

COMMERCIAL PRODUCTION OF GLOBE ARTICHOKE (CYNARA SCOLYMUS L.) IN-VITRO

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Abstract

This work was carried out in the Laboratory of tissue culture Arid Land Agriculture Research Unit (ALARU) Faculty of Agriculture Ain Shams University during the two autumn seasons of 2006 and 2008 in two separated experiments. First: Micropropagation of globe artichoke (*Cynara scolymus* L.).Second: Acclimatization of plantlets produced from tissue culture condition to the open field.

First experiment: data cleared that there is a relationship between the size of shoot-tip and the percentage of survival regenerated and the time needed to regenerate shoots however increasing the size of shoot tip explants from 2 to 5 cm led to a gradual increase in both survival and regenerated shoot percentage. And at the same time reduce the number of days needed to configured healthy shoots. Culturing the shoot tips explants in the media containing the ascorbic acid or citric acid led to a reduction of browning percentage.

Increasing the levels of both cytokinins (BA and Kn) from 2.5 to 10.0 mg/L was sufficient to enhance the capability of explants to produce more shoots especially at high level but unfortunately the highest level of both cytokinins (10.0 mg/L) led to obtain callus formation beside shoots production which is undesirable.

There are relationship between number of subculture and rates of shoots productions. Increasing the number of subcultures till fifth times increased gradually the number of shoot production and decreased shoot length.

IBA added to the media for rooting the regenerated shoots encouraged the root formation (number and length) than IAA treatments. On the other side addition of activated charcoal to the nutrient media is very important for enhancement root formation.

Concerning of the second experiment (acclimatization) data reported that culturing the plantlets produced from tissue culture in pots contained vermiculite either alone or in combination with other compounds gave the highest transplants survival percentage (60 - 90 %) than other substrate treatments.

INTRODUCTION

The globe artichoke (*Cynara scolymus* L.) which belongs to the family Compositae is considered one of the important vegetable crops grown in Egypt for local consumption and exportation. Artichoke is usually vegetatively propagated by crown division or offshoots because plants grown from seeds generally lack uniformity (Ibrahim *et. al.* 1981, Welbaum 1994). Traditional propagation usually enunciated with spread of diseases and develops a phytopathogenic situation which is 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 disease- free-plants which are of great value to artichoke growers (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 the practices of cultivation primarily by facilitating the production of healthy material and quicker multiplication (Ancora 1986). Moreover the absence of pathogens in the micro propagated plants could however be sufficient reason to justify better plant growth and hence a better yield. Browning of tissue is most severe in species that contain high levels of tannins or other hydroxyphenols. The browning results from the action of copper containing oxidase enzymes (e.g. polyphenoloxidaae and tryosinase) which are synthesized and/ or released due to wounding during the excision and sterilization of the tissue (Lerch 1981, Torres 1989). However the browning of tissue can be prevented by removing the phenolic compounds, modifying the redox potential, activating the phenolase enzymes and reducing the phenolase activity and/or substrate availability (Anderson *et. al.* 1982, Ammirato 1983). Nevertheless ascorbic acid and citric acid are the most commonly used as antioxidants and generally used at concentrations of 50-150 mg/l (Ancora 1986, Torres 1989)

In general the tissue culture micro propagation besides favoring restoration and genetic improvements of the cultivated varieties could lead to a worldwide increase in cultivation area.

Micro propagation techniques has many advantage therefore tissue culture technique was applied to evaluate its feasibility for a wider use. However through micro propagation different variation could be done in plantlets morphology these variants pose a problem for production of uniform true to type plants.

Therefore the objective of this work was undertaken to establish a rapid method of in vitro micropropagation for globe artichoke plantlets derived from in vitro conditions and compare to the field - derived - plants.

MATERIALS AND METHODS

This work was carried out in the Laboratory of tissue culture Arid Land Agriculture Research Unit (ALARU) Faculty of Agriculture Ain Shams University during the two autumn seasons of 2006 and 2008 in two separated experiments. First: Micropropagation of globe artichoke (*Cynara scolymus* L.). Second: Acclimatization of plantlets produced from tissue culture conditions to the open field.

First Experiment Micro propagation

1- Plant material

Crown pieces of globe artichoke (*Cynara scolymus* L.) cv. Herious were obtained from Horticulture Research Institute Agriculture Research Center Giza Egypt. These pieces were planted during the two autumn seasons of 2006 and 2008 in the open field of the Faculty of Agriculture Ain Shams University. All agricultural practices were used according to the recommendations of the Ministry of Agriculture. The new produced plants were used as the source of meristems materials needed for tissue culture study.

The main vegetative off shoots were obtained from the growing mother plants. These offshoots were collected in mid of March according to the method of Bekheet (1992). The shoots were washed under running tap water for 30 minutes and surface sterilized using 70% ethanol for 5 sec and 2% sodium hypochlorite for 20 min with two drops of a surfactant "Tween 20" were added. Shoot tips were excising at about 10 cm in length and culturing inside the Laminar Flow Hood and placed on the nutrient media.

2- Nutrient media

The basal nutrient media used in this experiment contained macro and microelements according to Murashige & Skoog (1962) which known as (M&S) medium supplemented with 5.0 mg/L kn plus IAA at 0.5 mg/L. The pH of the media was adjusted at 5.7 ± 0.1 before the addition of agar. The media were distributed into culture tubes where each tube contained 15 cm and sterilized by autoclaving at 121°C for 15 min.

3- - Treatments

(A) - Establishment stage

a- Size of explants

Shoot tip explants were excised inside the laminar Flow Hood at three sizes 2, 3 and 5 mm. All explants were excised on the same day and placed on the previous nutrient media. Data were recorded in the survival percentage (%) Percentage of shoot formation (%) and the number of days to configured branches.

b-Prevent the formation of browning

To prevent the browning formation in shoot tip explants the excised shoot tips (5 mm) were immersed for 30 second in a sterilized solution of the following contents:

- 1- Ascorbic acid (150 mg/l)
- 2- Citric acid (150 mg/L)
- 3)-Solution of ascorbic acid 150 mg mg/l + Citric acid (150 mg/l).

After that the treated shoot tip explants were cultured on the previous nutrient media of M&S. Data were recorded in the browning percentage (%) after 4 weeks from culturing.

(B) - Multiplication stage**Shoot formation****a- Effect of cytokinins**

Two different cytokinins i.e. kinetin (kn) and Benzyladenine (BA) were examined and added to M&S media at the concentrations of 0.0 (control) 2.5 5.0 and 10.0 mg /L. Data about number of shoots and shoot length were recorded after 4 weeks from cultured the shoot tips.

b- Number of subculture

This experiment was conducted to study the effect of consecutive sub-cultures on the number of shoot production. Shoot tips (5 mm in length) were cultured on jars containing 50 ml of M&S medium supplemented with 5 mg/L kn plus 0.5 mg/l IAA. The numbers and length of proliferated shoots were recorded after 4 weeks from culturing (first sub-culture). Shoots obtained from the first subculture were divided and cultured for another 4 weeks on the same previous fresh medium (second sub-culture). Seven sub-cultures being carried out in this experiment.

c- Root formation**a- Effect of auxins**

Uniform shoots (5 cm in length) obtained from the previous experiments were transferred to jars containing 50 ml medium consisted of half concentration of M&S medium plus different concentrations of two types of auxins i.e. IBA and IAA at the concentrations of 0.5 1.0 and 2.0 mg/l for each to study the best auxins and concentrations which encouraged root formation (number and length).

b- Effect of activated charcoal

Uniform shoots (5 cm in length) obtained from the previous experiments were transferred to jars (120 x 60 mm) containing 50 ml medium consisted of half

concentration of M&S medium plus 2.0 mg/L IBA in addition of 0.5 and 1.0 g/L activated charcoal to determine the effect of charcoal on root formation.

4- Culture conditions

All cultures were kept at constant temperature for $26 \pm 2^\circ\text{C}$ and sufficient fluorescent light of 1500 Lux for 16 hours photoperiod.

Second experiment Acclimatization

The most important stage in tissue culture is transferring plantlets from the aseptic cultural environment to the free-living environment and ultimately to the field. However acclimatization has been defined as process by which *in vitro* the plantlets adapts to environmental change. This process is necessary because *in vitro*- derived plantlets are neither adapted nor suited for *in vivo* conditions. Therefore this experiment was conducted to study the optimal transplanting medium for *in vitro* derived globe artichoke plantlets. The *in vitro* derived plantlets about 5cm in height were washed with current tap water and then disinfected by immersion in fungicide solution (topsin 1g/l) and transferred to plastic pots (7.5 x 10.5 cm.) containing one of the different media as following:

1. Peat moss.
2. Vermiculite.
3. Washed sand.
4. Peat. moss + washed sand (1:1).
5. Peat moss + Vermiculite (1:1).
6. Vermiculite + washed sand (1: 1).
7. Peat moss + Vermiculite + washed sand (1:1:1).

Each treatment consisted of 20 pots. Each pot contained one plantlet. Pots were then transferred to the greenhouse and covered with polyethylene sheets to maintain high relative humidity around plants. In addition spraying with water under the plastic sheets was carried out daily while the irrigation took place 3 times a week. Surviving plants were recorded after 4 weeks from transplanting.

5- Statistical analysis

Data were subjected to proper statistical analysis of variance procedure and means compared using the L.S.D. method at 5% level of significance according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

First Experiment: Micro propagation

(A)- Establishment stage

a- Size of explants

Results Indicate that there is a relationship between the size of shoot-tip and the percentage of survival regenerated shoots and the time needed to regenerate shoots as shown in Fig (1) however increasing the size of shoot tips led to a gradual increase in both survival and regenerated shoot percentage. And at the same time reduce the time needed to configured healthy shoots. Torres (1989) mentioned that the development rate of shoot tip explants was increased gradually with time but this rate was higher with increasing the size of shoot tip explants and vice versa. He also added that change the size of explants changes the levels of carbohydrates proteins and growth substances in the stock plant thus subsequently affecting the response of the explants *in vitro*.

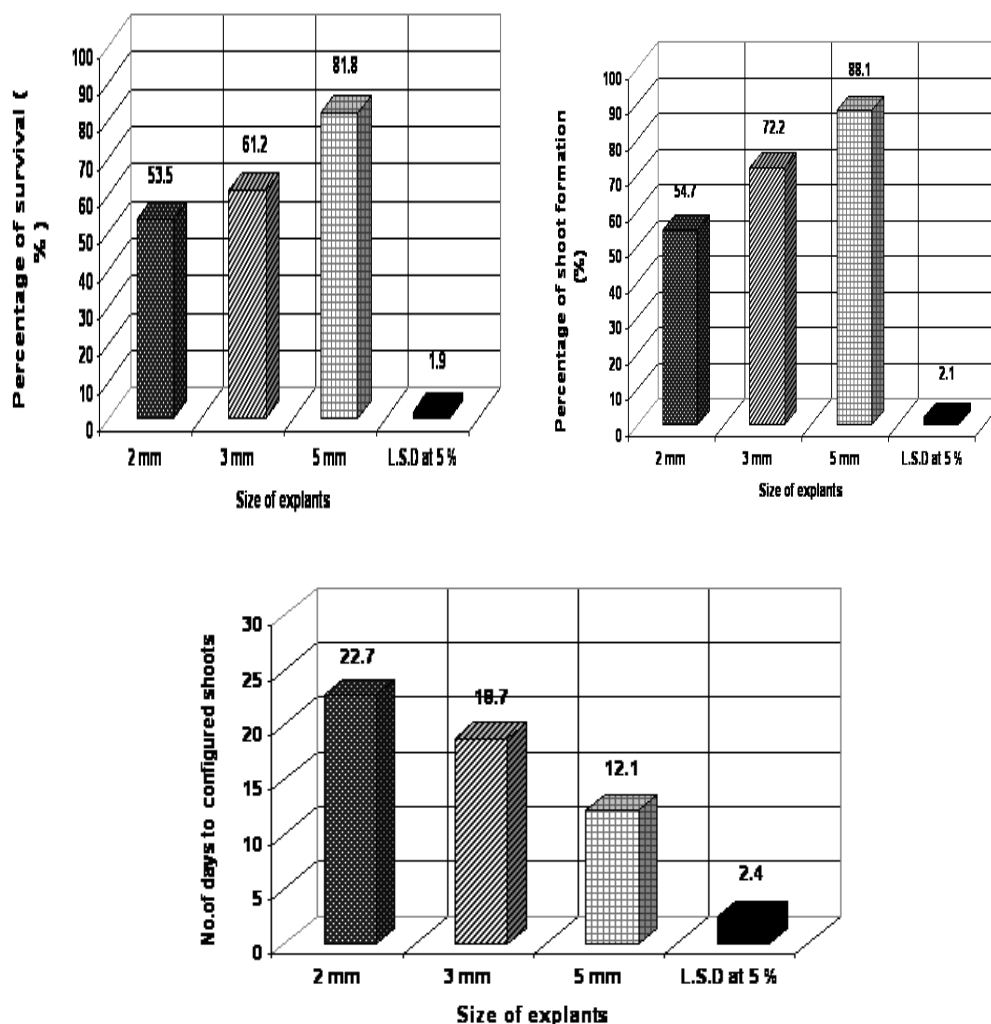
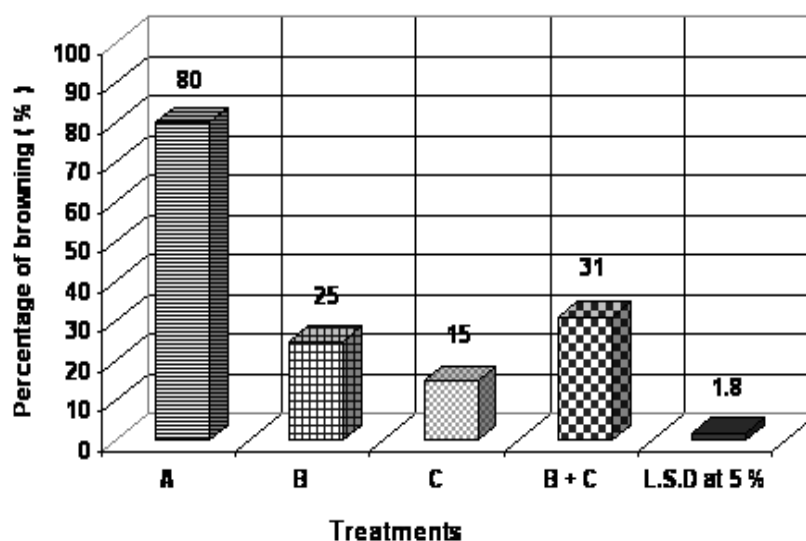


Fig. 1 Effect of the size of shoot tip explants on the percentage of survival regenerated shoots and the time needed to configured shoots

b-Prevent the formation of browning

It is clear from Fig.(2) that culturing the shoot tips explants in the media containing the two organic acids used in this experiment led to a reduction of browning percentage especially when these organic acids combined together compared with the medium free from the organic acids (control treatment) or used both of them alone. These results are in accordance with those obtained by (Ancora *et. al.* 1981) and (Ancora 1986, Torres 1989) who mentioned that ascorbic acid and citric acid are the most commonly used as antioxidants and preventing browning.



A: Control treatment

B: Ascorbic acid

C: Citric acid

Fig. 2. Effect of ascorbic acid and citric acid treatments on browning percentage in globe artichoke shoot tips cultured *in vitro*

2- Multiplication stage

A- Shoot formation

a- Effect of cytokinins

Multiple shoot formation rates varied with both concentrations and type of cytokinins (Fig 3). Increasing the levels of both cytokinins (BA and Kn) were sufficient to enhance the capability of explants to produce more shoots than control treatment. But Unfortunately the highest level of both cytokinins (10.0 mg/L) led to obtain callus formation beside shoots production which is undesirable. Some previous researchers supported results of our present experiment that there was direct relationship between the higher concentrations of cytokinins and the enhancement of shoots number. However presence of cytokinins in the media depressed the apical

dominance and consequently activated the axillary's buds which increase the opportunity to proliferate. Thus raising the concentrations of cytokinins increased the bud proliferation and the formation of multi apices plantlets (Torres (1989).). On the other side BA was significantly surpassed Kn in the number of shoots production (Fig. 3) These results coincided with Alphonse et al.(2002) on globe artichoke and El-Zeiny (1997 & 2002) on tomato and pepper respectively. But the contrary Kn treatments produced the longest shoots than the BA application as shown in Fig (6). these results were in agreement with some previous work (Anderson et al 1982 on strawberry Alphonse *et. al.* (2002) on glob artichoke and El- Zeiny (1997) and (2002) on tomato and pepper respectively.

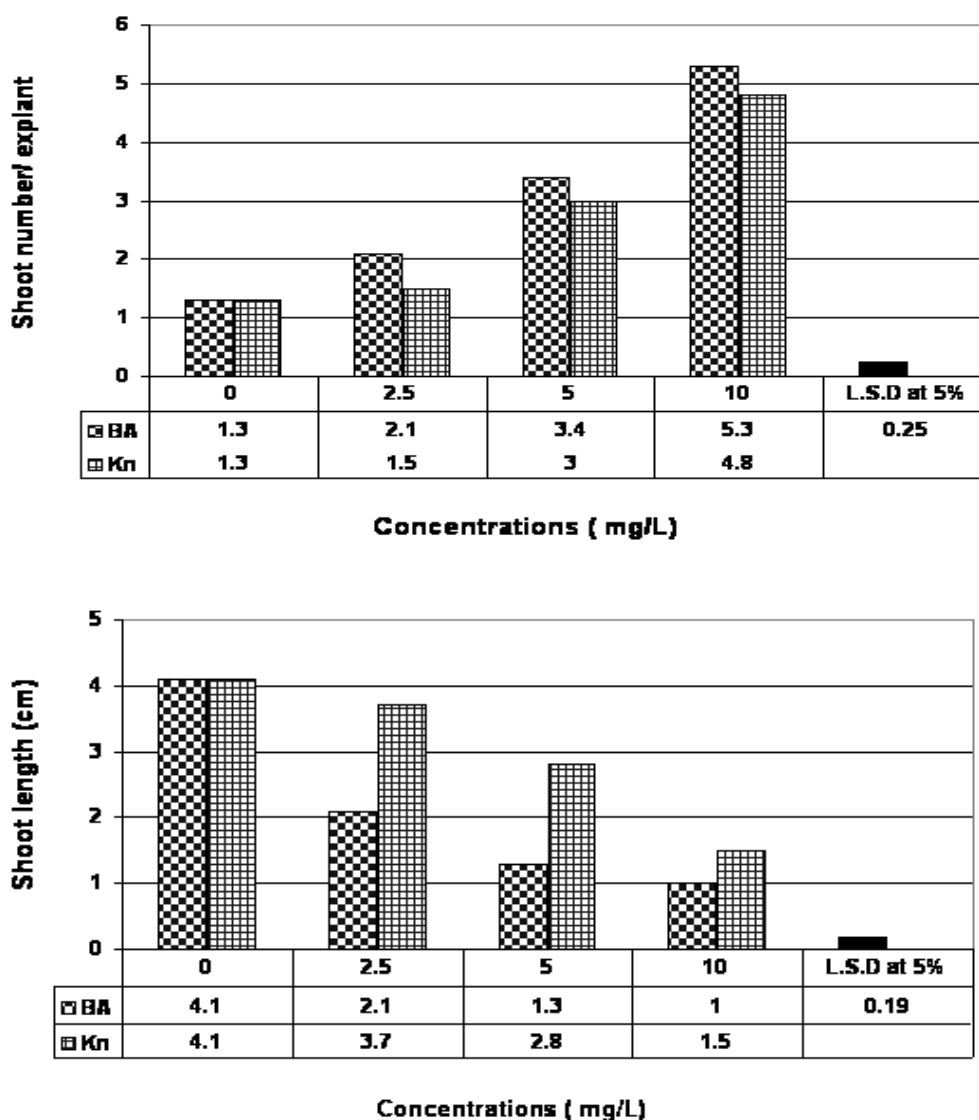


Fig 3. Effect of cytokinins on shoot number and shoot length production of globe artichoke developed from shoot tips culture.

b- Number of subculture

Data in Fig (4) showed that there are relationship between number of subculture and rates of shoots productions. Increasing the number of subcultures till fifth times increased gradually the number of shoot production and decrease shoot length. Multiple shoot formation rates declined eventually with increase in the number of subcultures in all treatments. So the best number of shoot production was obtained after culturing the shoot tips of globe artichoke five times in the nutrient media containing Kn at 5.0 mg / L plus 0.5 mg/1 IAA. These results were agreement with Ordas *et. al.* (1990) and Mehrez (1998)

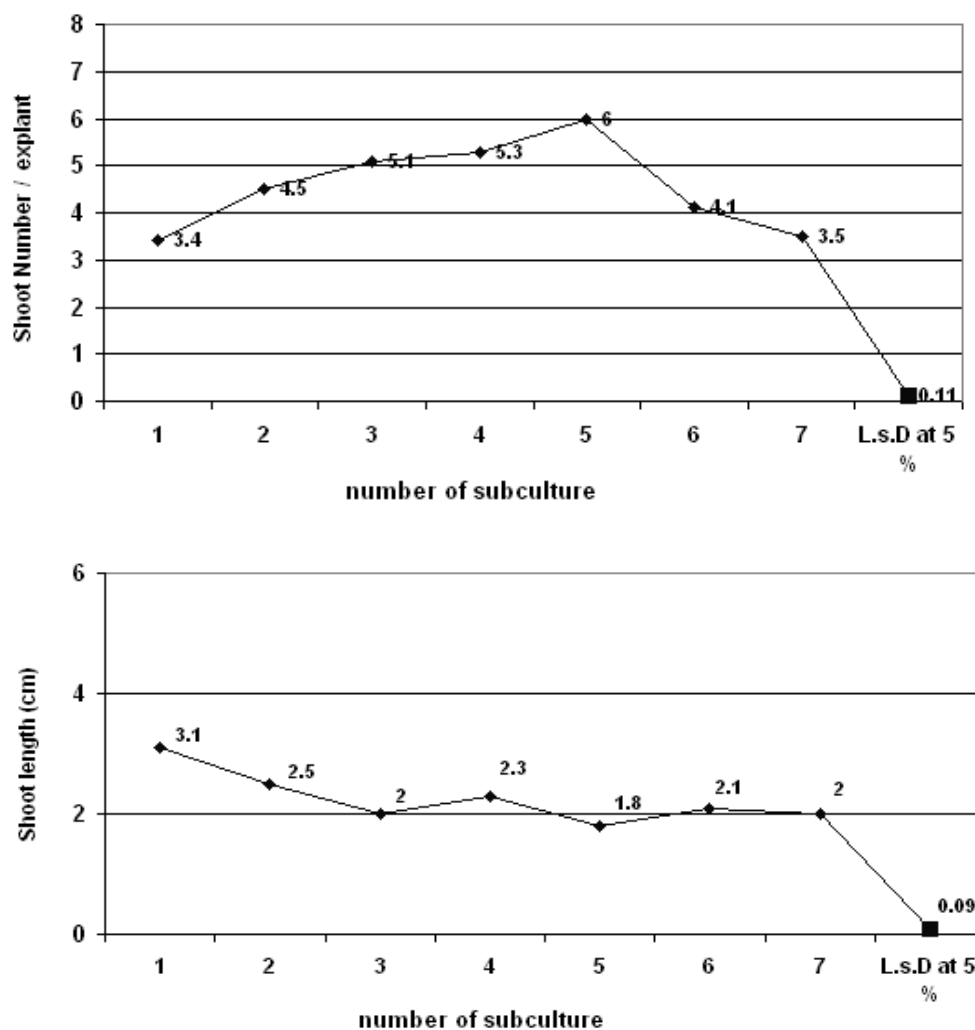


Fig 4. Effect of number of subcultures on the number and length of Shoots production of globe artichoke developed from shoot tip culture.

B- Root formation**a- Effect of auxins**

The estimation of percentage of root formation on globe artichoke shoots culture as affected by IBA and IAA cleared that the two levels of auxins gave rise to the explants up to 100 % for root formation. On the other hand IBA treatments encouraged the root formation (number and length) than IAA as shown in Fig (5). Similar Results were obtained by Upadhyaya et al (1985). Hartmann and Kester(1983) noted that IBA is most often recommended as a rooting agent because it less phytotoxic over a broader range of concentrations and active in more species than the other auxins such as IAA. On the other side IBA was significantly more stable than IAA to autoclaving so the majority of IAA could be degraded under the same concentration (Scott and Ellen 1990). From another point of view several investigators tried to relate the lower rooting ability of IAA to inhibitory effect of IAA induced ethylene. Mullins (1972) suggested that the promotive effect of auxins on induction of rooting are opposed by the inhibitory effect of auxins induced ethylene. Since IAA is more effective than IBA in inducing ethylene production.

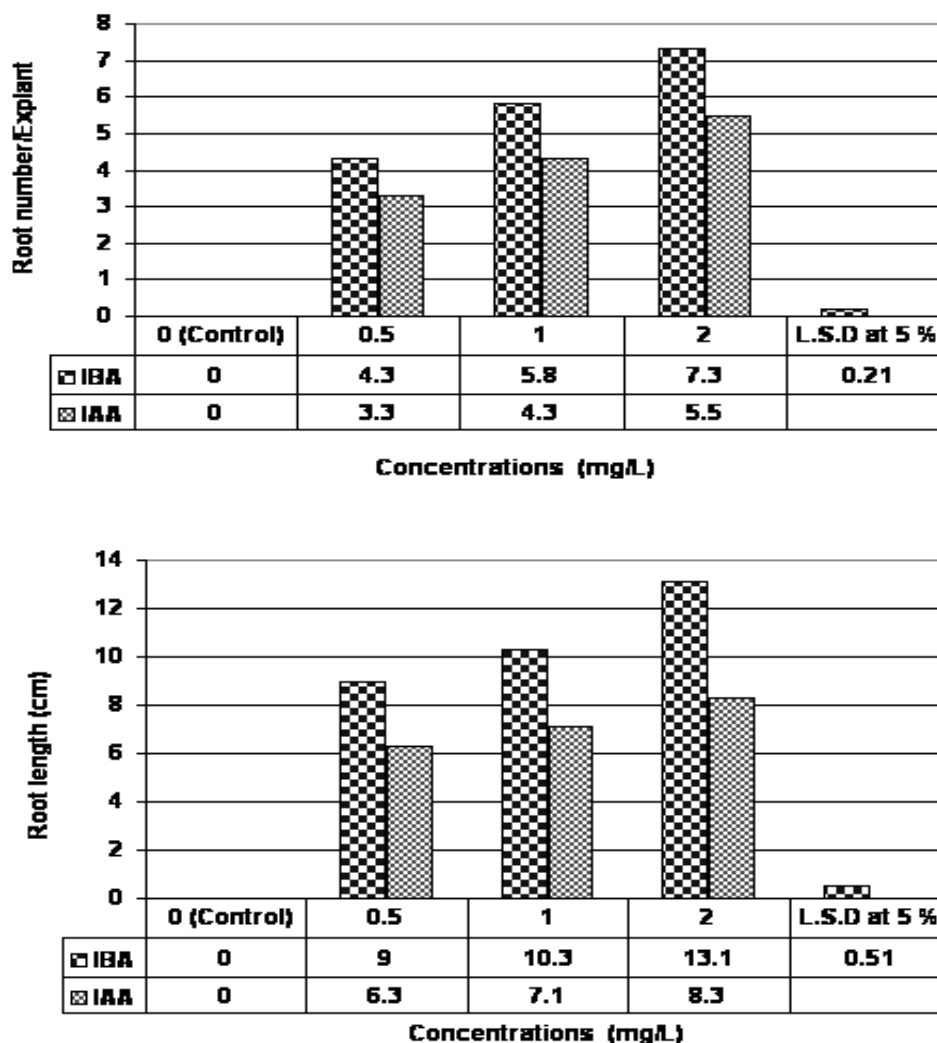


Fig 5. Effect of auxins on root number production of globe artichoke developed from shoot culture.

c- Effect of activated charcoal

Data in Fig (6) clear that addition of activated charcoal to the nutrient media is very important for encourage root formation comparing to the media free from activated charcoal. These results were agreement with those results obtained with Bigot and Foury (1984) and Ismaeel (1995) who illustrated that activated charcoal reduced the amount of phenolic browning that occurs in culture.

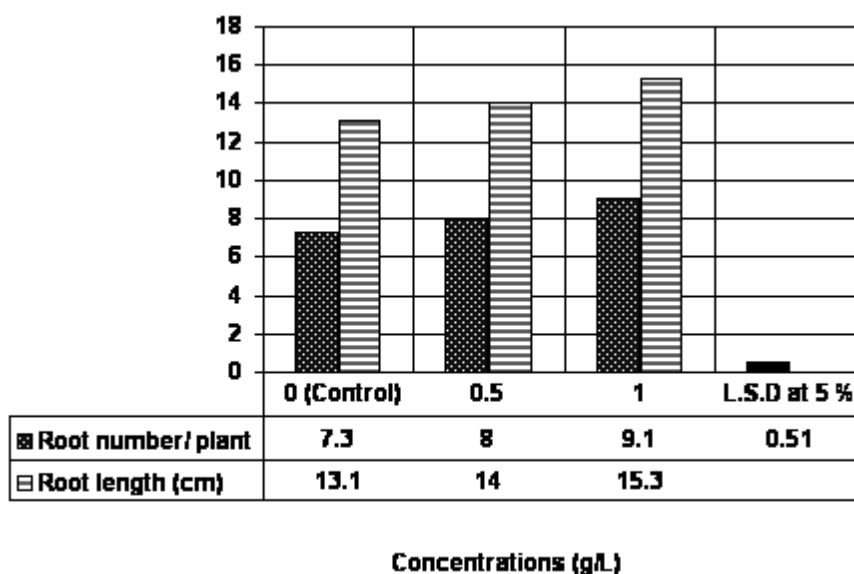


Fig 6. Effect of activated charcoal on root length and number of globe artichoke developed from shoots culture.

Second experiment: Acclimatization

Data in fig (7 and 8) reported that media contained vermiculite either alone or in combination with other compounds gave the highest transplants survival percentage ranged (65 – 90 %). On the other hand addition sand to the media for growing plantlets reduces the percentage of survival. Same results were happened with the results of Ismail (1995).

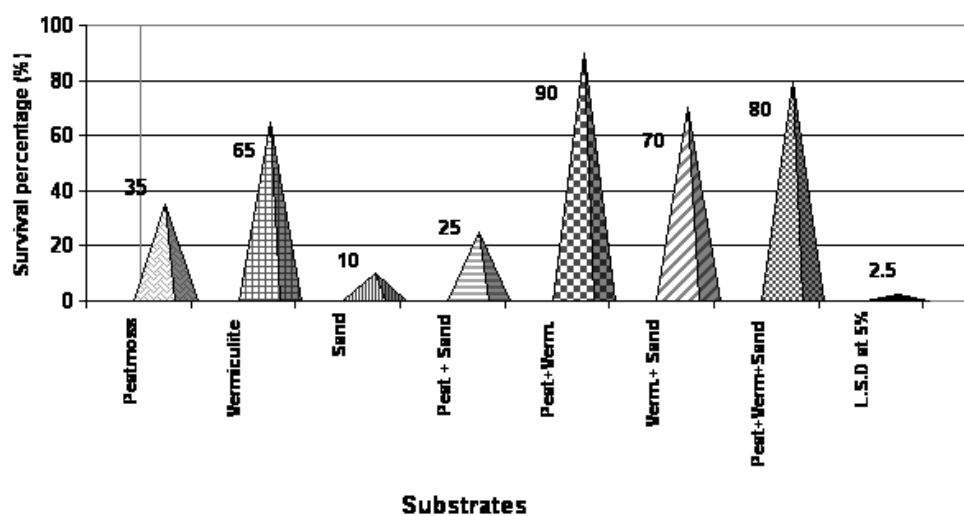


Fig 7. Effect of transplanting media on the percentage of survival of plantlets of globe artichoke (after 8 weeks of transplanting).



Fig 8. Ex vitro Acclimatized globe artichoke grown in pots containin peat moss and vermiculite.

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الإنتاج التجارى للخرشوف معمليا

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تم تنفيذ هذا العمل بمعمل زراعة الأنسجة- وحدة بحوث الزراعية بالاراضى القاحلة ، كلية الزراعة جامعة عين شمس خلال موسمى خريف 006 و 2008 في تجربتين منفصلتين :

التجربة الاولى: الاكثار الدقيق لنبات الخرشوف

التجربة الثانية : اقلمة النباتات الناتجة من زراعة الانسجة للحقل المفتوح.

التجربة الاولى: الاكثار الدقيق لنبات الخرشوف

اوضحت النتائج ان هناك علاقة بين حجم القمة النامية المنزرعة وكل من نسبة النجاح - عدد الأفرع الناتجة وكذلك المدة اللازمة لتكوين أفرع جديدة حيث وجد أن زيادة حجم القمة النامية المنزرعة من 2 الى 5 سم ادت الى زيادة تدريجية فى النسب السابقة وفى نفس الوقت قللت من عدد الايام لتكوين افرع جديدة.

كما وجد ايضا ان زراعة القمة النامية فى بيئة تحتوى على اى من حامض الاسكوربيك او الستريك يؤدى الى تقليل نسبة التلون باللون البنى خاصة عندما كانت البيئة تحتوى على خليط من كلا الحامضيين معا. كذلك وجد ايضا ان زيادة مستوى الستوكينينات (البنزيل ادنين او الكينتين) فى البيئة كان كافيا لتشجيع المنفصل النباتى (القمة النامية) لتكوين عدد وفير من الافرع الناتجة ولكن ادى التركيز المرتفع (10 ملليجرام/ لتر) الى تكوين كلس بجانب الافرع الناتجة.

لوحظ ان هناك علاقة بين عدد مرات النقل للمنفصل النباتى وعدد الافرع الناتجة حيث ان زيادة عدد مرات النقل حتى النقلة الخامسة يؤدى الى زيادة عدد الافرع الناتجة. ادى ايضا اضافة اندول حمض البيوترك لبيئة تجذير الافرع الناتجة الى تشجيع الجذور عددا وطولا مقارنة بمعاملات الاندول اسيتك اسد . كما يؤدى اضافة الفحم النشط لبيئات التجذير الى تشجيع التجذير.

التجربة الثانية: الاقلمة

اوضحت النتائج ان زراعة النبيتات الناتجة من زراعة الانسجة فى اصص محتوية على الفيرمكيوليت فقط و مختلطا مع بيئات اخرى اعطى اعلى نسبة نجاح (60 الى 90 %).