

IN VITRO MICROPROPAGATION OF GLOBE ARTICHOKE PLANT (*Cynara scolymus* L.):

1- EFFECT OF SODIUM HYPOCHLORITE CONCENTRATIONS, CYTOKININS AND SUBCULTURES NUMBER ON SHOOTS MULTIPLICATION RATE.

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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 long) in 70% ethanol for 5-10 seconds followed by 0.1% HgCl₂ (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 TDZ /l to Murashige and Skoog, 1962 (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. The Ancient Greeks and Romans considered artichokes as both a delicacy and an aphrodisiac. In 77CE, the Roman naturalist Pliny the Elder mentioned their use for nutritional and medicinal purposes, but it was most probably between 800 and 1500CE that the artichoke was domesticated and transformed, presumably in monastery gardens, into the plant which we know today (Prohens and Nuez, 2008). 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.

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 early globe artichoke plants in capitula production were separated in the mid 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% HgCl₂ (W/v) for 2 minutes and then rinsed very thorough in sterile distilled water.

2- Tissue culture media

The used nutrient medium in all experiments was Murashige and Skoog, 1962 (MS medium with vitamins mixture) supplemented with Glycine (2 mg/l), NaH₂PO₄. H₂O (50 mg /l), L-tyrosine (100 mg /l), Myo-inositol (100

mg /l), Adenine- hemi-sulphate (40 mg /l), Sucrose (40 g /l) and solidified with 7 g Agar agar at pH 5.8.

3- Establishment of artichoke tissue culture

3-1-Effect of 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 % for meristem tip cultures were recorded after 5 weeks.

3-2- Effect of MS medium 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- Effect of different cytokinin types and concentrations on in vitro multiplication

All small shoots which developed from the previous experiment were transferred to 200 ml jars containing 30 ml of hormone-free MS medium for 3 weeks. Then, the resultant small shoots were cultured into 200 ml jars contained 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- Effect of 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 shoots length were recorded at the end of each subculture (after 5 weeks).

6- Culture conditions

All culture media were adjusted to 5.8 before autoclaving and autoclaved for 20 min at 121 °C. The cultures of the different experiments were maintained in a growth chamber at 25 ± 2 °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 obtained data were analyzed statistically using Duncan's multiple range test (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 new 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	Weeks after culture	Characters		
		Shoots number	Leaves number	Shoots length (cm)
Control (MS only)	2	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
Control (MS only)	4	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
Control (MS only)	6	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).

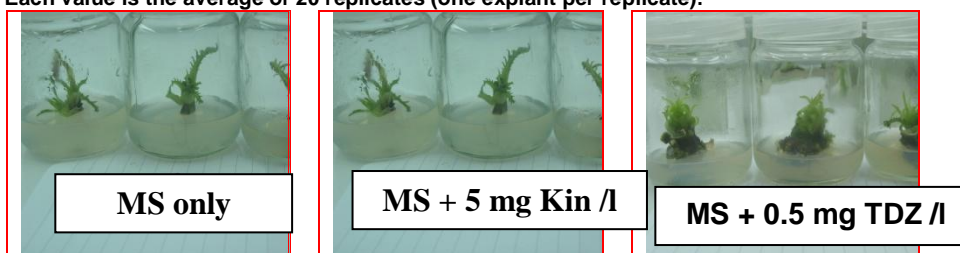


Fig. (1): Effect of MS medium supplemented with Kin at 5 mg/l or TDZ at 0.5 mg/l or hormone-free MS medium on development of globe artichoke explants.

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 Zip /l was the most effective one, since it recorded the highest number of

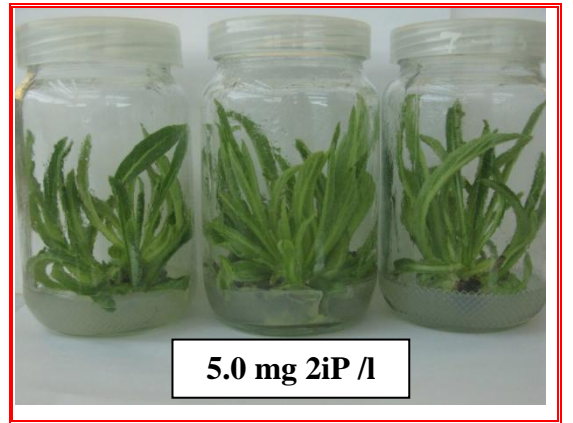
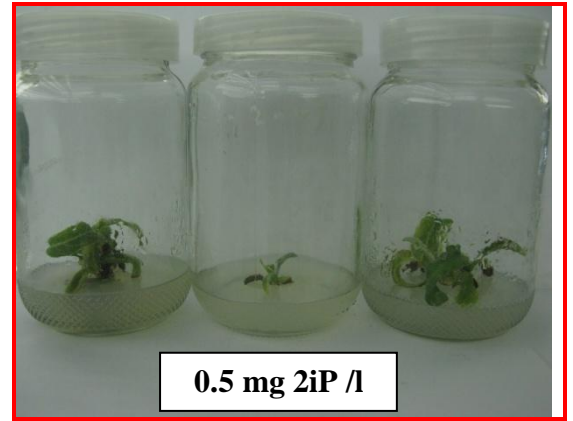
shoots and leaves as well as average shoots length (13.50, 37.83 and 5.33 cm, respectively) followed by 2ip at 2.5 mg/l (10.5, 30.8 and 2.83). The least treatment in this respect was control treatment, since registered 1.6, 4.27 and 1.9 cm for these characters, respectively. The other treatments gave 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).

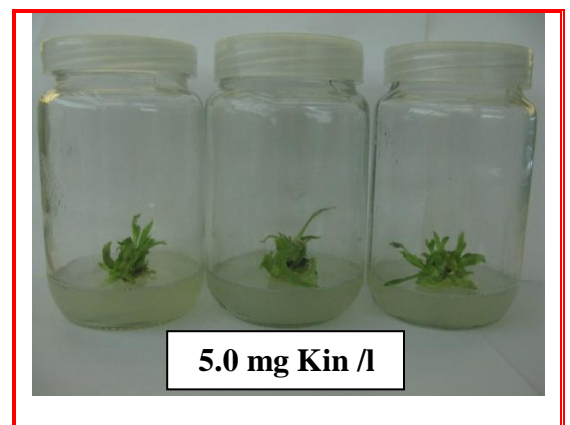
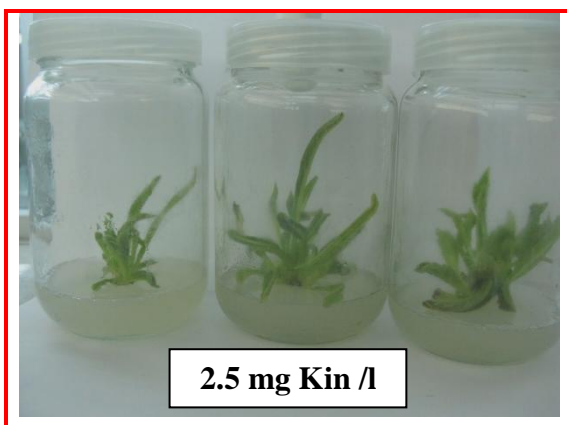
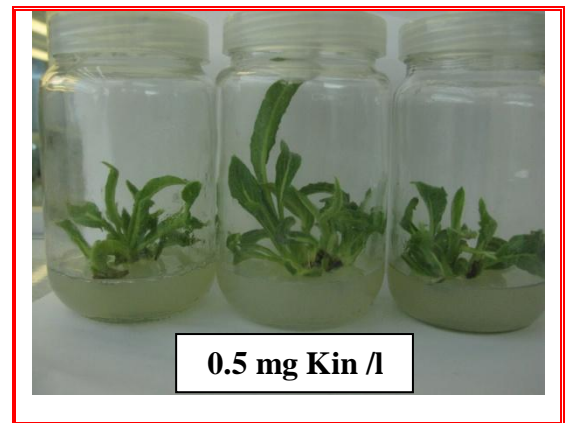
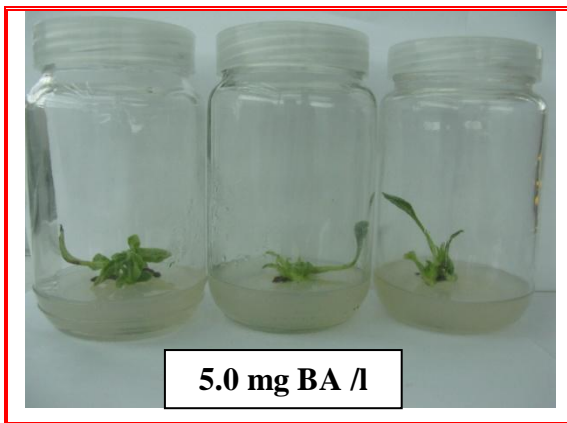
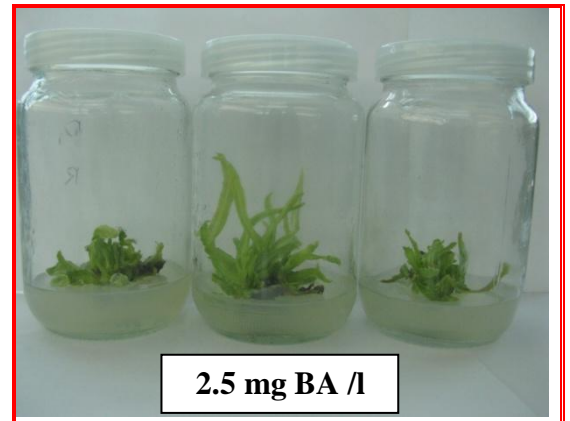
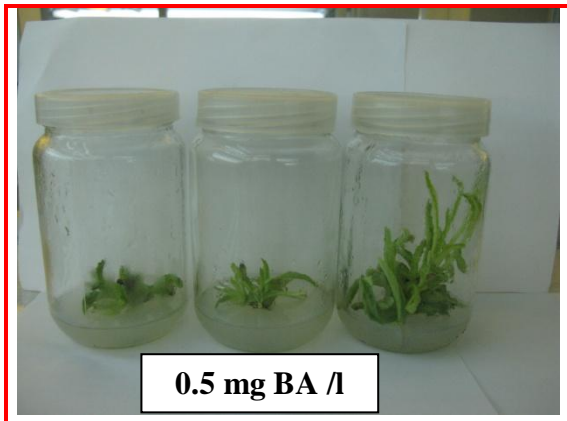
These results are in complete agreement with those obtained by **Ismaeel (1995)** who found increment in shoots multiplication rate using MS medium containing 5 mg Kin/l + 0.5 mg IAA/l IAA. However, this investigator pointed to a gradual multiplication rate and shoot length by increasing the concentration 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).





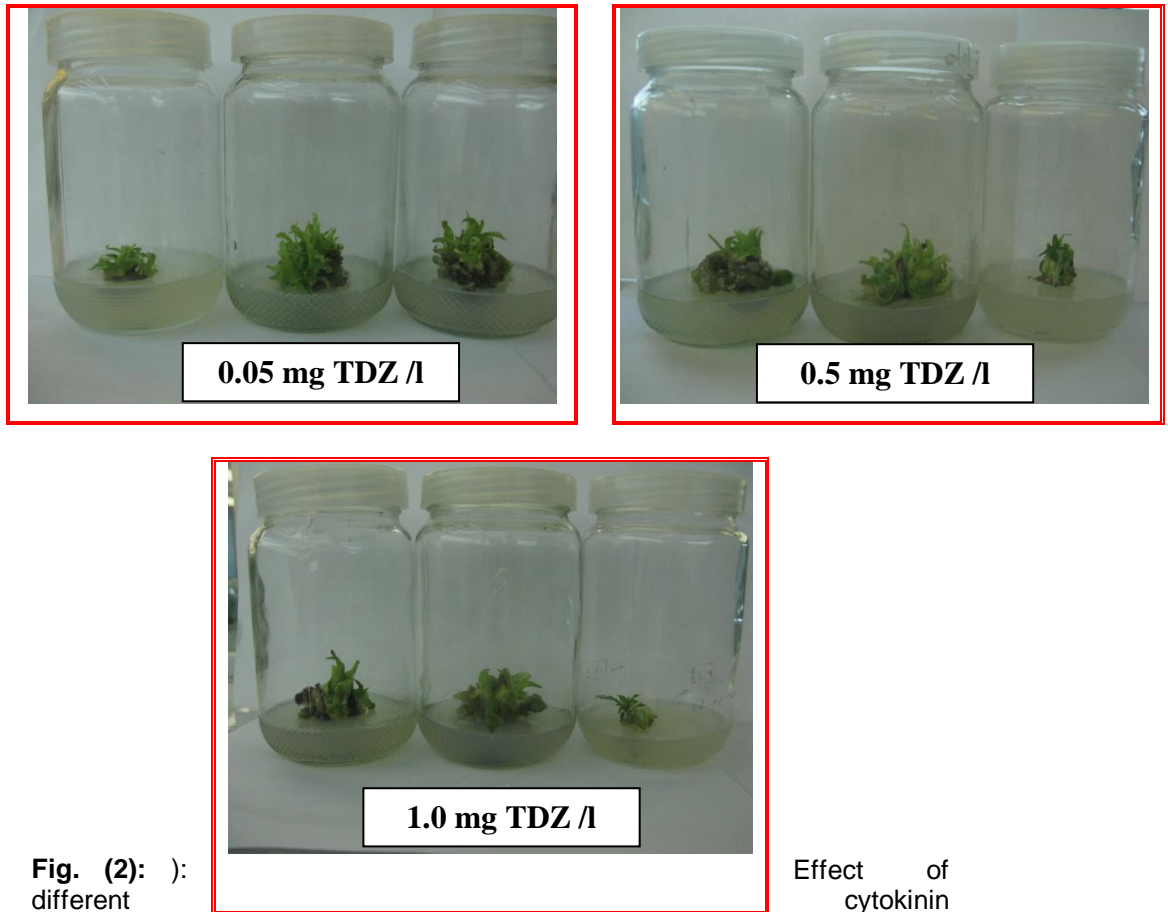


Fig. (2): Effect of different cytokinin types and concentrations on shoots and leaves number and average shoots length of globe artichoke explants cultured 5 weeks on MS medium.

4- Effect of subculturing numbers on multiplication rate

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 shoot 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)
1 st subculture	13.50 c	37.5 b	5.33 a
2 nd subculture	15.50 b	39.53 b	5.03 ab
3 rd subculture	18.40 a	45.33 a	5.16 ab
4 th subculture	19.13 a	47.16 a	5.40 a
5 th subculture	15.00 bc	40.26 b	4.00 a

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

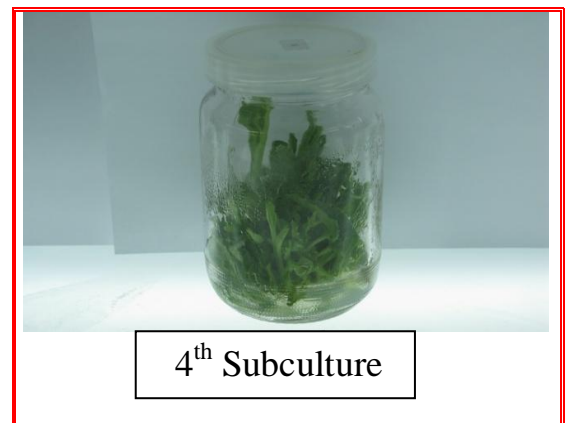
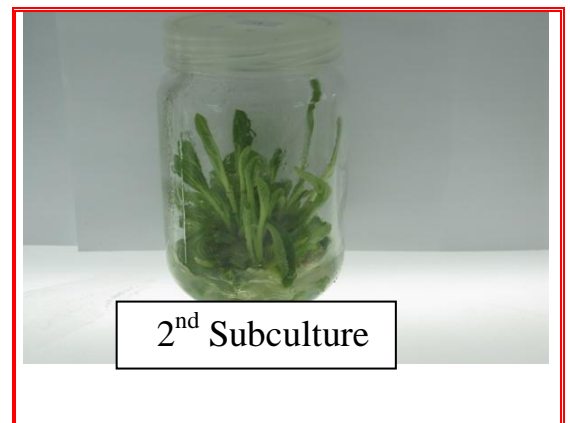




Fig. (3): subcultures shoots and

Effect of number of leaves

number and shoots length of globe artichoke cultured 4 weeks on MS medium supplemented with 5 mg 2iP /l.

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الإكثار الدقيق المعملّي لنبات الخرشوف. أ- مراحل التعقيم والتضاعف وإعادة الزراعة على أفضل بيئة تضاعف.

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تم تأسيس بروتوكول فعال لكل من تكوين مزارع نسجية معقمة وتضاعف النموات الخضرية للخرشوف باستخدام تقنية الإكثار المعملّي 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 1-2 سم في محلول 70% إيثانول لمدة 5-10 ثواني متبوعة بنقعها في 1,0% كلوريد زئبقيك لمدة دقيقتين ثم في محلول تركيزه 3% هيبوكلورايت الصوديوم لمدة 20 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 0,5 ملجم ثيديازيرون لكل لتر من بيئة موراشيغ 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، البنزاييل أدنين والكينتين بتركيزات (0,5، 2,5 و 5,0 ملجم لكل لتر من كل منهم) والثيديازيرون بتركيز (0,05، 0,5 و 1,0 ملجم لكل لتر) هذا بالإضافة إلى معاملة الكنترول (بيئة موراشيغ 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 موراشيغ وسكوج تحتوي على 5,0 ملجم أيزوبنتانيل أدنين + 1,0 ملجم أندول -3- حامض الخليك وذلك حتى المرة الرابعة ثم حدث تناقص لتلك الصفات المدروسة في المرة الخامسة من إعادة الزراعة على البيئة الجديدة.