

The Highest Population of Plantlets from Somatic Embryogenesis and Economical Evaluation of Cucumber Plant (*Cucumis sativus* L.) *In vitro*

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Abstract: Reliable protocol for in vitro propagation of most important cucumber hybrids, (Hana F1), grown in Egypt green house was established. Two type of explants, i. e., Shoot tips and cotyledons were used and two kind of cytokinins (TDZ and BA) with different concentrations from 0. 1 to 4 mg/L beside the control (without hormones) were compared. Adding two cytokinins treatments from 0. 0 to 2. 0 mg/L and 4. 0 mg/l to the media for culturing shoot tip and cotyledon, respectively gave 100% of direct shoots proliferation without calluses. High levels of both cytokinins, (4. 0 mg/L) obtained shoot and callus together with the shoot tip explant and 0. 1 to 2. 0 mg/L with the cotyledons explant, but at the same time the two tested cytokinins with any concentrations under treatments suppressed the development of root production. The morphology of callus obtained from cotyledons was differ than that driven from shoot tip culture, however, cotyledons explant produced, a compact green embryonic callus tissue which developed into direct shoot proliferation when transferred onto the embryo induction medium. Whlie shoot tip explant produce only yellow callus tissue and failed to regenerate to direct shoots which turned to brown colour and died after few days when transferred onto the embryo induction medium. The highest level (4. 0 mg/L) of both cytokinins produced the largest number of shoots. Shoot tip explant was significantly superior than cotyledon explant to gave the longest shoot and increasing the number of shoots per explant. TDA was the best kind of cytokinin to increase the number of shoots per explant but at the same time led to a reduction of the shoot length compared with BA. Tissue culture technique produce number of plantlets 10 times more than those obtained from the traditional method of propagation (Seed sowing) which obtained one plantles per one seed. plants produced from shoot tip or cotyledons culture when transplanted to the green house produced plants characterized with longest shoots with more leaf chlorophyll content, bigger leaf area, increase in fresh and dry weight of plant and at the same time obtained higher early and total yield than those driven from seed sowing. Economical comparing study was done to evaluate the price of seedling obtained by tissue culture method and traditional nursery method, the differences was calculated in terms of money according to the price of market. Tissue culture technique led to a reduction in the cost price of hybrid seedlings obtained, and we can say that the asexual propagation in vitro become the great benefit to cut price down and may be considered the best way to stop the increasing price of hybrid seeds year after year and this presented procedures can be replace the traditional nursery methods.

Keywords: Tissue culture, Propagation, Shoot tip, Cytokinins, Cotyledon. Embryogenesis

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crop in Egypt. In winter cucumber plants are cultivated under greenhouse using hybrid seeds which characterized by high productivity, but the seeds are very expensive. Due to the increasing price of hybrid seeds year after year, the asexual propagation by tissue culture methods became the great benefit. Also this technique can be used as an efficient method for vegetable plant propagation and to eliminate virus infection in clonal progeny^[1].

Nowadays, tissue culture technique can replace the traditional nursery methods and was considered the prime method for producing all asexual propagated cultivars. Further research efforts on such crop were acheived to gain the advantage of in vitro technique to produce mass of plantlets in a short period of time and to reduce cost accounts.

Since Malepazy and Nadolsky-Orezky^[2] reported the formation of somatic embryos in cucumber. Researchers have made remarkable progress in somatic embryogenesis. Somatic embryogenesis have been induced from various explants such as cotyledons, hybocotyle, true leaves or other explants^[3, 4].

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Cytokinins which are adenine derivatives are mainly concerned with cell divisions and shoot differentiation in the tissue culture. In seeking to follow the effect of cytokinins on shoot formation during the propagation of different plants, it is obvious that shoot formation depend to great extent on the type and concentrations of the used cytokinin. However (Thidiazuron) (TDZ); Benzyladenine (BA) and Kinetin (Kn) have been demonstrated to stimulate in vitro meristem and shoot formation in a wide range of species. High concentrations of cytokinins produced shoot growth and leaf expansion, while the low ones produced few lateral shoots^[5].

Plants grown in-vitro usually do not possess productive mechanism against desiccation, characterized with reduction in epicuticular waxes, impaired stomata function and a depression in photosynthetic competence^[6]. Moreover, plantlet leaf anatomy and physiology are affected in-vitro. Up till now very limited informations are available to answer the question of why tissue culture plants are more superior than conventional plants in growth and productive potentials. This may be contributed to the use of the true potential of tissue culture plants beside the appropriate management for their specific requirements^[7]. However, the main visual differences between the two planting materials are in their morphology and growth habits during their development^[8]. More observations on tissue culture plants, for example in strawberry, it tended to be more bigger in vegetative growth, higher in rate of photosynthetic and dry matter assimilation beside efficient physiologically leaf area and vigorous roots than conventional plants^[9].

The information at hand about the economical studies does not allow routine clonal propagation of all plants. Nevertheless sufficient data have been accumulated and guidelines can be offered, so that detailed steps for specific crops can be developed with a minimum of research. Routine employment of tissue cultures as an alternate method. Plants propagated by tissue culture technique may have economic significant before can provide wide spread benefits.

The objective of this work is to throw some light on different factors affect the regeneration ability in one of the most important cucumber hybrid (Hana F1) which grown in the green house and to put our hand on the best type and concentrations of cytokinins which produce much shoots throw shoot tips and cotyledon explant culture to reduce their cost price and also to have a full understand about their vegetative growth habits and total yield.

MATERIALS AND METHODS

This work was carried out in the Laboratory of tissue culture, Arid Land Agriculture Research and Services Center, Faculty of Agriculture Ain Shams University during 2004 and 2005 seasons in two separated experiments First: Micro propagation of cucumber hybrid (Hana F1), Second: Comparison study between growth and productivity of tissue culture and seed sowing plants.

First Experiment:

Micro Propagation: Seeds of cucumber hybrid (Hana F1) were sown on January 20 and 23 for the first and second seasons (2004 and 2005) respectively in the propagation trays containing a mixture of peat moss and vermiculate at ratio of 1:1 (v:v). The process of germination was done under green house conditions.

Preparation of Explant: After 3 days from germination, seedlings of the cucumber hybrid were removed from the trays and transferred to the tissue culture laboratory where roots were removed and the seedlings were washed several times with tap water. The remained seedlings were surface sterilized by immersing in 1% sodium hypochloride for 15 min then rinsed three times with sterilized distilled water. Excising and culturing the explants (shoot tips and cotyledons) were done inside the Laminar Flow Hood, then, the shoot tip explants were cut under microscope at about 0.2-0.5 mm in length while, cotyledon explants were cut into 10 discs (4 - 6 mm). All explants were excised on the same day and placed on nutrient media.

Nutrient Media: The basal nutrient media used contained macro and microelements according to Murashige and Skoog^[10] which known as (M&S) medium. 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.

Treatments: Two different cytokinins, i. e., thidiazuron (TDZ) and Benzyladenine (BA) were examined at the concentrations of 0.0 (control), 0.1, 1.0, 2.0 and 4.0 mg/L. The experiment design was a split split plot design with three replicates and each replicate was resembled by 10 tubes, as those explants (shoot tip and cotyledons) were located in the main plots. The two cytokinins (TDZ and BA) were randomly located in sub-plots while, the concentrations were located in sub sub plots.

Culture Conditions: The culture tubes were kept at constant temperature for 26± 2°C and sufficient fluorescent light of 1500 Lux for 16 hours photoperiod. Data were recorded after 14 days from culture on the percentage of morphogenetic characters, callus weight, shoot number/explant and shoot length.

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^[11].

Somatic Embryogenesis: Calluses obtained from the previous treatments were transferred onto embryo induction containing M&S medium plus 1.7 mg/l NAA and 2.3 mg/l BA according to Lou and Kako^[12].

Rooting and Acclimatization: The best shoots only (without callus) obtained from the previous treatments, were transferred to the rooting medium containing IBA at 2.0 mg/L. for 5 days. After that the complete plantlets produced were transferred directly from the culture tubes into plastic pots filled with peat-moss and vermiculate at ratio of 1:1 (v:v) and transferred to plastic house under mist for acclimatization. The plantlets were fertilized with Hoglands solution for a period of 7 days.

Second Experiment: Comparison Study Between Tissue Culture and Seed Sowing Plants: To have a full understand about the vegetative growth habits of tissue culture technique and their total yield in comparison to those of conventional plants (seed sowing), both the seedlings produced by tissue culture and by seed propagation were planted at the same time into plastic house in the Arid Land Agriculture Research and Services Center, Faculty of Agriculture Ain Shams University, Egypt, on February, 23 and 25 of 2004 and 2005 seasons respectively. The treatments were arranged randomly in 3 replicates using a randomized complete design where the seedlings were planted on ridge 1 m wide and spacing of 50 cm between rows and 50 cm between plants. Each replicate consisted of 20 plants. The plastic house were equipped with Agro-drip irrigation system. Other agriculture practices were done according to the recommendations of commercial greenhouse production.

Data Recorded:

Vegetative Growth: Data were recorded on plant height after 45 and 90 days from planting in the plastic house, total leaf chlorophyll using chlorophyll meter (SPAD), leaf area (cm²) using leaf area meter (LI-300-COR - Lincoln.), fresh and dry weight of plant (g). All of them were recorded at 45 days from planting.

Yield and Fruit Characters:

Early: yield (g/plant), measured as the weight of cucumber fruit during the two weeks of harvesting period. Total yield (Kg/plant) was recorded as the weight and number of all cucumber fruits harvested. Yield component measured as average fruit weight (gm).

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^[11].

Economical Evaluation: Comparing study was done to evaluate the price of seedling obtained by tissue culture method and traditional nursery method, the differences was calculated in terms of Egyptian money according to the price of market.

RESULTS AND DISCUSSIONS

First Experiment:

Morphogenesis: Cultured shoot tips explant on the basal medium free from cytokinins (control) gave 100% of complete plantlets (Shoot + root). while culturing cotyledons explant on the same media failed to regenerate anything and died after few days as presented in Table (1).

Adding the two tested cytokinins from 0.1 to 2.0 and 4.0 mg/L to the media for culturing shoot tip and cotyledon respectively gave 100% of direct shoots proliferation (Fig. A and B). Meanwhile forming shoot and callus together was obtained with the shoot tip explants when cultured on the media supplemented with high levels of cytokinins (4.0 mg/L).

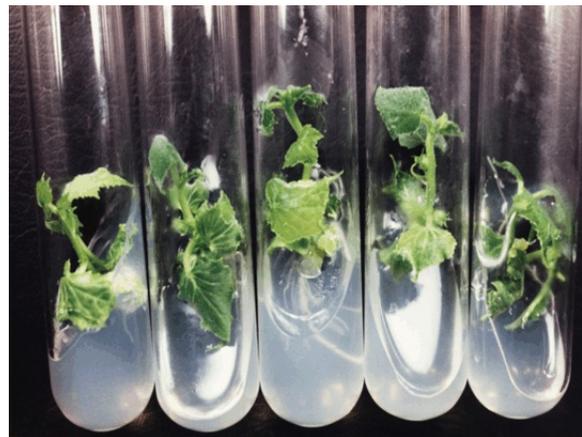


Fig. A: Direct shoot regeneration obtained from culturing shoot tip explant on M&S medium contained TDZ at 2.0 mg/L.

Table 1: Effect of cytokinins on the morphogenetic characters of shoot tips and cotyledons culture.

Treatments (mg/l)	Shoot formation		Root formation		Callus formation	
	Shoot tip	Cotyledon	Shoot tip	Cotyledon	Shoot tip	Cotyledon
Control	+	-	+	-	-	-
TDZ 0.1	+	+	-	-	-	+
1.0	+	+	-	-	-	+
2.0	+	+	-	-	-	+
4.0	+	+	-	-	+	-
BA 0.1	+	+	-	-	-	+
1.0	+	+	-	-	-	+
2.0	+	+	-	-	-	+
4.0	+	+	-	-	+	-

(+) formed(-) unformed



Fig. B: Direct shoot regeneration obtained from culturing cotyledon explants on M&S medium contained TDZ at 4.0 mg/L.

While cotyledons explant formed the same parameter with the media containing cytokinins at the concentrations ranged from 0.1 to 2.0 g/L. But at the same time the two tested cytokinins with any concentrations under treatments (0.1 to 4.0 mg/L) suppressed the development of root production as presented in Table (1). Our results are in agreement with many previous results on onion^[13] potato^[14] Tomato and cantaloup^[15, 16] which cleared that cytokinins have a great inhibitor effect on root initiation and the only success was for the development of shoots. Data in Fig. (1) summarize that the heaviest callus weight was formed from the cotyledon explants treated with TDZ compared with other treatments. Same results was obtained by Edriss *et al.*^[16], on melon and El-Zeiny^[17] on pepper.

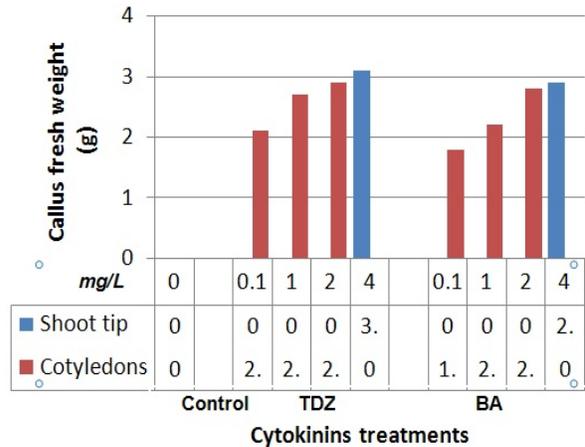


Fig. 1: Effect of cytokinins on callus weight of cucumber hybrids developed from shoot tips and cotyledons culture.

Somatic Embryogenesis: The morphology of callus obtained from cultured cotyledon explant was differ than that driven with shoot tip. However, cotyledons explant produced, a compact green embryonic callus tissue (Fig. C) which developed into direct shoot proliferation when transferred onto the embryo induction medium (Fig. D). While shoot tip explant produce only yellow callus tissue (Fig. E) which failed to regenerate to direct shoots and died after few days when transferred onto the embryo induction medium. The differences in the ability to undergo somatic embryogenesis may be due to differences in physiological development or to varying response to the stimulus of the same plant growth regulators.



Fig. C: Compact green embryonic callus tissue and green callus obtained from cultured cotyledon explants.



Fig. D: Shoot proliferation produced from cultured the embryonic cotyledon callus on the embryo induction medium.

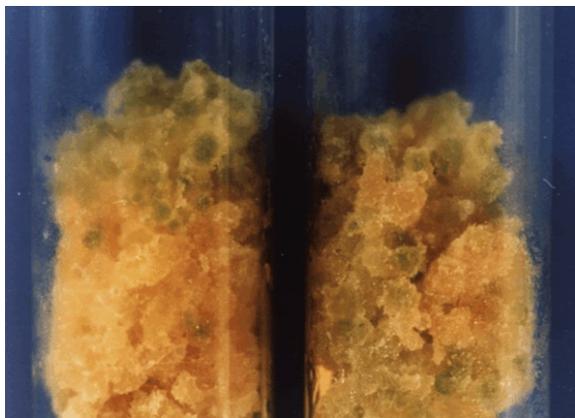


Fig. E: Yellow callus obtained from cultured shoot tip explants.

Our results is full agreement with the results of Lou and kako, ^[12] and Edriss *et al.*, ^[16]. The results about shoot regeneration from cultured cotyledons callus Table (2) record that, gradually increasing in the concentrations from 0. 1 to 2. 0 mg/L led to a significant increase in both embryonic callus percentage and number of regenerated shoots, so the maximum shoots were obtained with the level 2. 0 mg/L in both cytokinins (TDZ or BA). Data also clear that TDZ was significant superior than BA in giving highest percentage of embryonic callus or much number of regenerated shoots as presented in Table 2.

Data about shoot number per explants in Fig. 2 reflect that this character depended to a great extent on both the type of explants, cytokinins applied and the concentrations used. However, shoot tip explants was significantly superior than cotyledons to produce much number of shoots (Fig. 2A). Data also cleared that TDZ had a high dgree of activation in this parameter than BA treatment (Fig. 2B). These results coincided with those reported by Van Niewkerk *et al.* ^[18]; Fiola *et al.*, ^[19] and Edriss *et al.*, ^[16], who cleared that TDZ was better than BA in increasing the shoot number. On the other side, increasing the concentration from 0. 1 to 4. 0 mg/L was sufficient to enhance the capability of explants to produce more shoots than the control medium especially with the highest level (4. 0 mg/L) which produced the largest number of shoots (Fig. 2C). Some previous researchers, supported our present experiment, they mentioned that there was direct relationship between the higher concentrations of cytokinins and the increase in 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 increase the bud proliferation and the formation of multi apexes plantlets as reported by Anderson, ^[20] on strawberry; Edriss *et al.*, ^[15] on cantaloup; El-zeiny, ^[21] on tomato. Data about the interaction between treatments as presented in (Fig. 2G), it could be concluded that cultured shoot tip or cotyledons explant on media containing TDZ at 4. 0 mg /L was enough to produce the highest number of shoots which was 7 and 5 shoots per one shoot tip and cotyledons explant respectively compared with other treatments. Data in Fig. (3) about shoot length show that both of shoot tip explant and BA was the best kind of explant and cytokinin to increase shoot length (Fig. 3A and B). increasing gradually the concentration of cytokinins from 0. 1 to 4. 0 mg/L led to a reduction in this parameter. So, the shortest shoots were obtained from the highest levels of cytokinins i.e., 4. 0 mg/L (Fig. 3C). These results may be due to

Table 2: Percentage and number of shoots regeneration of cucumber hybrid developed from callus culture of shoot tips and cotyledons explants after transferring to the *embryo induction medium.

Treatmentsmg/l	% embryonic callus		Number of regenerated shoots	
	Shoot tip	cotyledon	Shoot tip	cotyledon
TDZ 0. 0	00. 0	00. 0	00. 0	00. 0
0. 1	00. 0	71. 0	00. 0	06. 2
1. 0	00. 0	81. 0	00. 0	07. 0
2. 0	00. 0	90. 0	00. 0	12. 4
4. 0	00. 0	00. 0	00. 0	00. 0
Mean	00. 0	48. 4	0. 0	05. 12
BA 0. 0	00. 0	00. 0	00. 0	00. 0
0. 1	00. 0	62. 0	00. 0	04. 7
1. 0	00. 0	68. 0	00. 0	05. 2
2. 0	00. 0	74. 0	00. 0	08. 1
4. 0	0. 0	00. 0	00. 0	00. 0
Mean	0. 0	40. 8	0. 0	03. 6
L. S. D. at 5%				
Cytokinins	00	0. 544	00	0. 067
Cocentrations	00	1. 176	00	0. 106
Interaction	00	0. 950	00	0. 087

* Ebryo induction media contained M&S medium plus 1. 7 mg/l NAA and 2. 3 mg/l BA accerding to Lou and Kako (1994).

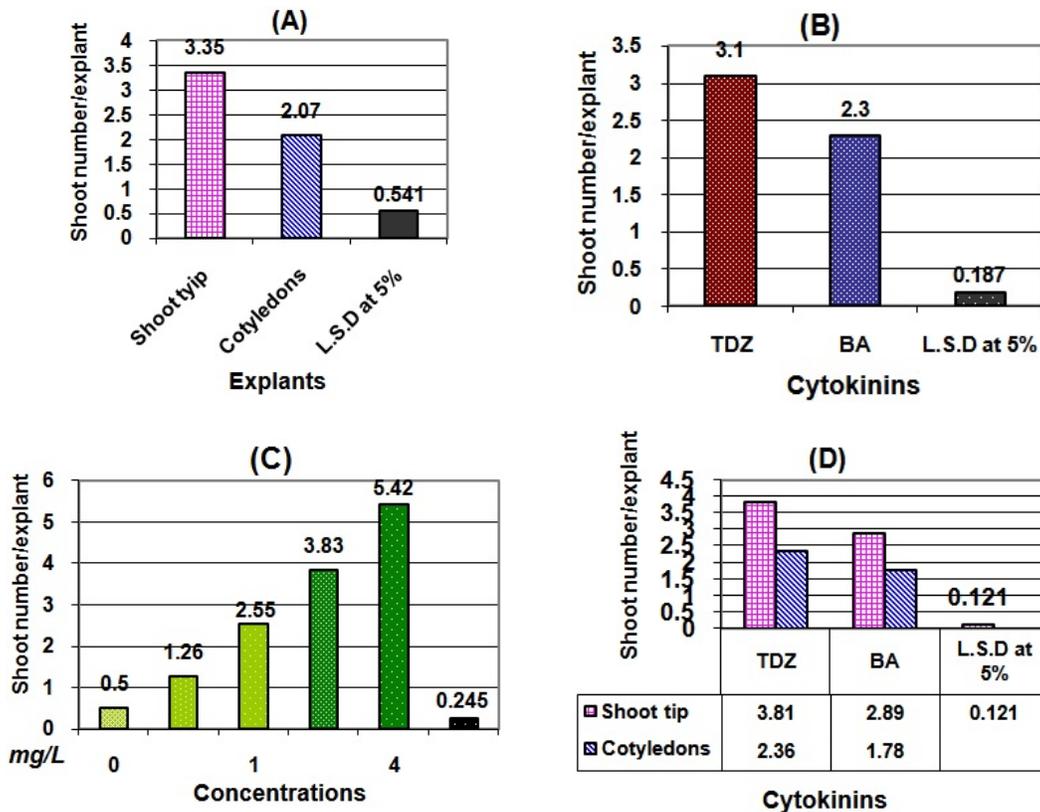


Fig. 2: Effect of cytokinins on shoots number of cucumber hybrid developed from cultured shoot tip and cotyledons explants.

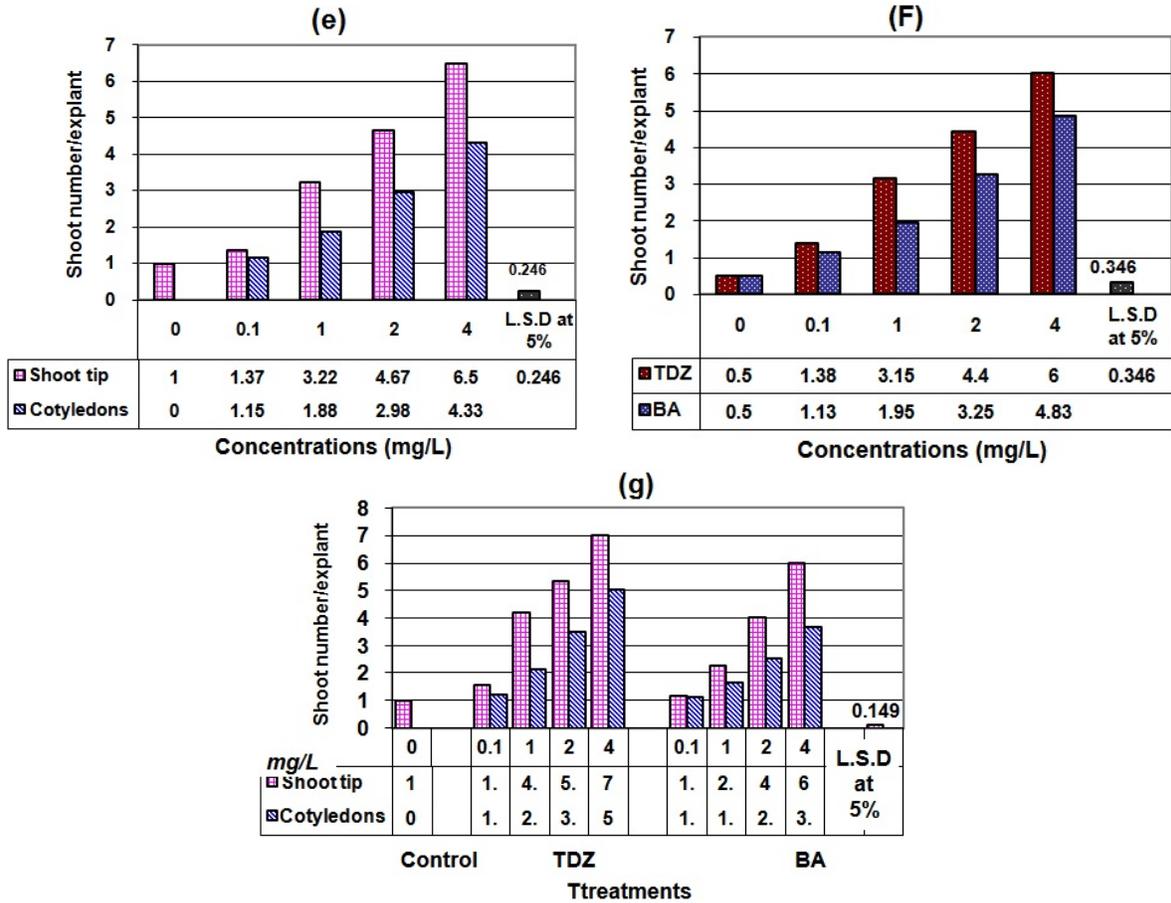


Fig. 2: Effect of cytokinins on shoots number of cucumber hybrid developed from cultured shoot tip and cotyledons explants.

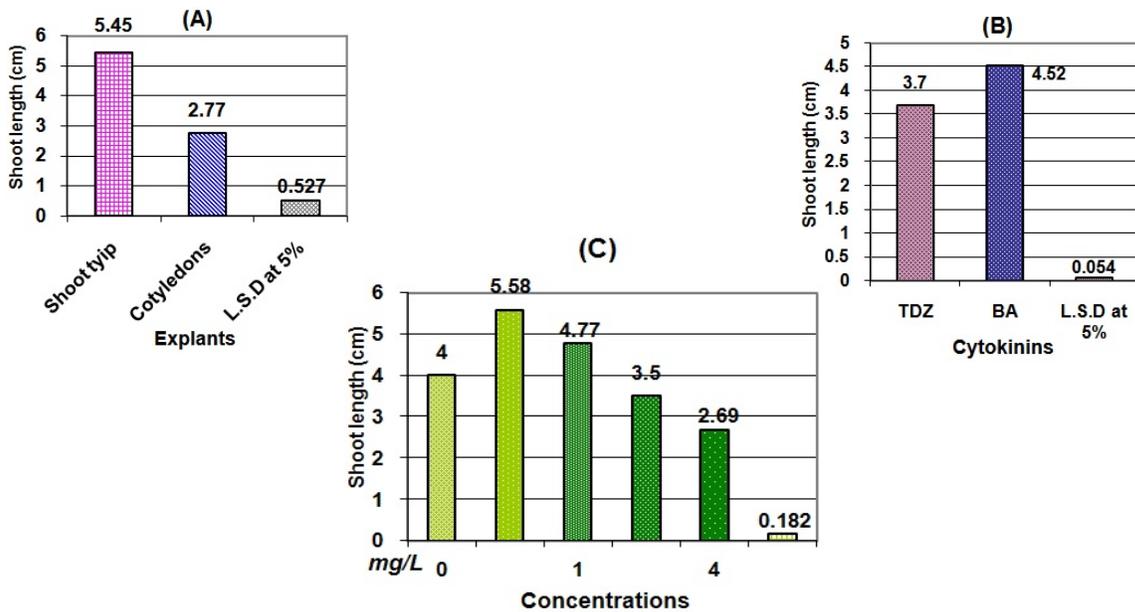


Fig. 3: Effect of cytokinins on shoots length of cucumber hybrid developed from cultured shoot tip and cotyledons explants.

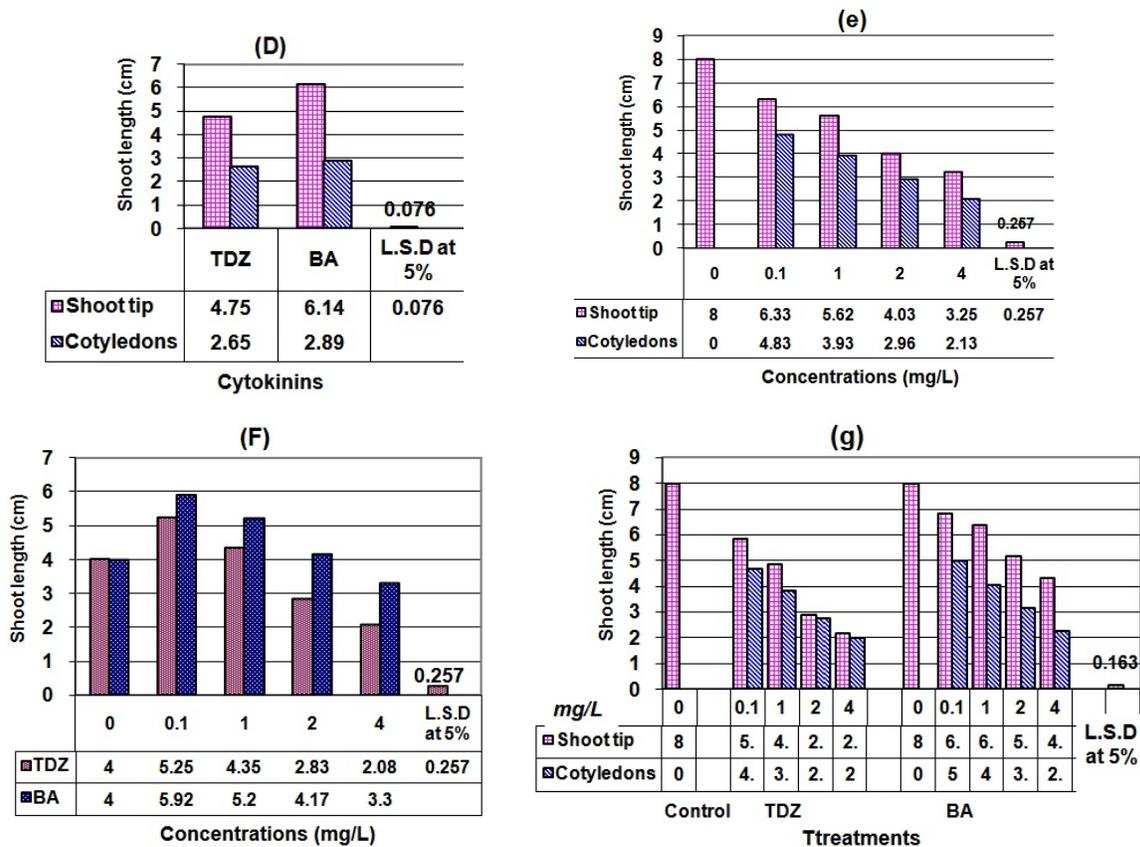


Fig. 3: Effect of cytokinins on shoots length of cucumber hybrid developed from cultured shoot tip and cotyledons explants.

the much number of obtained shoots with the highest level of concentration (4.0 mg/L) as shown before in Fig. 2C that it utilize nutrient from the media, and slow down nutrient uptake by shoot growth causing less in shoot length^[22]. Also data cleared that added BA treatment to the media for culturing shoot tip explants significantly produced the longest shoots than the TDZ applications (Fig. 3D). The trend of these results was in agreement with some previous work by Anderson,^[20] and El-Zeiny,^[17, 21]. It could be mentioned from the interaction between treatments that culturing shoot tip explant on medium free from hormones (Control) produced the longest shoot (8 cm) compared with media supplemented with TDZ or BA at any concentrations under investigation (Fig. 3G).

Rooting and Acclimatization: All shoots obtained from the previous treatments when cultured to the rooting media containing 2.0 mg/L IBA was enough to form 100% roots (data not tabulated), hence complete plantlets were obtained (Fig. F). Data about plant acclimatization cleared that the survival percentage was about 85% and 80% from plants driven from shoot tip and cotyledon respectively. (data not tabulated).

Conclusion: From the previous results of the first experiments we can concluded that using tissue culture technique was sufficient to encouraged the capability of explants to produce much number of plantlets especially when cultured in the media containing TDZ at 4.0 mg/L which gave number of plantlets from both shoot tip and cotyledon (7 and 5 respectively) 10 times more than the control treatment (without hormones) or those seedlings obtained from standard propagation i.e., seed sowing which contained one shoot only per seedling.

Second Experiment: Comparison Between Tissue Culture and Seed Sowing Plants:

Vegetative growth: The presented data in Figs. 4, 5 and 6 show that there were significant differences in the morphological characters of plants were detect among the methods of propagation however, micropropagated plants produced vigorous vegetative growth than the other plants produced from traditional propagation. Data about plant height, (Fig. 4) clear that seedlings exerted from shoot tips or cotyledons culture after 45 days from transplanting to the green house were significantly shorter than those obtained from seed sowing. While data recorded after 90 days from

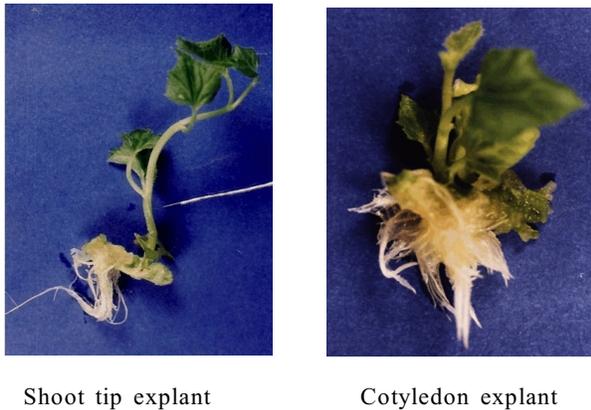


Fig. F: Complete plantlets obtained from tissue culture technique.

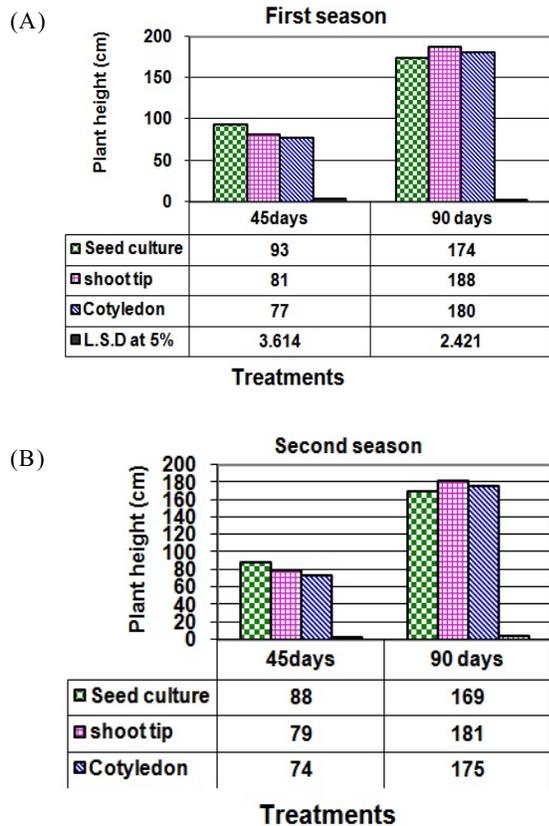


Fig. 4: Comparison between tissue culture and seed sowing on plant height of cucumber after 45 and 90 days from transplanting to the green house.

transplanting recorded that tissue culture plants were more superior than the conventional plants. At 45 days from transplanting the tissue culture plants were shorter than the conventional plants due to the increase in branch number which were formed in young tissue culture plantlets compared with the conventional plants

which produced only one shoot per seedling. While the differences in the rate of growth between tissue culture and those of conventional one after 90 days from planting clear that this rate of increase in tissue culture plants surpassed the conventional ones. similar conclusion was reported and supported our results, by (Bigot, and Foury^[23]) and Robinson *et al.*^[3], which pointed that tissue culture plants were found to be more superior than conventional ones in growth rate. Values presented in Figs (5 and 6) reveal that significant increase was occurred in the leaf area, total chlorophyll, fresh and dry weight of the cucumber plants produced from tissue culture as compared with those obtained from conventional plants. Same trend was obtained with Swartz *et al.*^[24], Grout *et al.*^[25], Alphonse *et al.*^[26], and El-Zeiny^[16] who suggested that tissue culture technique produced plants characterized with wide leaf area and contained high amount of chlorophyll than those plants obtained from traditional method.

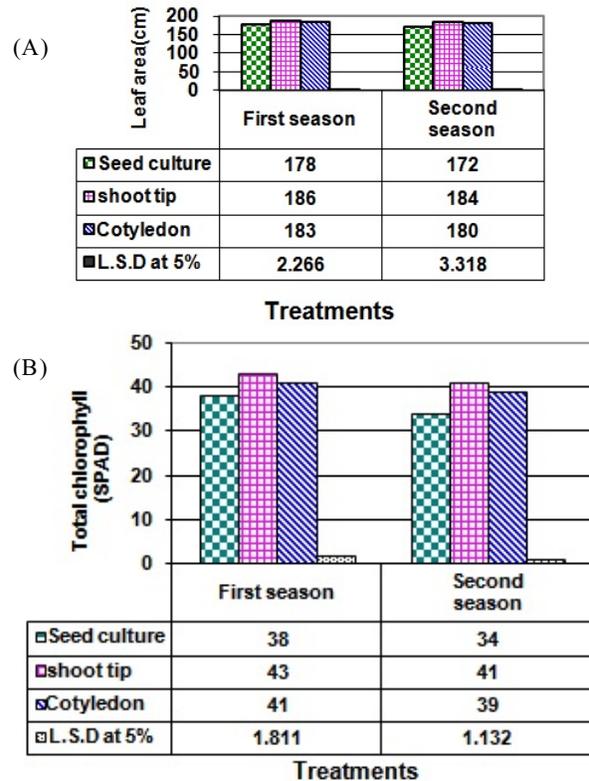


Fig. 5: Comparison between tissue culture and seed sowing on Leaf area and total chlorophyll of cucumber plants after 45 days from transplanting to the green house.

Early and Total Yield: Data presented in Figs. (7 and 8) exhibit that the tissue culture plants were significantly superior than the conventional plants in producing higher yield expressed as early and, total yield, per plant, number of fruit per plant and average fruit weight in the two seasons. These results may be

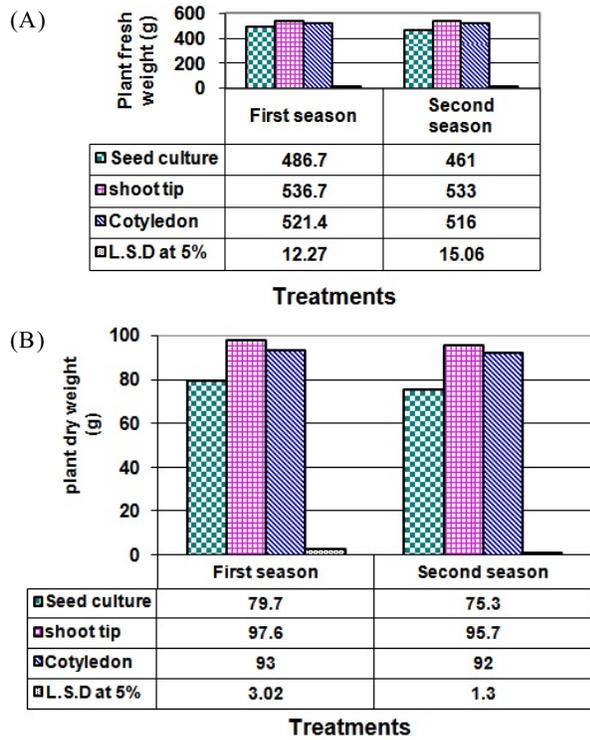


Fig. 6: Comparison between tissue culture and seed sowing on fresh and dry weight of cucumber plant after 45 days from transplanting to the green house.

due to the previous results of tissue culture plants which possessed much shoot number per plant and width leaf area with high leaf chlorophyll content that induced more photosynthetic rates. This in turn built high yield of carbohydrates which gave rise to more cell division and enlargement inducing more vegetative vigorous tissue culture plants, this reflect to produce more total yield than those of conventional plants^[7, 17].

Economical Evaluation: Comparing study was done to evaluate the price of seedling obtained by tissue culture method and traditional nursery method, the differences was calculated in terms of money according to the price of market. These values were presented in Table (3) which calculate in details the total cost of 5000 plantlets derived from tissue culture(Shoot tipd + Cotyledons) cycle period (45 days) compared by produce 500 seedlings from cucumber hybrid (hand F1) by conventional way.

The data in Tables (3 and 4) indicate that the total cost price of tissue culture technique reached 2097. 5 Egyptian pounds meanwhile it was 449. 25 through out traditional nursery method. But at the same time because of tissue culture technique produced number of

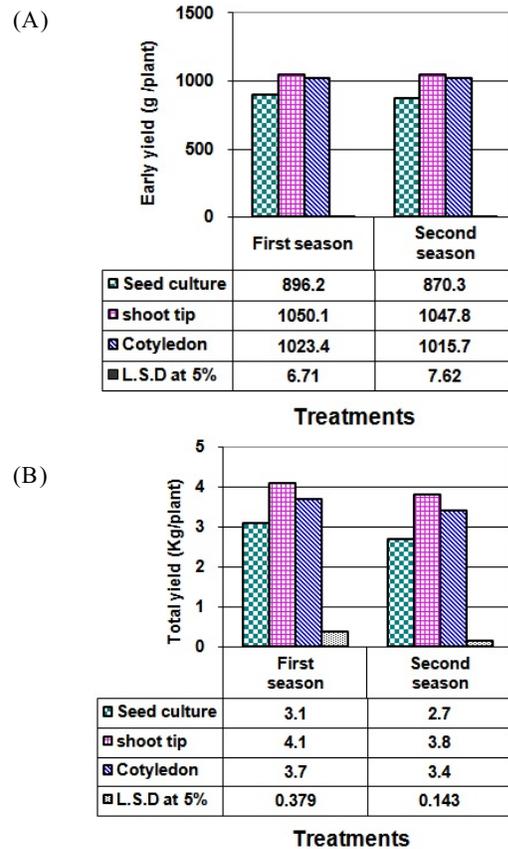


Fig. 7: Comparison between tissue culture and seed sowing on early and total yield of cucumber plants growth under the green house conditions.

Table 3: Total cost, profit and economical analysis of plantlets obtained by tissue culture and seedlings produced via the traditional nursery methods. Conventional methods.

Items	Tissue culture	Conventional
Seed Stock	500	500
Price per unit (pound)	0. 65	0. 65
Total cost (pound)	325	325
*Total seedling production per plant	5000	500
Netted pots	5000	500
Price per unit (pound)	0. 06	0. 06
Total cost (pound)	300	30
Substrates /seedlings (kg)	225	24. 5
Price per unit / liter (pound)	0. 5	0. 5
Total cost (pound)	112. 5	12. 25
Foliar spray (cm)	200	40
Price per unit	0. 05	0. 05
Total cost (pound)	10	2
Pesticides (pound)	0	10
Labor cost /45 days (Pound)	450	45
Lab rental / 45 days (Pound)	500	0
Green house rental / area (Pound)	250	25
Media chemicals (Pound)	150	0
Total cost (pound)	2097. 5	449. 25

* Tissue culture technique produce number of plantlets 10 times more than the traditional methods as mentioned before.

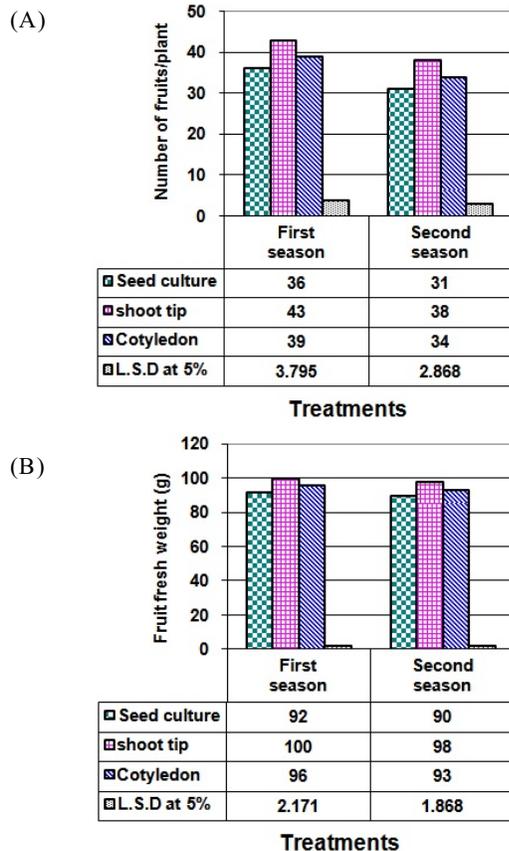


Fig. 8: Comparison between tissue culture and seed sowing on average fruit number per plant and fruit fresh weight of cucumber plants grown under the green house conditions.

Table 4: Total cost and profit (net income) of cucumber seedling production by tissue and conventional methods

Parameters (Pound)	Tissue culture	Conventional
Total seedling production per plant	5000	500
Total cost price of seedlings	2097. 25	449. 25
% Looses	10	2
Net seedling production	4500	490
Cost price of one seedling	0. 47	0. 92
Price of sold one seedling	1. 5	1. 5
Profit (net income) for sold one seedling	1. 03	0. 58
Total profit (net income) for sold all seedlings	4635	284. 2

plantlets 10 times more than the traditional propagation per one seed i.e., 5000 plantlets compared with 500 plant obtained by the seed sowing culture. This in turn reduced the cost price of one seedling from 1.3 L. E to 0.5 L.E as presented in Table (4). So the total income profitable for sold the plantlets via tissue culture technique reached 4635 L. E compared with 284. 2 L. E produced by conventional method.

General Conclusion: We can concluded that very encouraging to use tissue culture technique because it produced much number of plantlets which led to a reduction in the cost price of hybrid cucumber seedlings obtained, and we can say that the asexual propagation in vitro become the great benefit to cut price down and may be considered the best way to stop the increasing price of hybrid seeds year after year and this presented procedures can be replace the traditional nursery methods. On the other hand tissue culture propagation increasing the early and total yield and enhancement the quality of cucumbrt fruits as compared with the traditional method of propagation.

REFERENCES

1. Quak, F., 1977. Meristem culture and virus free plants: Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. Springer Verlag, New York, pp: 598-616.
2. Malepazy, S. and A. Nadolsky-Orezky, 1983. In vitro culture of cucumis sativus L. Regeneration of plantlets from callus formed by leaf explants. Pflanzenphysiologie, 111: 273-276.
3. Chee, P.P., 1992. Initiation and maturation of somatic embryos of squash (Cucurbita pepo). Hort Science, 27: 59-60.
4. Chee, P.P. and D.M. Tricoli, 1988. Somatic embryogenesis and plant regeneration from cell suspension culture of Cucumis sativus L. Plant Cell Rep., 7: 274-277.
5. Razdan, M.K., 1994. An Introduction to Plant Tissue Culture. Oxford and IBH Published Co. PVI, LTD, New Delhi, pp: 99-115.
6. Preece, J.E., 1992. Practical regulation of woody plant growth and development using biotechnology. Acta Hort., 300: 8-9.
7. Robinson, J.C., C. Fraser and K. Eckstein, 1993. A field comparison of conventional suckers with tissue culture banana planting material over three crop cycle. J. Hort. Sci., 68: 831-836.
8. Drew, R.A. and M.K. Smith, 1990. Field evaluation of tissue culture banana in south eastern queens land. Aus. J. Export. Agric., 30: 569-570.
9. Eckstein, K. and J.C. Robinson, 1993. Dry matter Production, photosynthesis and transpiration of tissue culture and sucker banana planting material. J. Hort. Sci., 68: 215-219.
10. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. plant., 15: 473-479.
11. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. Sixth Edition, IowaState Univ. Press, Amer., Iowa. USA.
12. Lou, H. and S. Kako, 1994. Somatic embryogenesis and plant regeneration in cucumber. Hort Science, 29: 906-909.

13. Hussey, G., 1978. In vitro propagation of the onion by axillary and adventitious shoot proliferation. *Scientia Hort.*, 9: 227-236.
14. Novak, F.J., J. Zadina, V. Horakova and I. Moskova, 1980. The Effect of growth regulators on meristem tip development and in vitro multiplication of *Solanum tuberosum* L. *Potato Res.*, 23: 155-166.
15. Edriss, M.H., T. El-Abd, S. Shanan and O. A. El-zeiny, 1992. Complete plantlet avoid callus formation from tomato hybrid Alex 61 using shoot tip culture technique. *J. Agr. Sci. Mansoura. Univ.*, 17(2): 91-96.
16. Edriss, M.H., A.F. Abou hadid and O.A. El-zeiny, 1995. Plant regeneration and somatic embryogenesis from shoot tip and cotyledon of *Cucumis melon* (Galia) *Acta Hort.* No, 434.
17. El-Zeiny O.A.H., 2002. Using tissue culture as a tool for increasing the productivity of seedlings and total yield of some pepper hybrids. *Arab Univ. J. Agric. Ain Shams Univ. Cairo*, 10: 273-285.
18. Van Niewkerk, J.P., R.H. Zimmerman and I. Fordham, 1986. Thidiazuron stimulation of apple shoot proliferation *in vitro*. *Hort Science*, 21: 516-518.
19. Fiola, J.A., M.A. Hassan, H.J. Swartz and R.H. Bors, 1990. Effect of thidiazuron, light fluence rates on in vitro shoot organogenesis from excised *rubus* cotyledons and leaves. *Plant Cell Tissue and Organ Culture*, 20: 223-228.
20. Anderson, H., 1993. How do micropropagation strawberries grow? *Grower*, 12: 16-22
21. El-Zeiny O.A.H., 1997. Tissue Culture study on tomato. Ph. D. Thesis Facult. Of Agriculture. Al-Azhar Univ. Cairo., pp: 73-74.
22. Barghchi, M. and P. G. Alderson, 1993. In vitro propagation of *Pistacia vera* L. From Seedlings tissue culture. *J. Hort, Sci.*, 58: 435-445.
23. Bigot, C. and L. Foury, 1984. In vitro propagation of globe artichoke (*Cynara scolymus* L.) from seeds : field comparison of some clones with their parent lines. *Agronomie*, 4: 699.
24. Swartz, H.J., G.J. Galleta and R. H. Zimmerman, 1983. Field performance and phenotypic stability of tissue culture propagated blackberries. *J. Amerc. Soc. Hort. Sci.*, 108: 285-290.
25. Grout, J.M., E.R. Paul and D.K. Wildung, 1986. Influence of tissue culture and leaf-bud propagation on the growth habit of blueberry. *J. Amer. Soc. Hort. Sci.* 111: 372-375.
26. Alphonse, M., M.A. Badawi and T.M. Abd-Elal, 2002. Physiological studies on the in vitro micropropagation of some artichoke genotypes 11-Rooting, adaptation and field production stages compared to seed and vegetative propagation methods. *Egypt. J. Hort.*, 29(32): 397-419.