J. Plant Production, Mansoura Univ., Vol. 3 (7): 2237 - 2248, 2012

MICROPROPAGATION OF GLOBE ARTICHOKE PLANT (*Cynara scolymus* L.).
1- EFFECT OF SODIUM HYPOCHLORITE CONCENTRATIONS, CYTOKININS AND SUBCULTURES NUMBER ON SHOOTS MULTIPLICATION RATE.

Dawa, K. K. [*]; W. A. El-Saady [*]; M. E. El-Denary [**] and I. M. Abo-Elglagel [**].

[*]Vegetables and Ornamentals Dept., Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

[**]Potato and Vegetatively Propagated Veget., Hort. Res. Inst., Agric. Res. Center, Egypt.

ABSTRACT

An efficient protocol for both development of aseptic tissue cultures and shoots regeneration for *Cynara scolymus* L. cv. Herious was established by using *in vitro* micropropagation technique. Meristem tips of good young offshoots from selected globe artichoke plants were used as explants. Dipping shoot tips (1-2 cm length) in 70% ethanol for 5-10 seconds followed by 0.1% $HgCl_2$ (W/v) for 2 minutes and then in 3% sodium hypochlorite for 20 minutes was the most effective sterilizing and disinfectant treatment for surviving the majority of meristem tip cultures after 5 weeks of culturing. In the establishment stage, adding 0.5 mg thidiazuron (TDZ) /l to MS basal medium gave the best values for both shoots and leaves number but registered middle values for average shoots length compared to the other treatments. Concerning the multiplication stage, among the different cytokinin types and concentrations used such as 2iP, BA and Kin (at 0.5, 2.5 and 5.0 mg /l for each) and TDZ (at 0.05, 0.5 and 1.0 mg /l) in addition to control treatment (hormone-free MS medium), 2iP with all concentrations recorded the highest values in this respect in terms of shoots and leaves number and average shoots length. The multiplication rate of proliferated globe artichoke shoots and average shoots length were markedly increased with increasing subcultures number on MS basal medium augmented with 5.0 mg 2iP /l + 1.0 mg IAA /l till the fourth subculture then declined thereafter during the fifth one.

Keywords: Globe artichoke, *Cynara scolymus*, Micropropagation, *in vitro* multiplication, Cytokinins.

INTRODUCTION

Globe artichoke (*Cynara scolymus* L.) is a small genus belonging to the Asteraceae family (Wiklund, 1992); it comprises seven species native to the Mediterranean Sea basin. It is a perennial, frost sensitive and cross pollinated vegetable. Globe artichoke heads or capitula, which are immature composite inflorescences, are the edible part of the plant and are used worldwide as fresh, frozen or canned delicacy (Sidrach *et al*. 2005).

Globe artichoke plays an important role in human nutrition, particularly in the Mediterranean diet. Since its flower heads are prepared for different value-added products, such as salad and jam. Moreover, leaf extracts are widely

used alone or in association with other herbs for embittering alcoholic and soft drink and to prepare herbal teas or herbal medicine products. The extracts from the leaf and capitula can also be used for the preparation of beauty creams and milk coagulants used in the preparation of traditional cheese. Furthermore, it has been demonstrated that artichoke is a promising source of oil from seeds, both with respect to quality and quantity, while the residual flour following oil extraction can be used as component of animal feed, due to its high proteins content. The oil has (i) a high and well balanced content of oleic and linoleic acids, (ii) a low content of free acids, peroxides, saturated and linoleic acids, and (iii) a favourable-tocopherol content, which provides a good level of protection against oxidation (Maccarone *et al*. 1999). For all these, the Ancient Greeks and Romans considered artichokes as both a delicacy and an aphrodisiac.

Globe artichoke contributes significantly to the Mediterranean agricultural economy, with an annual production of about 750 Mt (more than 60% of global production) from over 80kha of cultivated land. Italy is the leading world producer (about 470 Mt), followed by Spain (188 Mt), France (52.5 Mt) and Greece (35 Mt). In southern Europe, artichoke production is an important component of regional economic stability and social development and, thanks to its long growth cycle, its cultivation provides employment almost the whole year round. Globe artichoke is also cultivated, although to a lesser extent, in the Near East (Turkey and Iran), North Africa (Egypt, Morocco, Algeria, Tunisia), South America (Argentina, Chile and Peru), and the United States (mainly in California), and its cultivation is spreading in China (55 Mt in 2005) (FAO data 2005: http://faostat.fao.org/).

Outside of Europe, the most commonly grown cultivars are ‘Bamafsigi’, ‘Baladi’ and ‘Violet de Provence’ (the Nile delta region), the Turkish early ‘Sakiz’ and late ‘Bayrampasa’, grown, respectively on the Aegean coast and in Marmara (Ercan *et al*. 2004). In Algeria, Morocco and Tunisia, most cultivars have been introduced from other Mediterranean countries.

In Egypt, globe artichoke cultivated area was 21203.42 feddan in 2010, which produced 215534 ton with an average of 10.165 ton/feddan (FAO data 2010: http://faostat.fao.org/).

Globe artichoke propagation can be made by sexual (seeds) or asexual (vegetative propagation) means. Since sexual propagation produces high level of heterogeneity, therefore, it often not used at commercial scale. Also, artichoke is traditionally propagated vegetatively by offshoots and crown segments and this type of propagation presents some inconveniences such as; transmission of diseases through the mother plants used in propagation, low multiplication rate (around 5 offshoots for plant per year), disuniformity due to the physiological differences among shoots resultant from the same mother plant, elevated costs of matrix implantation and low possibility of mechanization.

The micropropagation offers an alternative method for large, health, homogeneous and disease-free plants production of globe artichoke (Ancora *et al*. 1981, Pecaut and Dumas de Vaulx, 1983 and Rossi and Paoli, 1992) supporting *in vitro* multiplication of selected genotypes. Moreover,

propagation through meristem culture has been widely applied in globe artichoke late types that producing capitula only during spring and early summer than early types that produce capitula between autumn and spring. As well as, the obtained mother virus free plants may represent a source for the production of sanitary controlled propagative material. Furthermore, plants obtained in this way have shown improved field performance with respect to both qualitative and quantitative traits, and this can compensate for the higher cost of the planting material (Saccardo *et al*. 2007).

The aim of this work was to evaluate the effect of sodium hypochlorite concentrations to control explant contaminations, improving the multiplication rate of globe artichoke shoots on different MS culture media and the effect of subcultures number on shoots multiplication rate.

MATERIALS AND METHODS

1. Plant material and preparation of explants

Good offshoots (10-15 cm long and 3-4 cm diameter) developed from healthy and strong globe artichoke plants which characterized with early production of the immature composite inflorescences called heads or capitula were separated in the middle of March (Bekheet, 1992) from these mother plants and used as the source of explants. These offshoots were put in glass container containing a few amount of concentrated soap solution and were thoroughly washed under running tap water for 30 minutes (outside the culture cabinet). After this step, the shortened offshoots were transferred to inside the culture cabinet, since they were trimmed and shortened then surface sterilized by immersion in 70 % ethanol for 5-10 seconds followed by a treatment with 0.1% $HgCl_2$ (W/v) for 2 minutes and then rinsed very thorough in sterile distilled water.

2. Tissue culture media

The used basal nutrient medium in all experiments was Murashige and Skoog, 1962 (MS medium with vitamins mixture) supplemented with Glysine (2 mg/l), NaH2PO4. H2O (50 mg /l), L-tyrosine (100 mg /1), Myo-inositol (100 mg /1), Adenine- hemi-sulphate (40 mg /l), Sucrose (40 g /l) and solidified with 7 g Agar agar /l (w/v) at pH 5.8.

3. Establishment of artichoke tissue culture

3.1. Sodium hypochlorite concentrations

Different concentrations of sodium hypochlorite (0, 1, 2 and 3%) were used for 20 minutes with several drops of wetting agent (Tween 20). Thereafter, they were rinsed several times in sterile distilled water. Finally, the explants were kept in sterile antioxidant solution containing ascorbic and citric acid at 150 ppm each (El-Saady, 2000). Meristem tips at 1-2 mm in length were excised with a small part of submeristematic tissues under a binocular and cultured in 200 ml jars containing 30 ml of MS medium with vitamins supplemented with the above mentioned additives plus 0.5 mg TDZ /l and 1.0 mg IAA /l. Data in terms of survival and contamination percentage for meristem tip cultures were recorded after 5 weeks.

3.2. Cytokinin type

Meristem tips which sterilized with the best sterilization treatment as previously mentioned were cultured into 200 ml jars, containing 30 ml of MS medium augmented with 5 mg Kin /l or 0.5 mg TDZ /l or hormone-free MS medium as a control and each treatment consist of 20 replicates. Data as a number of shoots and leaves /explant and shoots length were registered after 2, 4 and 6 weeks.

4. Different cytokinin types and concentrations on in vitro multiplication

All small shoots which developed from the previous experiment were transferred to 300 ml jars containing 30 ml of hormone-free MS medium for 3 weeks. Then, the resultant small shoots were cultured into 300 ml jars contained 40 ml of MS medium amended with different cytokinin types (2iP, BA and Kin) at different concentrations (0.5, 2.5 and 5.0 mg /l for each) and TDZ (0.05, 0.5 and 1.0 mg /l) plus control treatment (hormone-free MS medium). Each treatment consisted of 15 replicates. Data of culturing as a number of shoots and leaves /explant and shoots length were recorded after 5 weeks.

5. Subculturing numbers on multiplication rate

Shoots (4-6 cm in length) obtained from the best treatment of the previous multiplication stage were subcultured individually in jars contained 40 ml of MS medium supplemented with the best cytokinin type and concentration obtained from the previous multiplication experiment. Multiplication rate in terms of number of shoots and leaves /explant and average shoots length were recorded at the end of each subculture (after 5 weeks). Each subculture was represented by 20 cultured jars, one shoot each.

6. Culture conditions

All culture media were adjusted to pH 5.8 before autoclaving and autoclaved for 20 minutes at 121 ^{o}C and 1.2 kg /cm2. The cultures of the different experiments were maintained in a growth chamber at 25 ± 2 ^{o}C and exposed to 16 hr photoperiod at an intensity of 2000 Lux from white fluorescent light lamp during establishment and multiplication stages.

7- Experimental design and statistical analysis

All experiments were designed in completely randomized design and the analysis of variance (ANOVA) was performed to test the significance of the differences between treatments. When significant differences were found ($P \leq 0.05$), a multiple comparison test of means (Duncan's multiple range test) was calculated (SPSS, 2001).

RESULTS AND DISCUSSION

1. Establishment of tissue culture

1.1. Effect of sodium hypochlorite concentrations

Data presented in Table (1) show the effect of sodium hypochlorite concentrations on survival and contamination percentage of globe artichoke shoot tip explants cultured 5 weeks on MS medium supplemented with 0.5 mg TDZ /l and 1.0 mg IAA /l.

It is clear from this data that there are significant differences between treatments and control concerning the survival and contamination

percentage. Since, the disinfectant treatment consisting 3% sodium hypochlorite gave the best results in this concern (85% survival and 15% contamination), followed by the treatment comprising 2% sodium hypochlorite (75% survival and 25% contamination) compared to the lowest survival and contamination percentage (0% and 100%, respectively) for control treatment. The obtained results go in line with the findings of Alphonse *et al*. (2002) who reported that dipping meristem tips of some artichoke genotypes in 70% ethanol + 1.5% sodium hypochlorite + mixture of streptomycin and gentamycin at 50 mg /l of each was the most effective sterilizing and disinfectant treatment for the survival of all tested artichoke genotypes after 4 weeks of culturing on MS medium. Also, such results are in agreement with those obtained by Elia *et al*. (2007) on the early artichoke cultivar 'violet du provence' who had attained the lowest level of shoot apices mortality after they had immersed them as explants in 2.5% sodium hypochlorite solution for 20 min.

Table (1): Effect of sodium hypochlorite (NaClO) concentrations on the percentage of survival and contaminated meristem tip cultures of globe artichoke after 5 weeks of culture.

NaClO concentration	Survival (%)	Contamination (%)
0 % sodium hypochlorite (Control)	0 c	100 a
1 % sodium hypochlorite	32 b	68 b
2 % sodium hypochlorite	75 a	25 c
3 % sodium hypochlorite	85 a	15 c

Each value is the average of 10 replicates (one explant per replicate).
Values within each column followed by the same letter are not significantly different by Duncan's multiple range test (p=0.05).

2. Effect of MS medium type

In regard to the effect of MS medium augmented with kin at 5 mg /l or TDZ at 0.5 mg /l on shoots and leaves number and shoots length at 2, 4 and 6 weeks after culturing, the obtained and presented results in Table (2) and Fig. (1) indicated that the MS medium supplied with TDZ at 0.5 mg /l was the best treatment in this connection. Whereas, the control treatment gave the lowest results in this regard. The MS medium provided with 5 mg /l kin recorded middle results especially with shoots and leaves number regardless of the shoots length, since, it gave the highest values for this parameter .

These results are in general agreement with those found by El-Saady (2000) on artichoke cv. Balady who mentioned that TDZ gave the best results for shoots and leaves number compared with the other cytokinin treatments in this respect. Likewise, these results are consistent with those of Bhuiyan *et al*. (2009) on taro.

The increment in number of shoots and leaves and average shoots length might be due to effect of cytokinin type on cells division and formation of axillary buds through activating DNA synthesis. This in turn promotes the growth of lateral buds and shoots formation (Tisserat, 1985, Pierik, 1987 and Torres, 1989).

Table (2): Effect of MS medium supplemented with Kin at 5 mg/l or TDZ at 0.5 mg/l or hormone-free MS medium on development of globe artichoke explants.

Treatments	Characters		
	Shoots number	Leaves number	Shoots length (cm)
2 weeks after			
Control (MS only)	1.00 c	1.66 b	1.00c
MS + Kin at 5 mg/l	1.40 b	4.06 a	1.97 a
MS + TDZ at 0.5 mg/l	1.80 a	5.00 a	1.34 b
4 weeks after			
Control (MS only)	1.00 b	2.66 c	1.17 c
MS + Kin at 5 mg/l	1.60 ab	5.20 b	2.78 a
MS + TDZ at 0.5 mg/l	2.43 a	10.77 a	2.24 b
6 weeks after			
Control (MS only)	1.33 b	2.67 c	1.33 c
MS + Kin at 5 mg/l	1.73 ab	7.44 b	3.15 a
MS + TDZ at 0.5 mg/l	2.77 a	13.00 a	2.24 b

Each value is the average of 20 replicates (one explant per replicate).

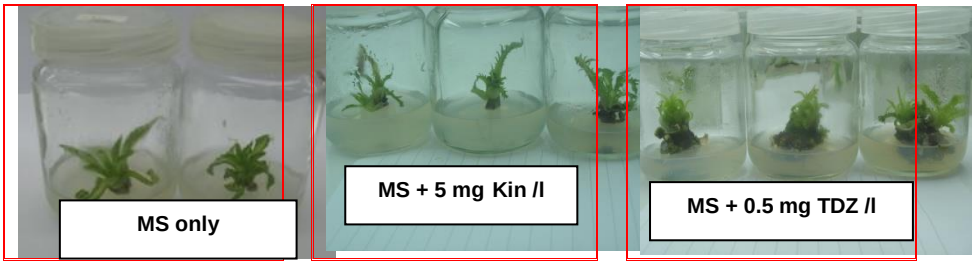


Fig. (1): Effect of MS medium supplemented with Kin at 5 mg/l or TDZ at 0.5 mg/l or hormone-free MS medium on development of globe artichoke explants.

3- Effect of different cytokinin types and concentrations on in vitro multiplication

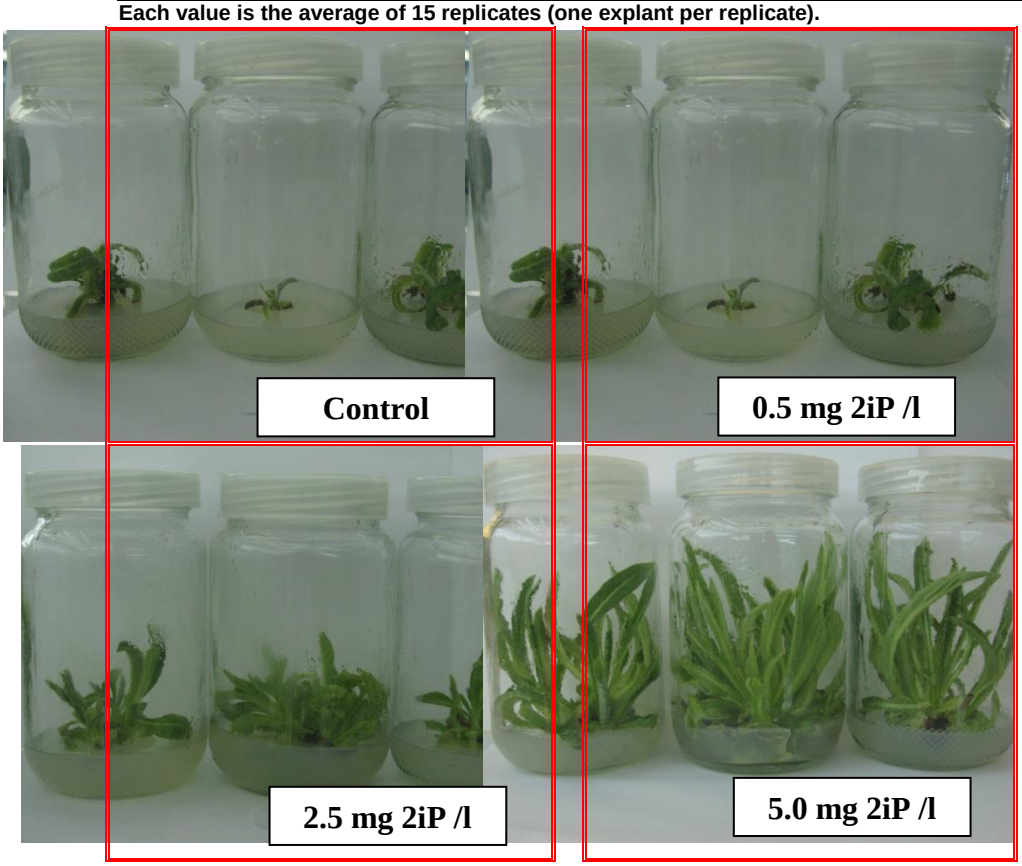
Results presented in Table (3) and illustrated in Fig. (2) clearly indicated that among the tested cytokinin types and concentrations, the treatment of 5 mg 2ip /l was the most effective one, since it recorded the highest number of shoots and leaves as well as average shoots length (13.50, 37.83 and 5.33 cm, respectively) followed by 2ip at 2.5 mg /l (10.5, 30.8 and 2.83). The least treatment in this respect was control treatment, since registered 1.6, 4.27 and 1.9 cm for these characters, respectively. The other treatments gave middle values between these two extremes. Generally, application of different cytokinin types and concentrations to MS medium recorded higher multiplication rate as compared to the control treatment (hormone-free MS medium).

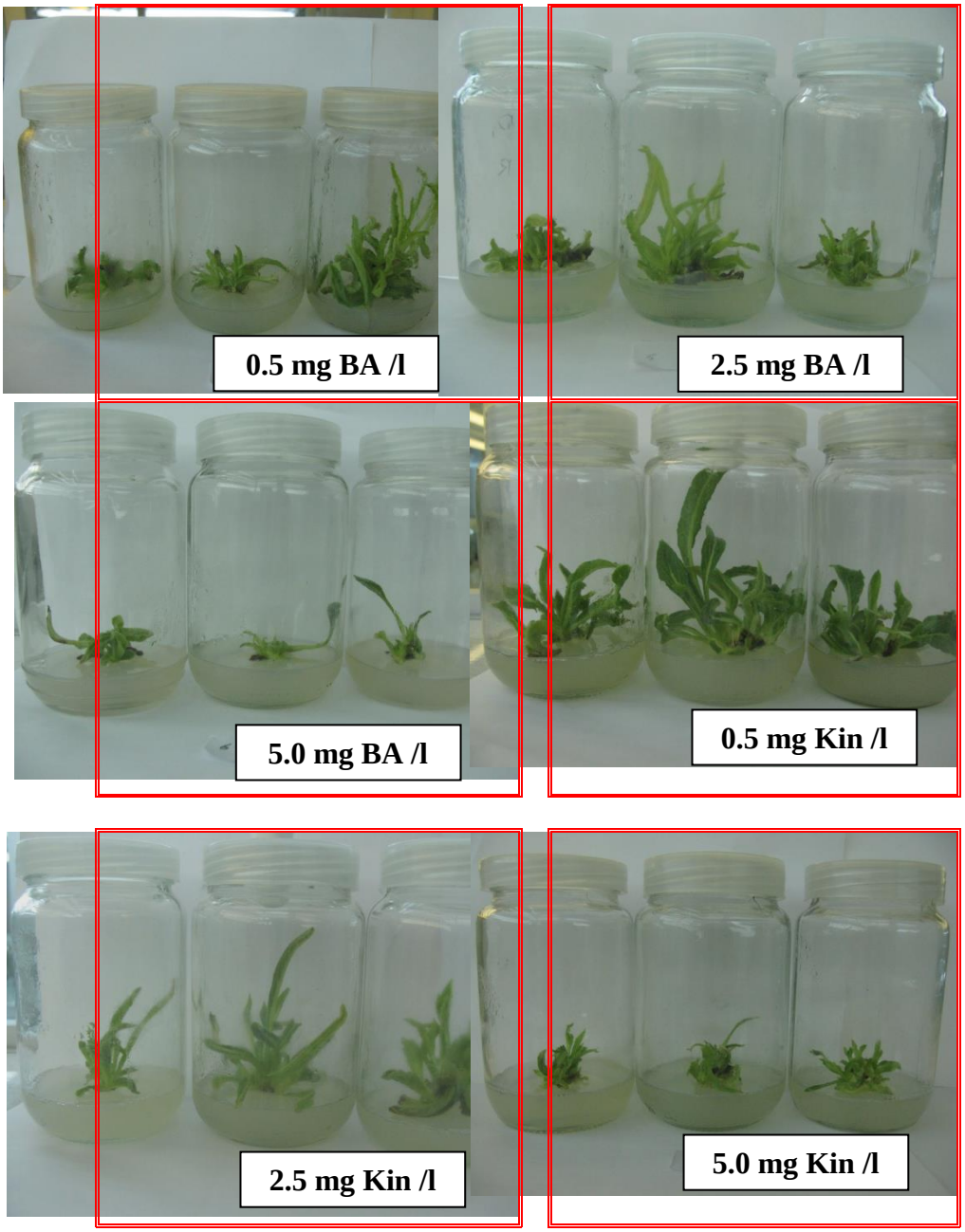
These results are in complete agreement with those obtained by Ismaeel (1995) who found increment in shoots multiplication rate using MS medium containing 5 mg Kin /l + 0.5 mg IAA /l. However, this investigator pointed to a gradual multiplication rate and shoot length by increasing the concentration of BA. Also, similar results were obtained by El-Saady (2000) using different cytokinin types and concentrations on artichoke cv. Balady.

Table (3): Effect of different cytokinin types and concentrations on shoots and leaves number and average shoots length of globe artichoke explants cultured 5 weeks on MS medium.

Treatments		Measurements		
		Shoots number	Leaves number	Shoots length (cm)
control		1.60 i	4.27 k	1.90 d
2ip	(0.5 mg/l)	7.40 e	19.60 c	2.47 c
	(2.5 mg/l)	10.50 b	30.80 b	2.83 b
	(5.0 mg/l)	13.50 a	37.83 a	5.33 a
BA	(0.5 mg/l)	7.87 d	16.40 e	2.50 c
	(2.5 mg/l)	8.40 c	17.30 d	2.53 c
	(5.0 mg/l)	5.20 h	11.10 i	2.37 c
Kin	(0.5 mg/l)	6.10 g	17.40 b	2.67 bc
	(2.5 mg/l)	6.60 f	19.30 c	2.90 b
	(5.0 mg/l)	7.60 de	15.20 f	1.90 d
TDZ	(0.05 mg/l)	6.60 f	13.10 g	1.77 d
	(0.5 mg/l)	5.97 g	11.77 h	1.83 d
	(1.0 mg/l)	5.20 h	10.40 j	1.90 d

Each value is the average of 15 replicates (one explant per replicate).




0.5 mg BA /l
2.5 mg BA /l
5.0 mg BA /l
0.5 mg Kin /l
2.5 mg Kin /l
5.0 mg Kin /l

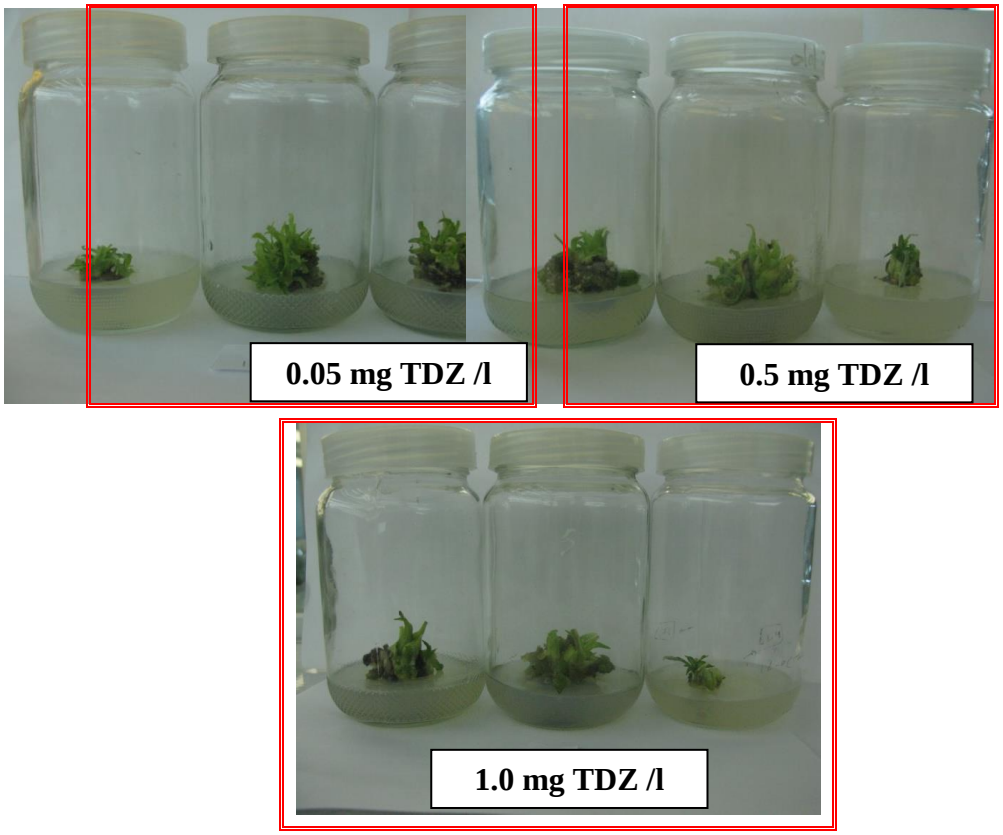


Fig. (2): Effect of different cytokinin types and concentrations on shoots and leaves number and average shoots length of globe artichoke explants cultured 5 weeks on MS medium.

4- Effect of subculturing numbers on multiplication rate

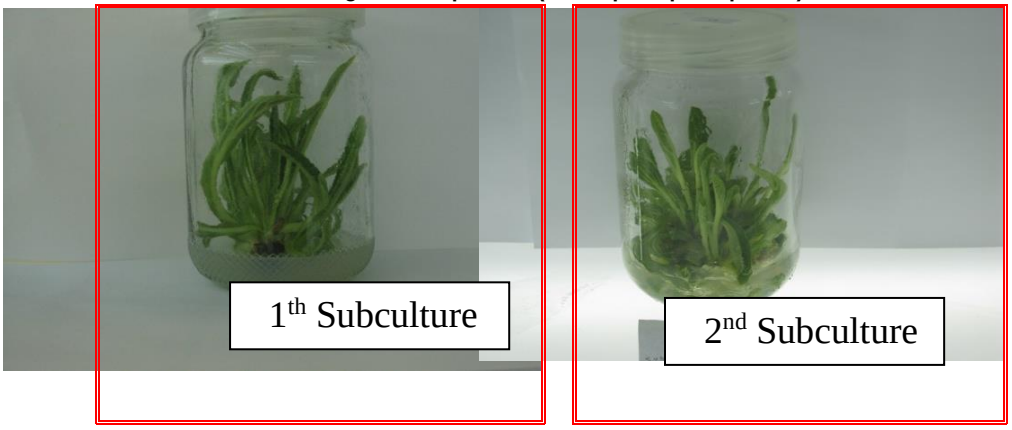
Data in Table (4) and Fig (3) show the effect of subcultures number on improvement of multiplication rate of globe artichoke shoots. Data indicated that increasing number of subcultures gradually increased number of shoots and leaves as well as shoots length till 4th subculture, which recorded the highest shoots numbers (19.13) with highest leaves number (47.16) and longest shoots (5.40 cm), and then declined thereafter.

These obtained results go in line with the findings of El-saady (2000) on artichoke. He pointed out that shoots multiplication rate gradually increased with increasing the number of subcultures till the fourth subculture, and then declined thereafter. Also, Alphonse *et al*. (2002) noticed that, number of shoots and leaves and shoost length were increased as the number of subculture increased till third subculture on MS medium supplemented with 1.0 mg IAA /l + 5.0 mg Kin /l on artichoke cv. Violetto then declined in the subsequent ones.

Table (4): Effect of subcultures number on multiplication rate improvement of globe artichoke shoots after each 4 weeks of culture on fresh MS medium supplemented with 5 mg 2iP /l.

Treatments	multiplication rate		
	Shoots number	Leaves umber	shoots length (cm)
1st subculture	13.50 c	37.5 b	5.33 a
2nd subculture	15.50 b	39.53 b	5.03 ab
3rd subculture	18.40 a	45.33 a	5.16 ab
4th subculture	19.13 a	47.16 a	5.40 a
5th subculture	15.00 bc	40.26 b	4.00 a

Each value is the average of 20 replicates (one explant per replicate).



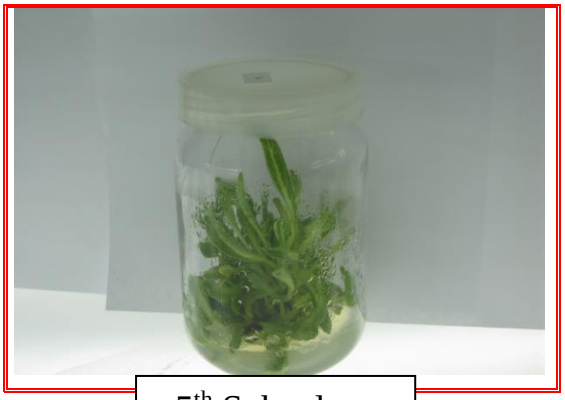


Fig. (3): Effect of subcultures number on shoots and leaves number and shoots length of globe artichoke cultured 4 weeks on MS medium supplemented with 5 mg 2iP /l.

REFERENCES

Alphonse. M, M. A. Badawi and T. M. Abd-Elal (2002): physiological studies on the *in vitro* micropropagation of some Artichoke genotypes. 1- Sterailization, multiplication and elongation stages. Egypt. J. Hort. 29, No.3-4 pp. 377-395.

Ancora, G., M.L. Bell-Donini and L. Cuozzo (1981): Globe artichoke plants from shoot apices through rapid *in vitro* micropropagation. Sci. Hort., 14: 207–213.

Bekheet, S. A (1992): Studies on micro-propagation of globe artichoke through tissue culture techniques. M. Sc. Thesis, Hort. Dept., Faculty of Agriculture, Ain Shams University.

Bhuiyan, M. K. R; M. M. Hossain and M. Zakaria (2009): Effect of Thidiazuron and Benzyl amino purine on *in vitro* multiple shoot development in *Colocasia esculenta* ((L.) Schott) cultivar "Bilashi"

Elia. A, G. Conversa; C. Montervino and C. Lotti (2007): micro-propagation of the early Artichoke cultivar ‹Violet du provence › Acta Hort. 630: 127-134.

El-Saady, W. A. M (2000): Studies on Globe artichoke plant propagation by tissue culture technique. M. Sc. Thesis Fac. Agric., Mansoura Univ., Egypt, 92 P

Ercan, N., A. N. Onus, E. Polat, F. Ayar, M. Temirkaynak, and A. S. Şensoy (2004): Determination of optimum GA3 concentrations and awakening irrigation time for globe artichoke (*Cynara scolymus* L. cv. Sakýz) grown in mediterranean region of Turkey. Acta Hort. 660:197-200.

Ismaeel, Sh. A. (1995): Performance of globe artichoke plants developed from tissue culture under low plastic tunnel. M. Sc. Thesis, Hort. Dept., Fac. of Agric., Ain Shams Univ.

Maccarone, E., B. Fallico, F. Fanella, G. Mauromicale, SA. Raccuia and S. Foti (1999): Possible alternative utilization of Cynara spp. II. Chemical characterization of their grain oil. INDUSTRIAL CROP PRODUCTION 10, 229-237

Murashige, T. and F. Skoog (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473 -497

Pecaut, P. and R. Dumas de Vaulx (1983): Virus-free clones of globe artichoke (*Cynara scolymus* L.) obtained after *in vitro* propagation. Acta Hort., 131: 303–9

Pierik, R. L. M. (1987): *In vitro* culture of higher plants. 2nd ed., Martinus Nijhoff Publishers. Dordrecht, The Netherlands.

Rossi, V. and De. Paoli (1992): Micropropagation of artichoke (*Cynara scolymus*). In: Bajaj, Y.P.S. (ed.), Biotechnology in Agriculture and Forestry, Vol. 19. High-Tech and Micropropagation III. Springer-Verlag Berlin, Heidelberg.

Saccardo, F., F. Micozzi, G. Di Lernia, C. Piccioni, M. Barba and M. A. Pagnotta (2007): Virus free artichoke germplasm: quali-quantitative response of globe artichoke. Acta Hort. 730:375-379.

Sidrach, L., F. Garcia-Canovas, J. Tudela and J. Rodriguez-Lopez (2005): Purification of cynarases from artichoke (Cynara scolymus L.): enzymatic properties of cynarase A. PHYTOCHEMISTRY, 66, 41-49.

SPSS, (2001): SPSS for Windows Version: 12. Syntax Reference Guide. SPSS Inc., Chicago, IL, USA.

Tisserat, B. (1985): Embryogenesis, organogenesis and plant regeneration. In: Plant cell culture. (ed. R. A. Dixon) pp. 79-105, IRL-Press. Oxford, England.

Torres, K. C. (1989): Tissue culture techniques for Horticultural crops. An Avi. Book, Van Nostrand Rein Hold. New York, pp. 52-65.

Wiklund, A. (1992): The genus Cynara L. (Asteraceae-Cardueae). Bot. J. Linn. Soc. 109: 75-123.

الإكثار الدقيق لنبات الخرشوف.

أ- تأثير تركيزات هيبوكلورايت الصوديوم، السيتوكينينات وعدد مرات إعادة الزراعة على معدل تضاعف الأفرع الخضرية.

كوثر كامل ضوه * ، وليد على السعدي * ، محمد عراقي الديناري ** و إبراهيم محمد أبو الجلاجل **

*** قسم الخضر والزينة، كلية الزراعة، جامعة المنصورة، المنصورة، مصر.**

**** قسم بحوث البطاطس ومحاصيل الخضر خضرية التكاثر، معهد بحوث البساتين، مركز البحوث الزراعية، الدقي، مصر.**

تم تأسيس بروتوكول فعال لكل من تكوين مزارع نسيجية معقمة وتضاعف النموات الخضرية للخرشوف باستخدام تقنية الإكثار المعملي الدقيق. ولقد استخدمت القمم المرستيمية المجهزة من خلفات صغيرة جيدة متحصل عليها من نباتات خرشوف منتخبة كأجزاء نباتية. ولقد وجد أن غمس

القمم النامية التي بطول يتراوح ما بين ١-٢ سم في محلول ٧٠% إيثانول لمدة ٥-١٠ تواني متبوعة بنقعها في ٠،١% كلوريد زئبقيك لمدة دقيقتين ثم في محلول تركيزه ٣% هيبوكلورايت الصوديوم لمدة ٢٠ دقيقة مع الرج المستمر كانت معاملة التعقيم الأكثر فعالية في بقاء غالبية مزارع القمم المرستيمية حية وجيدة غير ملوثة خلال فترة الخمس أسابيع من زراعتها. في مرحلة البداية، وجد أن إضافة ٠،٥ ملجم ثيديازيرون لكل لتر من بيئة موراشيج وسكوج الأساسية قد أعطى أفضل القيم لكل من عدد الأفرع الخضرية والأوراق الناتجة من كل جزء نباتي منزرع بالإضافة إلى قيم متوسطة بالنسبة لطول الأفرع الخضرية النامية. أما فيما يتعلق بمرحلة التضاعف، فإنه من بين الأنواع والتركيزات المختلفة للسيتوكينينات المستخدمة مثل الأيزوبنتانيل أدنين، البنزايل أدنين والكينتين بتركيزات (٠،٥، ٢,٥ و ٥,٠ ملجم لكل لتر من كل منهم) والثيديازيرون بتركيز (٠،٠٥، ٠,٥ و ١,٠ ملجم لكل لتر) هذا بالإضافة إلى معاملة الكنترول (بيئة موراشيج وسكوج خالية من الهرمون)، وجد أن الأيزوبنتانيل أدنين بجميع تركيزاته قد سجل القيم الأعلى في عدد الأفرع الخضرية والأوراق الناتجة وكذلك لمتوسط طول تلك الأفرع الخضرية. هذا ولقد زاد معدل تضاعف الأفرع الخضرية للخرشوف وكذلك طول تلك الأفرع بشكل ملحوظ مع زيادة عدد مرات إعادة زراعة الأفرع الخضرية على بيئة جديدة من موراشيج وسكوج تحتوي على ٥,٠ ملجم أيزوبنتانيل أدنين + ١,٠ ملجم أندول -٣- حامض الخليك وذلك حتى المرة الرابعة ثم حدث تناقص لتلك الصفات المدروسة في المرة الخامسة من إعادة الزراعة علي البيئة الجديدة.

قام بتحكيم البحث

أ.د / حسام السعيد عبد النبى	**كلية الزراعة – جامعة المنصورة**
أ.د / المهدى ابراهيم على متولى	**كلية الزراعة – جامعة كفر الشيخ**