximum value at the eighth week for all explant (Data not shown). Whereas there were different only in the rate of weekly increasing of calli fresh weight; as 'Balady' was the better compared with 'Fuza' and 'Alba', respectively (**Figs. B and C**).

CONCLUSION

An efficient protocol for calli induction and production for Jerusalem artichoke cultivars (i.e., Balady, Fuza and Alba) has been developed. Calli cultures were successfully induced from stem explant cultured on MS medium fortified with 2 mg L^{-1} NAA and 1 mg L^{-1} BA for all examined cultivars. It was quite suitable medium for callus induction from stem explants of the different cultivars, where it gave the best results compared to the other tested media. Stem explants showed the most efficiency to initiate calli in contrast to the other examined explants. Due to the nutritional, medicinal and in-

dustrial important of inulin in recent years; further studies should be done to evaluate inulin accumulation in calli cultures of the three mentioned cultivars under the current investigation. In addition, great efforts must be exerted to enhance inulin accumulation from their calli cultures for large scale production by tissue cultures techniques.

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تعزير نمو الكالس في ثلاثة أصناف من الطرطوفة

[155]

نعمه عبدالمنعم عبدالله^{1*} - محمد إمام رجب² - صلاح محمود المنياوي² - حسين سيد طه¹

1- قسم التكنولوجيا الحيوية النباتية - المركز القومي للبحوث - الدقى - الجيزة - مصر
2- قسم البساتين - كلية الزراعة - جامعة عين شمس - ص.ب. 68 - حدائق شبرا 11241 - القاهرة - مصر

*Corresponding author: mohamedragab99@hotmail.com

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الموجز

الأصناف. الأوزان الطازجة للكالس إزدادت أسبوعيا حتى وصلت أعلى قيمة لها عند الأسبوع الثامن لكل المنفصلات المنزرعة على أفضل بيئة لنشوء الكالس. الصنف البلدى سجل أفضل النتائج لنشوء وإنتاج الكالس بالمقارنة بالفيزواوالألبا. تفوق منفصل الساق على منفصلات الجذر والورق لكل الأصناف المختبرة. وكانت بيئة موراشيچ وسكوج المدعمة ب 1 مجم/لتر بنزيرل أدنينين + 2 مجم/لتر نفتالين حمض الخليك أنسب بيئة لنشوء الكالس ولتعزير نمو وإنتاج الكالس من منفصل الساق لكل الأصناف مقارنة بباقي البيئات المختبرة.

الكلمات الدالة: هليانسس تيويروسس، المركبة، كالس، منحنى نمو الكالس

أجريت هذه الدراسة بهدف تأسيس بروتوكول يمكن تطبيقه لنشوء وإنتاج الكالس باستخدام منفصلات مختلفة لأصناف الطرطوفة الثلاثة (بلدى، فيوزا، ألبا)، حيث تم زراعة منفصلات الورق والساق والجذر من نباتات ناتجة فى المعمل على بيئة موراشيچ وسكوج مزودة بتوليفات مختلفة من البنزيرل أدنينين ونفتالين حمض الخليك. أعلى نسبة لنشوء الكالس (100%) سجلت مع كل البيئات عدا بيئة موراشيچ وسكوج الخالية من منظمتا النمو وكذلك المضاف لها 1 مجم/لتر بنزيرل أدنينين. أقصى قيمة للوزن الطازج للكالس تم الحصول عليها بزراعة منفصلات الساق على بيئة موراشيچ وسكوج المضاف لها 1 مجم/لتر بنزيرل أدنينين + 2 مجم/لتر نفتالين حمض الخليك لكل