Zagazig J. Agric. Res., Vol. 36 No. (6) 2009 1259-1272

SHOOT REGENERATION IN TISSUE CULTURES OF GLOBE ARTICHOKE (*Cynara scolymus* L.)

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Accepted 21/12 /2009

ABSTRACT: This investigation was conducted to study the effect of different concentrations of kinetin (0.0, 0.5, 1.0, 2.0, 3.0 and 4.0mg/l) on the morphogenic respone of globe artichoke through tissue culture technique.

All used kinetin concentrations caused a marked significant effect on both number of shoots and leaves per explant at 15,30 and 45 days of culture except number of leaves/ explant of 15 days of culture, as well as the fresh weight of shoots and average dry weight of shoot at 45 days of culture.

Kinetin at 0.5mg/l followed by kinetin at 2.0 mg/l. recoded the highest values of both number of shoots and leaves per explant. On the other hand, kinetin at 1mg/l being the most effective and favourable treatment for increasing the number of leaves per shoot. Moreover, kinetin at 0.5 mg/l was found to be the optimum concentration regarding fresh weight of shoots.

In addition, the maximum values of average dry weight of shoot were more achieved via application of kinetin to MS-medium at a concentration of 3.0 mg/L.

Key words: Artichoke, tissue culture, kinetine, MS-medium, seedlings, shoots.

INTRODUCTION

Globe artichoke (*Cynara scolymus* L.) is considered as one of the most important non traditional vegetable crops for local consumption and export in Egypt. Artichoke is usually propagated vegetatively by crown divison or offshoots, because the plants grown from seeds generally lack uniformity (Ibrahim *et al.*, 1981; Welbaum, 1994; Abd-Elall, 2003).

In addition, traditional propagation favours spread of diseases and develops a phytopathogenic situation hard to control, since field- grown plants can be easily attacked by several pathogen agents such as viruses, bacteria, fungi, nematodes and insects. However, *in vitro* culture allows the production of diseases free plants, which are of great value to artichoke (Rossi and De-Paoli, 1992).

The use of tissue culture methods is expected to help in solving some problems of genetic improvement in the globe artichoke and practices of cultivation, primarily by facilitating the production of healthy material and quicker multiplication (Ancora, 1986). Moreover, the absence of pathogens in the micropropagated plants, could however be sufficient reason to justify better plant growth and hence a better yield.

In general, the tissue culture micropropagation, beside favoring restoration and genetic improvements of the cultivated varieties, could lead to a worldwide increase in cultivation area.

On the other hand, kinetin is one of the cytokinins which are of special importance in plant tissue culture. The cytokinins are generally added to a culture medium to stimulate cell division to induce shoot formation and axillary shoot proliferation (Gamborg *et al.,* 1976). Moreover, Lauzer and Vieth (1990) found that the presence of kinetin in the basal multiplication medium resulted in higher number of shoots per explant than in kinetin free medium. Moreover; Krishnamoorthy (1981) reported that the number of cell divison increased proportionally to the concentration of added cytokinins, when auxin is not a limiting factor. George (1993) stated that cytokinins are very effective in promoting direct or indirect shoot intiation.

In addition, Dixon and Gonzales (1994) found that the presence of high levels of cytokinins during multiplication stage produced high number of shoots. Gamborg and Shyluk (1981) came to similar conclusion.

Similarly, Abd-El all (2003) on globe artichoke, found that the greatest number of shoots was produced in MS-medium supplemented with 1mg/l IAA+ 5 mg/l kinetin, followed by 1 mg/l IAA + 2.5 mg/l kinetin. He, also added that, the lowest number of

shoots was produced in hormone free medium (the control treatment). El-Shabasi (2007) stated that, in globe artichoke, the highest number of shoots per explant and fresh weight culture were resulted from using multiplication medium supplemented with 2.0 mg/l. kinetin + 1.0 mg/l IAA. Howeover, average fresh weight of shoot was not significantly affected by different concentrations of kinetin or 2ip.

Therefore, the present work was conducted to study the effect of different concentrations of kinetin on the morphogenic responses of globe artichoke through tissue culture technique (*in vitro* propagation).

MATERIALS AND METHODS

This experiment was conducted during the period from 2006 to 2008 in Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Zagazig University, to study the effect of different concentrations of kinetin on the morphogenic responses of globe artichoke (***Cynara scolymus*** L.) *in vitro* propagation through tissue culture technique. The Herious cv. plants were used as a source of explants.

Plant Material

The main vegetative shoots, which were about 10-15 cm in length offshoots were taken from selected and vigorously growing mother plants of Herious cv. These offshoots were obtained from the Experimental Farm of Faculty of Agriculture, Moshtohor, Benha University.

Preparation of Explants for Culture

The offshoots were carried out by removing the basal parts to a length till 5cm, the outer leaves were discarded, and the inner leaves were shortened.

The shoot tips were surrounded by 2-3 pairs of leaves and shortened to 2-3cm in length. The explants were washed under running tap water for 1 hour, and the superficial disinfected by dipping in ethanol (70%) for 30 second, followed by immersion in 30% (v/v) commercial bleach containing 5.25% sodium hypochlorite plus tween 20 (2 drops / 100 ml.) for 20 minute with agitation. Moreover, the explants were then rinsed three times using sterile distilled water. The shoot tips were then immerged in 0.1% mercuric chloride for 5 minute and

rinsed again several times using sterilized distilled water. The shoot tips were trimmed to 3-4 mm in length before inoculation on the nutrient medium. In order to avoid their oxidation, disinfected shoot tips were maintained in a sterile solution of 150 mg/l citric acid, and 100 mg/l ascorbic acid.

In order to obtain sufficient stocks of plant materials, needed for the followed experiment in establishment *in vitro* shoot culture, the sterile shoot tips (3-4 mm in length) were cultured for one month on basal MS (Murashig and Skoog, 1962), Table 1 medium supplemented with 2.0 mg/l 2ip, 0.5 mg/l IAA, 40 gm/l sucrose,and 7.0 gm/l agar (Mohamed, 1998). These shoot tips were subcultured for another one month on fresh MS medium contained 1.0mg/l kinetin.

The Treatments

This experiment included six concentrations of kinetin; i.e., 0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l added to the nutrient media, to study their effect on the morphogenic responses of globe artichoke shoot tips. The solid MS nutrient medium containing 30 gm/l sucrose, and 8 gm/l agar was used in this experiment. Uniform *in vitro* derived shoots were individually placed in 400 ml culture jars containing 50 ml of the various six treatments media.

These treatments were arranged in a complete randomized design system with four replicates. Each replicate contained three culture jars.

The cultures were maintained at 25±2°C and 16 hours photoperiods provided by white fluorescent lights at an intensity of 1000 lux.

Data Recorded

Data were recorded at 15, 30 and 45 days after culture as follows:

1 Number of shoots per explants,

2 Number of leaves per explants,

3 Number of leaves per shoot, and

4 Average shoot length (cm).

On the other hand, at 45 days of culture, the following data were recorded:

1 Fresh weight of shoots (g),

2 Dry weight of shoots (g),

3 Average fresh weight of shoot (g), and

4 Average dry weight of shoot (g).

Statistical Analysis

All collected data were subjected to statistical analysis of variance according to Snedecor

Table 1. Chemical composition of the used Murashige and Skoog basal nutrient medium

Consitituents	Concentration (mg/l)
Macronutrients:	
NH_4NO_3	1650
$KN\ O_3$	1900
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Micronutrients:	
$MnSO_4.4H_2O$	22.3
$ZnSO_4.4H_2O$	8.6
H_3BO_3	6.2
KI	0.83
$Na_2MO_4.H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Iron:	
Na_2EDTA	37.25
$FeSO_4.7H_2O$	27.85
Vitamins:	
Glycine	2.0
Nicotinic acid	0.5
Pyridoxine-HCl	0.5
Thyamine-HCl	

and Cochran (1980). The differences between various treatments means were tested by L.S.D. at 0.05 and 0.01 levels.

RESULTS AND DISCUSSION

Effect of Kinetin Concentration on Shoot Multiplication

The effect of different kinetin (kin) concentrations on number of shoots and leaves per explant, number of leaves per shoot as well as mean shoot length after 15,30 and 45 days of culture are presented in Table 2 and Fig. 1.

The obtained results reveale that kinetin treatments caused a marked significant effect on both number of shoots and leaves per explant and number of leaves per shoot in the three ages of culture, except number of leaves per explant at 15 days after culture and number of leaves per shoot at 30 days after

Table 2. Effect of kinetin concentration on the morphogenic responses of globe artichoke

Kinetin Concentration (mg/L)	No.of Shoots / explant			No. of leaves/ explant			No. of leaves/ shoot			Average shoot length (cm)		
	Days after culture											
	15	30	45	15	30	45	15	30	45	15	30	45
0.0	2.50	3.25	3.00	9.00	15.00	14.50	3.44	4.38	5.17	2.50	2.50	1.98
0.5	5.50	6.00	5.67	18.00	28.00	45.68	3.23	4.64	8.06	2.75	3.63	2.25
1.0	3.75	4.25	4.67	16.75	22.75	38.00	4.48	5.60	8.15	2.50	2.25	1.71
2.0	4.50	5.25	6.33	14.75	21.50	31.00	3.25	4.13	4.97	2.88	2.75	2.50
3.0	2.50	2.25	4.00	9.50	9.75	22.68	3.85	3.42	5.69	2.50	2.25	3.63
4.0	3.75	4.25	4.00	12.75	20.75	32.68	3.36	4.96	8.29	2.13	2.00	2.03
L.S.D at 0.05 level	1.70**	1.91**	1.85**	N.S	8.91**	15.32**	0.72**	N.S	2.51*	N.S	N.S	N.S

N.S., *,**:not significant , significant at 0.05 and highly at 0.01 level of significance , respectively.

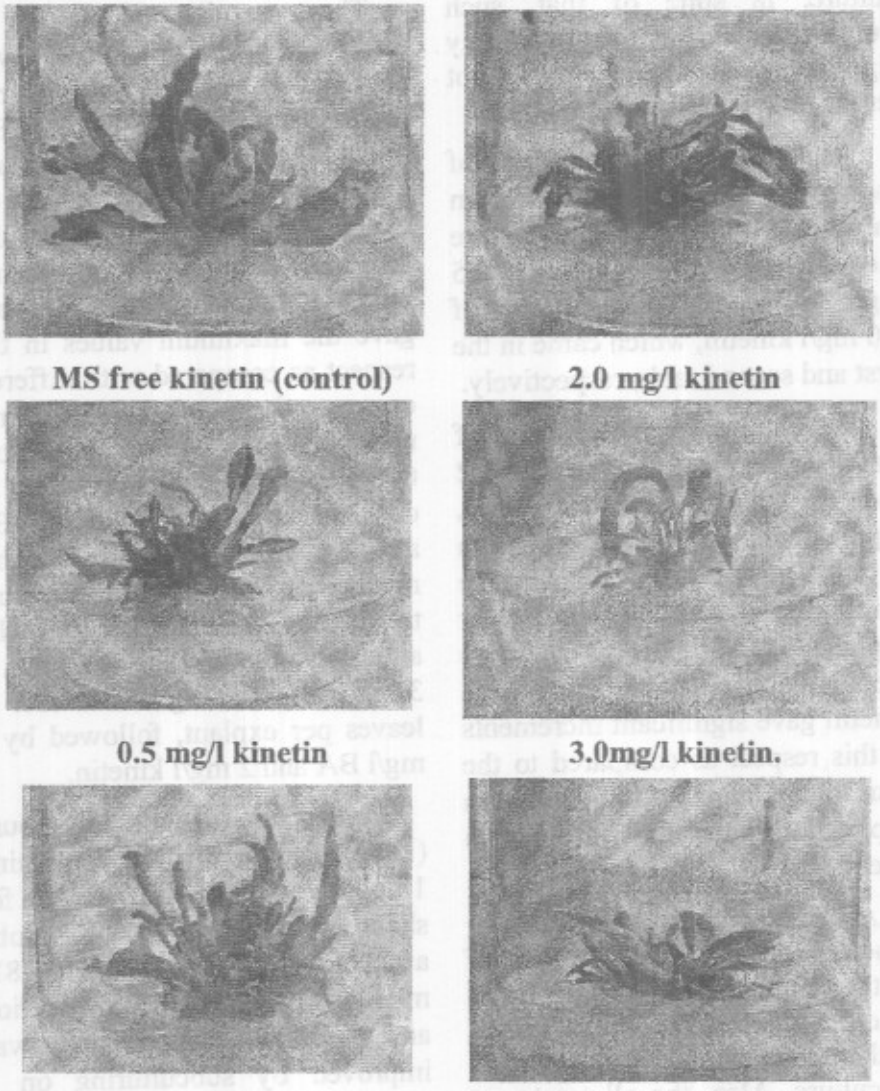

Fig. 1. Effect of kinetin concentration on the morphogenic responses of globe artichoke

culture. In spite of that, such treatments did not reflect any significant effect on average shoot length at the three ages of culture.

Concerning the number of shoots per explant, the maximum values were obtained with culture medium containing kinetin at 0.5 mg/l, followed by the treatment of 2.0 mg/l kinetin, which came in the first and second rank, respectively.

With regared to number of leaves per explant, data in Table 2 and Fig. 1 revealed also that, supplementation of MS-medium with 0.5 mg/l kinetin produced the maximum number of leaves per explant. Also, using culture medium containing 1 or 2 mg/l kinetin gave significant increments in this respect as compared to the control treatment (without kinetin application) particularly at 45 days after culture.

As for number of leaves per shoot, in general, the treatment of culture medium containing 1mg/l kinetin produced the highest values in this respect at 15,30and 45 days of culture. Also, the all treatment of other kinetin concentrations, except 2 and 3 mg/l, showed some increments of leaf number per shoot at 45 days of culture as compared with the control treatment.

These results are similar to those of Mohamed (1998) who supplied MS-medium during multiplication stage with BA, kinetin or 2ip (each at 0, 1, 2 or 3 mg/l) and found that, culturing the explants on a medium containing kinetin at 3 mg/l significantly increased the number of shoots and gave the maximum values in this respect as compared with different concentrations of all hormones under study, as well as the control treatment. Moreover, all concentrations of kinetin and BA at 2 and 3 mg/l produced higher number of shoots than the control treatment (Without hormones). He added also that the use of kinetin at 3 mg/l gave the highest number of leaves per explant, followed by 3 mg/l BA and 2 mg/l kinetin.

Furthermore, Bigot and Foury (1984) used a medium containing 1mg/l kinetin + 0.1 mg/l NAA for shoot multiplication of globe artichoke. Ancora *et al.* (1981) mentioned that shoot proliferation and orerall development was improved by subculturing on a MS-medium with a reduced concentration (5mg/l) of kinetin plus 0.5 mg/l IAA. The results compared with those obtained using MS-medium supplemented with 10 mg/l kin. and 0.5 mg/l IAA showed that, together with a

better growth of the shoots, a further increase in the proliferation rate was obtained.

Also, El-Gizawy *et al.* (1993) studied the effect of different concentrations of IAA and kinetin. On nultiplication of globe artichoke. They found that, the addition of both IAA and kinetin significantly improved and increased the rates of shoots and leaves proliferation. They also found that the best results of shoots proliferation were recorded with media containing 0.5 mg/l IAA when combined with either 5 or 10 mg/l kinetin. Similarly, Abd-Elall (2003) found that the greatest number of shoots was produced in MS-medium supplemented with 1mg/l IAA + 5mg/l kinetin, followed by the treatment of 1mg/l IAA + 2.5 mg/l kinetin. He added also that, the lowest number of shoots was produced in hormon free medium (control).

Lauzer and Vieth (1990) found that the presence of kinetin in the basal multiplication medium resulted in a higher number of shoots per explant than when not present. Moncousin (1981) stated that the proliferation phase of *in vitro* culture of globe artichoke required high cytokinin levels with additional tyrosine phenylalanine and without auxin. In a study on *in vitro* shoot multiplication by Okasha *et al.* (1996) a propagules derived and grew on the culture medium of 5mg/l kin + 0.5 mg/l IAA were divided into two batches. The first batch was subcultured on to the same medium. The second batch of propagules was subcultured on MS-medium supplemented with 0.5, 1.0, or 2.5 mg/l BA. It was observed that the best shoot numbers were achived with culture medium contaning 2.5 mg/l BA which showed better results than the other two concentrations of BA. However, it was found that shoots obtained from this treatment of BA were fascinated. In general, results revealed that subculturing the propagules, on medium contained 5 mg/l kinetin + 0.5 mg/l IAA gave better results of shoot development.

In addition, Brutti *et al.* (2000) studied the effect of diverse cytokinins on *in vitro* multiplication of globe artichoke. They found that with 6-benzyl aminopurine (BAP) a multiplication rate was obtained greater to that with kinetin and 2ip. However, BAP alone or combined with kinetin or 2ip showed a lower percentage of normal rosette shoots. Using a combination of 2 mg/l kinetin and 10 mg/l 2ip,

normal rosettes with a monthly multiplication rate of 3.0 were obtained. El-Shabasi (2007) stated that the highest number of shoots per explant were resulted from using multiplication medium supplemented with 2.0 mg/l kinetin + 1.0 mg/l IAA. Pierik (1987) mentioned that cytokinins are often used to stimulate growth and development. Kinetin, BAP (6-benzylamino purine), 2ip (N^6-(2-isopentyl) adenine), and PBA [6-(benzylamino)-9- (2-tetrahydropyranyl)-H- purine] are being in common use. They usually promote cell division, especially if added together with an auxin.

In higher concentrations they can induce adventitious shoot formation,but root formation generally is inhibited they promote axillary shoot formation by decreasing apical dominance. In addition, George (1993) stated that cytokinins are very effective in promoting direct or indirect shoot initiation. They are used for this purpose in combination with auxins. A balance between auxin and cytokinin normally gives the most effective organogenesis. Gamborg *et al.* (1976) mentioned that the cell division are stimulated by addition of cytokinins to culture media. Krishnamoorthy (1981). reported that the number of cell division increase proportionaly to the concentrations of added cytokinins when auxin is not a limiting factor. Moreover, Dixon and Gonzales (1994) found that the presence of high levels of cytokinins during multiplication stage produces high number of shoots. Gamborg and Shyluk (1981) come to similar conclusion.

In the present experiment, shoot multiplication which occurred through a proliferation of shoot tips cultured on MS- medium with kinetin alone (without auxin) may be attributed to the sufficient level of endogenous auxin which occurring in explanted tissues in the previously experiment.

From the previously mentioned results, it could be suggested that, supplementation of globe artichoke multiplication medium by kinetin alone at medium concentration (0.5 – 2.0 mg/l) were favourable to induce shoot development.

Effect of Kinetin Concentration on the Fresh and Dry Weight of Shoots

The effect of kinetin concentration on the fresh and dry weight of shoots of globe artichoke, cultured for 45 days *in vitro*, is presented in Table 3. It is

obvious that the multiplication medium which containing 0.5 mg/l kinetin recorded the highest value of fresh weight of shoots. This result was parallel with those of both shoots and leaves number per explant Table 2. Such data also, reveale that different used kinetin concentrations did not cause any significant effect on the dry weight of shoots and average fresh weight of shoot. Concerning average dry weight of shoot, data in such table reveale that the maximum values were obtained via culture medium containing kinetin at a concentration of 3.0 mg/l. In this connection, El-Shabasi (2007) found that the highest values of fresh weight of culture were resulted from using multiplication medium supplemented with 2.0 mg/l kinetin + 1.0 mg/l IAA. However, average fresh weight of shoot was not significantly affected by different concentrations of kinetin or 2ip.

Table 3. Effect of kinetin concentrations on fresh and dry weight of shoots after 45 day of culture

Kinetin Concentration (mg/l)	Weight of shoots (g)		Average weight of shoot (g)	
	Fresh	Dry	Fresh	Dry
0.0	1.84	0.33	0.56	0.11
0.5	2.88	0.57	0.52	0.10
1.0	1.94	0.59	0.43	0.13
2.0	1.59	0.40	0.28	0.07
3.0	1.78	0.83	0.44	0.21
4.0	1.17	0.48	0.31	0.12
L.S.D at 0.05 level	0.99*	N.S	N.S	0.08*

N.S. and *: not significant and significant at 0.05 level of significance, respectively.

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إنتـــاج الأفـــرع فـــى مـــزارع أنســـجـــة الخـــرشـــوف

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أجرى هذا البحث لدراسة تأثير تركيزات مختلفة من الكينتين(صفر، ٠,٥، ١، ٢، ٣، ٤ ملليجرام /لتر) على الاستجابة المورفولوجية للخرشوف من خلال تقنية زراعة الأنسجة.

ولقد أوضحت النتائج المتحصل عليها، أن جميع تركيزات الكينتين المضافة لبيئة موارشيج وسكوج(MS-medium) . أظهرت تأثيراً معنوياً على كل من عدد الأفرع والأوراق لكل منفصل نباتي عند ١٥ ،٣٠، ٤٥ يوم من الزراعة عدا عدد الأوراق لكل منفصل نباتى عند ١٥ يوم من الزراعة وبالإضافة إلى ذلك الوزن الطازج للأفرع ومتوسط الوزن الجاف للفرع بعد ٤٥ يوم من الزراعة، وقد نتجت أعلى قيم لكل فى عدد الأفرع والأوراق لكل منفصل نباتى من بيئة الزراعة المحتـــوية علـــى الكينتين بتركيز ٠,٥ ملليجرام/لتر، يليها اســـتخدام التـــركيـــز ٢ملليجرام/لتر.

ومن ناحية أخرى، كانت بيئة النمو المحتوية على ١ مليجرام/لتر كينتين هى المعاملة الأكثر تأثيرا وفعالية فى زيادة عدد الأوراق لكل فرع، وبالنسبة للوزن الطازج للأفرع، فقد أعطى تركيز ٠,٥ملليجرام/لتر من الكينتين أعلى القيم.

وبالإضافة إلى ذلك، فقد كانت أقصى قيم لمتوسط الوزن الجاف للأفرع أكثر وضوحا من خلال إضافة الكينتين لبيئة النمو بتركيز ٣ملليجرام/لتر.