J. of Plant Production, Mansoura Univ.,Vol 11 (8): 727-732, 2020

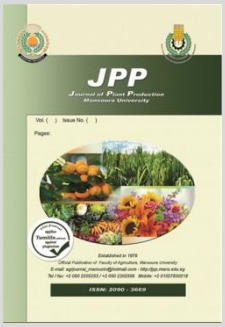
Journal of Plant Production

Journal homepage: www.jpp.mans.edu.eg
Available online at: www.jpp.journals.ekb.eg

An Efficient Protocol for Direct Organogenesis of Pepper (*Capsicum annuum*)

Abo Shama, H. M.*; E. S. Amer and I. M. Abu Hussein

University of Sadat City.,Genetic Engineering and, Biotechnology Institute, Plant Biotechnology Department ,Egypt.



ABSTRACT

A simple and efficient plantlet regeneration protocol was established for Pepper (*Capsicum annum* L.) achieved through direct organogenesis from Leaves and cotyledons explants. Leaves were taken from 21 day- old *in vitro* plantlets and and cultured on Murashige and Skoog (1962) MS medium supplemented with different growth regulators. Thidiazuran (TDZ)or Benzyl adenine (BA) with Indole 3-acetic acid (IAA) containing medium was tested. Prolific direct adventitious shoot regeneration occurred on most of the tested growth regulators. The best response in terms of frequency of shoots number per explant (10.5) was obtained from cotyledon cultured on 1.0 mg/L TDZ in combination with 0.5 mg/L IAA. To elongate the shoots it were cultured on MS medium supplemented with various concentrations of gibberellic acid (GA_3) in combination with 0.25 mg/L Kinetin (KIN). The highest shoot length (2.2 cm) was achieved on MS medium supplemented with 0.5 mg/L GA_3 and 0.25 mg/L KIN. Regenerated shoots were excised to root. The highest number of root/shoot was obtained with quarter MS salt medium supplemented with 1.0 mg/L indole-3- butyric acid (IBA) with high length of root (4.60). plantlets were finally transferred to mixture of peatmoss, vermiculite and sand at equal volume 1:1:1.The plantlets showed 80 % survival after 30 days during acclimatization and transplanting.

Keywords: *Capsicum annuum*, direct regeneration, cotyledon, *in vitro* culture, acclimatization, adventitious shoots.

INTRODUCTION

The sweet pepper (*Capsicum annuum* L.) is one of the most popular vegetable crops used by consumers. Pepper fruits represent an important part of the fresh vegetable market in Egypt and also significant worldwide in the segment of condiments, spices and salt preserves. Plants are a dicotyledonous and short-lived perennial herb of the family Solanaceae. The genus has about 25 wild and 5 domesticated species. However, *Capsicum annuum* is considered as the economically most important species of the genus. The most recent of FAO statistics indicate that in Egypt more than 637760 tonnes of fresh fruit is produced on 41303 hectares (FAOSTAT, 2016). Most of this area is grown with imported seeds at high cost, which is a burden on the national Egyptian economy. The sweet pepper is cultivated over a wide range of climate and soil conditions in both temperate and tropical regions of the world.

In conventional Propagation of pepper seeds are generally utilized. This method has some disadvantages such as: low of germination, short viability period and high risk of catching various diseases. Furthermore, pepper is sensitive to many pests and pathogens including fungi, viruses, bacteria and nematodes and extreme climatic conditions especially temperature extremes which are limiting factors for its production (Agrawal *et al.*, 1988; Christopher and Rajam , 1994). In order to improve propagation of the commercial cultivars of this specie and to meet the increasing demands for pepper, more reliable propagation approaches for mass multiplication are needed. Tissue culture methods provide a way to asexually multiply pepper plants as the plants lack natural vegetative propagation. Several tissue culture techniques for micropropagation of pepper have been reported from different explants, including hypocotyls, leaf, stem apex , cotyledon, root, and zygotic embryo (Sanatombi and Sharma 2008b;Batista *et al.*, 2013) and induced somatic embryogenesis (Aboshama 2011,Gatz 2014 ;). many of the *in vitro* conditions set for a specific cultivar of the plant proved inappropriate for proper micropropagation of other cultivars.

The aim of this study is to develop an efficient protocol for plant regeneration of pepper cultivar (top star) that planting in Egypt through direct organogenesis and subsequent multiplication, elongation, rooting and acclimatization with high survival of plantlets.

MATERIALS AND METHODES

This study was carried out at Plant Biotechnology Department, Genetic Engineering and Biotechnology Institute, University of Sadat City, Egypt, during the years of 2015 to 2018. Commercially *Capsicum annuum* cv. top star (Japanese origin) was supplied from Seeds Company (Saif Gaara Company, Egypt) for *in vitro* regeneration studies. All experiments were repeated two times during the years of 2015 to 2018.

Establishment of *in vitro* cultures:

Seeds of *Capsicum annuum* L. cv. top star were soaked for 5 hours in distilled water and surface sterilized with 50% commercial clorox (5 % NaOCl) for 10 minutes along with 2-3 drops of Tween 20. Seeds were rinsed three times with sterile distilled water. Seeds were cultured on

* **Corresponding author.**
E-mail address: haroun.aboushama@gebri.usc.edu.eg
DOI: 10.21608/jpp.2020.112903

Murashige and Skoog (1962) MS medium supplemented with 3% sucrose, 0.7 % Agar. After 21 days from culture, leaves and cotyledons were separated from *in vitro* germinated plants and used as explants for organogenesis induction. The medium pH was adjusted to 5.7-5.8 and autoclaved at 121°C and 1.2 kg/cm2 air pressure for 20 min. All cultures were maintained at 25 ± 20C under florescent light (2000 LUX) and 16-h / 8 dark cycles.

BA and IAA concentrations on shoot induction.

Cotyledon and leaf explants of *in vitro* germinated plants of *Capsicum annuum* cv. top star were cultured on MS medium supplemented with 3% sucrose + 0.7 % Agar with different concentration of Benzyl adenine (BA) at (0.0, 1.0, 2.0,3.0, 4.0 and 5.0 mg/L) and Indole 3-acetic acid (IAA) at(0.0, 0.5 and 1.0 mg/L) to evaluate their effect on shoot bud induction. Three replicates were designed and each replicate was one jar (150 ml) cultured with three explants. The subculture on the same medium after 20 days. Percentage of explants response of shoot induction and numbers of shoot per explants were counted after 40 days from culture on induction medium.

TDZ and IAA concentrations on shoot induction.

Cotyledon and leaf explants were cultured on MS medium supplemented with 3% sucrose + 0.7 % Agar with different concentration of Thidiazuran (TDZ) at (0.0,0.5,1.0 and 2.0mg/L) and (IAA) at (0.0, 0.5 and 1.0 mg/L) to evaluate their effect on shoot bud induction. Three replicates designed and each replicate one jar (150 ml) cultured with three explants. The subculture on the same medium after 20 days. percentage of explants response of shoot induction and numbers of shoot were counted after 40 days from culture on induction medium.

shoot elongation.

To evaluate shoot elongation, cluster of shoots were cultured in MS medium containing 3% sucrose +0.7% Agar + 0.25 mg/L Kinetin (KIN) with different concentrations of gibberellic acid (GA3) at (0.0,0.5 and 1.0 mg/L). Three replicates were designed and each replicate three jars (150 ml) cultured with one cluster. The Subculture on the same medium as described in previous experiment. Data were recorded for shoot lengths (cm) after 40 days from culture.

Root formation and acclimatization

For rooting formation, the shoot at length of (2.0 to 2.5 / cm) was individually cultured on rooting media consisting of quarter, half or full strength MS salts medium and each of which supplemented with 3.0 g/L activated charcoal (AC) + 2 % sucrose with different concentrations of indole-3- butyric acid (IBA) (0.0 , 0.5 and 1.0 mg/L). Numbers of roots/ shoot and length of roots were evaluated after 4 weeks. Three replicates were designed and each of replicate consisted of three tubes (100 ml) cultured by one explant.

For acclimatization, plantlets were carefully washed in running tap water to remove the substrate and then transferred to pots containing mixture of peatmoss, vermiculite and sand at equal volume (1:1:1). Pots were covered with plastic bags to maintain high relative humidity around the plants in a greenhouse. A solution of half strength MS salts medium was added to the pots to enhance the development of plants. Percentage of survival was recorded after 30 days from transplanting.

Analysis Statistical.

All experiments were carried in completely randomized design with two ways analysis of variance and the mean values were compared using the Fisher's least significant difference test (LSD test) at 5% according to the method described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Effect of BA and IAA concentrations on shoot induction.

The present investigation show that it was possible to obtain high frequency of shoot bud regeneration directly from leaf and cotyledon explants of Pepper. Initially, the leaf and cotyledon explants on the responded treatments of growth regulators enlarged and developed globular like structures at end of the leaf or cotyledon from intact stem within 2 weeks of culturing. After 40 days from culturing, all shoots were directly proliferated from petioles Fig1. This result was in agreement with the report presented by Gayathri *et al.,* (2015) which showed that multiple shoot formation was observed after 14 days from leaf culture of *Capsicum Chinense* Jacq. (naga king chili).

Fig .1. Direct organogenesis from leaf cultured on MS medium supplemented with 4.0 mg/L BA + 0.5 mg/L IAA

Table 1 shows the main effect of growth regulators on number of shoots per explants. The results showed that there was no shoot initiation in the absence of BA . Data indicated that the main effect of BA show that, BA significantly increased shoot initiation . The highest mean number of shoots per explant (5.58) was recorded using cotyledon at 4.0 mg/l BA , followed by (5.15) at 5.0 mg/L BA without significant differences , while the lowest mean number of shoots per explants was recorded (1.08) at 1.0 mg/L BA .

Table 1. Effect of BA and IAA concentrations on direct shoot bud proliferation from cotyledon and leaf culture of pepper.

Treatments (mg/l)	Mean Number of shoot per explant													
	Leaves							Cotyledon						
BA / IAA	0.0	1.0	2.0	3.0	4.0	5.0	Means (A)	0.0	1.0	2.0	3.0	4.0	5.0	Means (A)
0.0	0.0	0.0	0.83	2.63	3.73	3.10	1.75	0.0	0.0	1.86	3.50	5.43	4.96	2.66
0.5	0.0	0.0	1.73	3.16	5.40	3.40	2.31	0.0	1.30	2.50	4.86	7.26	6.96	3.83
1.0	0.0	1.20	2.30	3.30	3.30	3.73	2.32	0.0	1.86	2.50	3.73	4.06	3.50	2.63
Means (B)	0.0	0.46	1.62	3.03	4.14	3.41		0.0	1.08	2.30	4.03	5.58	5.15	
LSD	A 0.51		B 0.36			A&B 0.88		A 0.48		B 0.34			A&B 0.84	

And leaf explant was recorded the highest mean number of shoots per explant (4.14) at 4.0 mg/L BA , followed by(3.4) at 5.0 mg/L BA with significant differences, while the lowest mean number was recorded (0.46) at 1.0 mg/LBA. These results are in harmony with those of reported by Sanatombi and Sharma (2008a) showed that the maximum bud induction of the tested cultivars of *Capsicum annuum* when cultured on MS medium containing BA compared with those of control and other treatments. Cell division, shoot multiplication and axillary bud formation can be promoted by BA(Deepa *et al.*2018).The effect of cytokinins on stimulation both of cell division and also promotion growth of axillary shoots has been discussed by George *et al.*,(2008).

As for the interaction, a higher mean number of shoots /explant was recorded (7.26) in cotyledon at 4.0 mg/l BA combined with 0.5 mg/L IAA followed by (6.96) at 5 mg/L BA combined with 0.5 mg/L IAA without significant differences . leaf explant recorded the highest mean number of shoots per explants (5.4) at 4.0 mg/L BA combined with 0.5 mg/L IAA (Table1).This result was agreed with Mezghani *et al.*, (2007) they found that the maximum shoot bud induction of *Capsicum annuum* using cotyledons as explant cultured on BA in combination with IAA compared with those of control and other treatments. Auxins are using to increase the activating enzyme that break down starch and has the ability to move the active leading to increased proliferation of organogenesis(Taiz and Zeiger2009).The role of auxin in multiplications has been described by Majda and Robert(2018).

Fig 2 (A, B) refers to the variation in response percentage of cotyledon and leaves to shoot formation. The best percentage obtained at concentration 4.0 and 5.0 mg/L BA with 0.0 and 0.5 mg/L IAA . Roots formed on Leaves in absence of BA.

Effect of TDZ and IAA concentrations on shoot induction .

Data in Table 2 show the main effect of growth regulators on number of shoots per explants. The results showed that there was no shoot initiation in the absence of growth regulators. Data indicated that the main effect of TDZ show that, TDZ significantly increased these characters. The highest mean number of shoots per explant (9.71) was recorded using cotyledon at 1.0 mg/l TDZ , followed by (7.92) at 2.0 mg/l TDZ with significant differences, while the lowest mean number of shoots per explants was recorded (5.13) at 0.5 mg/l TDZ compared to leaf explant that recorded the highest mean number of shoots per explant (9.0) at 1.0 mg/L TDZ , followed by(6.74) at 2.0 mg/L TDZ with significant differences, while the lowest mean number was recorded (4.53) at 0.5 mg/LTDZ.

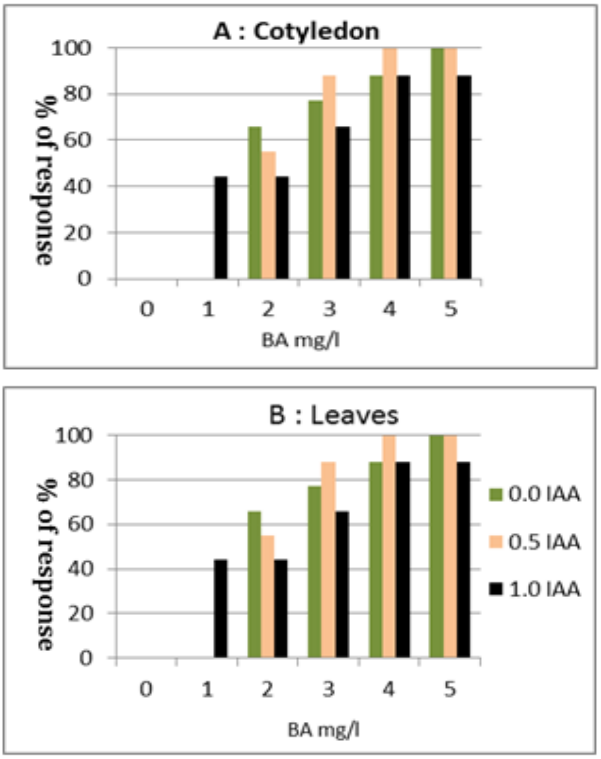


Fig .2. percentage of response to organogenesis induction from (A) cotyledon (B) leaves of pepper

Table 2. Effect of TDZ and IAA concentrations on mean number of shoot / explant of Pepper.

Treatments (mg/l)	Mean number of shoots/ explant									
	Leaves					Cotyledon				
TDZ IAA	0.0	0.5	1.0	2.0	Means (A)	0.0	0.5	1.0	2.0	Means (A)
0.0	0.00	4.46	9.4	7.10	5.28	0.00	4.93	9.86	8.33	5.80
0.5	0.00	5.23	10.1	7.46	5.72	0.00	5.76	10.5	8.56	6.25
1.0	0.00	3.90	7.43	5.66	4.27	0.00	4.70	8.70	6.86	5.09
Means (B)	0.00	4.53	9.00	6.74		0.00	5.13	9.71	7.92	
LSD	A	0.45	B	0.39	A x B 0.78	A	0.65	B	0.56	A x B 1.13

The results are in full agreement with those previously reported by many workers who found that shoot proliferation dependent to a great extent on kind and concentration of cytokines used. Otroshy *et al.*, (2011) tested the effect of some plant growth regulators cytokinins such as TDZ at different concentrations (0, 1.0, 2.0 mg/l) and some plant growth regulators auxins such as IBA, IAA and NAA at different concentrations (0.0, 0.2 and 0.5 mg/l) via individually or in combination on shoot formation of *Capsicum annuum*. L. reported that.

As for the interaction, a higher mean number of shoots /explant was recorded (10.5) in cotyledon with 1.0 mg/l TDZ combined with 0.5 mg/L IAA compared to leaf explant that recorded the highest mean number of shoots per explants (10.1) at 1.0 mg/L TDZ combined with 0.5 mg/L IAA (Table1). The results are in agreement with those previously reported by many workers who found that shoot proliferation depended to a great extent on kind and concentration of cytokinin used plus auxin Khan *et al.*, (2011) revealed that MS medium supplemented with

cytokinins such as BA and TDZ at different concentrations (0.0, 0.5, 2.5, 5.0, 7.5 and 10.0 µM /L) affected positively on buds induction from single nodes of *Capsicum annuum* cv. PusaJwala. The maximum shoot induction achieved when single node of *Capsicum annuum* cv. PusaJwala were cultured on MS media augmented with 5.0 µM /L of BA and 2.5 µM /L of TDZ compared with those of control and other treatments.. The shoot proliferation depends upon the balance of cytokinins and auxins the addition of lowest levels concentration of NAA used affect well the initiation of grapevine *in vitro* (Tapia and Read, 1998).

All explants were responded to shoot formation except in absence of BA.

Fig .3. proliferation shoot on cotyledons explant cultured on MS medium + 1.0 mg/L TDZ

Effect of different concentration of GA_3 on shoot elongation of pepper cultivar Top star .

Data in Table 3 and fig 4 showed the effect of GA_3 on the morphogenetic characteristics of pepper cultivar Top star. It was clear that shoot length (cm) as parameter indicating the morphogenesis and growth were dependent to a great extent on concentration of GA_3. The results indicated that the effect of GA_3 on enhancing morphogenetic characteristics was dependent on the GA_3 concentration on shoot length (cm) parameter of pepper cultivars recorded the highest values, when cultured on MS medium supplemented with 0.5 mg/ L of GA_3 plus 0.25 mg /L kin in comparison with those of control and other treatments. The results are in full agreement with those previously reported that shoot proliferation depended to a great extent on kind and concentration of cytokinins and gibberellins used as Hyde and Phillips,(1996) studied the effect of some plant growth regulators such as NAA and GA_3 at different concentrations from 0.1 up to 1.0 mg / L via individually or in combination on shoot elongation of Chile pepper *Capsicum annuum* L. illustrated that the highest shoot length (cm) attained with the micro-shoots of Chile pepper *Capsicum annuum* L were cultured on MS media supplied with 2.0 mg /L of NAA and GA_3 compared with those control and other treatments. Also, Arous *et al.*, (2001) studied the effect of some plant growth regulators such as (BAP at 1.0 mg /L plus NAA at 0.5 mg/L) and BAP at 1.0 mg /L , NAA at 0.5 mg/L plus GA_3 at 0.5 mg /L) on shoot elongation of Tunisian chili (*Capsicum annuum* L.). Indicated that adding 0.5 mg /L of GA_3 to the growth MS medium complemented with BAP and NAA at 1.0 and 0.5 mg /L respectively caused the highest shoot length (cm) of Tunisian chili (*Capsicum annuum* L.) compared with MS media without gibberellic acid.

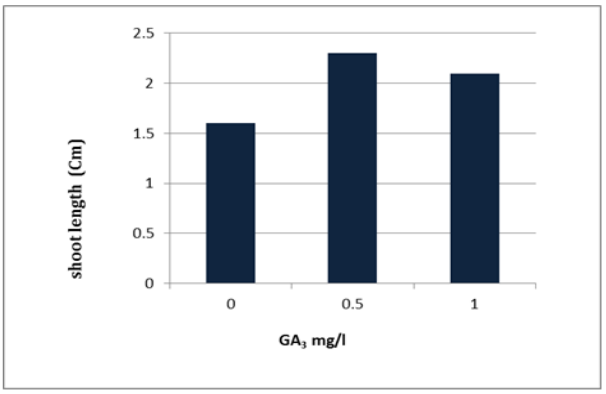


Fig .4. Effect of GA_3 concentrations on shoot length (cm)

Fig .5. Shoot elongation of pepper on MS medium + GA_3 1 mg/L + 0.25 mg /L kin .

Root formation and acclimatization

The obtained shoots of tested pepper cultivar (Top star) from the previous proliferation medium were transferred to rooting media containing different strength (quarter , half and full strength) of the basal MS medium supplemented with Indole butyric acid (IBA) at concentrations of 0.0 , 0.5 and 1.0 mg / L. all strength salts MS medium supplemented with auxin aiming to induce roots. Data in Table (4)indicated the effect of all strengths MS medium augmented with three concentrations of IBA enhanced roots number and root length (cm) of shoot pepper compared with control. Quarter MS medium supplemented with IBA at 1.0 mg /L possessed the maximum roots number (4.33) compared with different strength of MS medium and another concentration of IBA .on the other hand, adding 1.0 mg /L of IBA to the quarter MS medium recorded the highest shoot length (4.6 cm)

followed by (4.33 cm) at 0.5 mg/L IBA with quarter MS salts medium without significant differences compared with those of strength MS media and another concentration of IBA. cultivar top star noticed in relation to its responses to impact of three types of strength MS medium and all concentrations of IBA on root morphogenetic characteristics.

Table 3. Effect of MS salts medium at different strength with IBA concentrations on root formation of pepper.

Treatments	Number of roots				Length of roots			
MS IBA(mg/l)	1/4	1/2	1/1	Means (A)	1/4	1/2	1/1	Means (A)
0.0	1.36	1.03	0.40	0.93	1.66	1.46	0.56	1.23
0.5	3.33	2.66	1.36	2.45	4.33	2.50	1.26	2.70
1.0	4.33	2.03	2.66	3.01	4.60	3.40	1.53	3.17
Means(B)	3.01	1.91	1.47		3.53	2.45	1.12	
LSD	A 0.62	B 0.62	A x B 1.08		A 0.93	B 0.93	A x B 1.61	

The results are in agreement with those previously reported by many workers as follows Hailu *et al.,* (2015) experimented the effect of auxins as IBA and IAA at different concentrations (0.0, 0.25, 0.5, 0.75 and 1.0 mg /L) individually on rooting of Elite Ethiopian Hot Pepper (*Capsicum annuum* L.) Cultivar: marekofana. Found that MS medium supplemented with 0.5 mg /L of IBA gained the maximum roots number and the highest of root length (cm) and shoot length (cm) of Elite Ethiopian Hot Pepper (*Capsicum annuum* L.) Cultivar: marekofana in comparison with those control and other treatments. Also, Gayathri *et al.,* (2015) tested the effect of IBA at different concentration (0.0, 3.68, 6.71 and 7.36 µM /L) on rooting of *Capsicum Chinense*Jacq. (naga king chili).Stated that adding 7.36 µM /L of IBA to the growth MS media possessed the upper root formation percentage, the maximum roots number and the highest root length (cm) in comparison with those control and other treatments. In addition, Hyde and Phillips, (1996) established that adding NAA from 0.1 up to 1.0 mg /L to the growth half strength MS media enhanced the rooting of Chile pepper *Capsicum annuum* L compared with MS medium without any auxins . Culturing the micro-shoots of Chile pepper *Capsicum annuum* L. on MS containing NAA at 0.1, 0.5 and 1.0 mg /L for one month possessed the lowest root formation percentage while subculturing the micro shoots of Chile pepper *Capsicum annuum* L on MS medium complemented with 0.1 mg /L of NAA gave the highest root formation percentage compared with those control and other treatments. The use of auxins and many other factors and changes in the rooting environment have been described in order to enhance the rooting of micro cuttings (Brand and Lineberger, 1986). And also, the lack of rooting morphogenesis may be due to lack of cell sensitivity to respond to morphogenesis even though auxin may present in abundance (Hartmann *et al.,* 1997).

Similarly, auxins are involved the process of adventitious root formation. In many woody plants, IBA is commonly used to promote root initiation (Torrey, 1976).

After 30 days from acclimatization the plantlets grew vigorously with high percentage of plant survival (80%) was achieved during this phase of culture. The transplanted plants had established well in pots Fig (6 B).

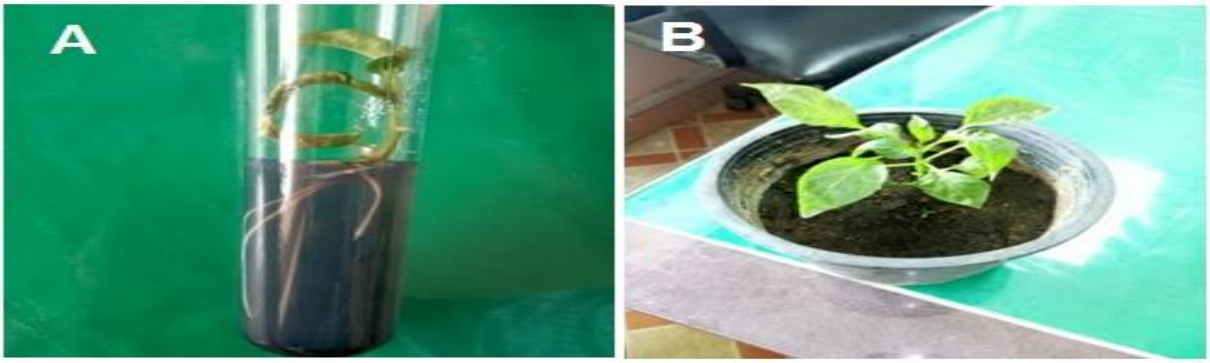


Fig .6. A: Rooting of shoots regenerated from leaf of *Capsicum annuum* L on rooting medium consisted of MS medium enriched with 1.0mg/L IBA and 3.0 g/L (AC). B: Acclimatization of plantlet that are driven from direct organogenesis of pepper

REFERENCES

Aboshama, H.M.S (2011). Direct Somatic Embryogenesis of Pepper (*Capsicum annuum* L.).World Journal of Agricultural Sciences 7 (6): 755-762, 2011

Agarwal S.; Chandra N.; Kothari S. L. Shoot tip culture of pepper for micropropagation. CurrSci 57: 1347–1349; 1988.

Arous.S, Boussaïd.M and Marrakchi.M (2001) Plant regeneration from zygotic embryo hypocotyls of Tunisian chili (*Capsicum annuum* L.).J.Appl. Hort., 3(1):17-22, January-June, 2001

Batista, D.S., Dias, L.L.C., Macedo, A.F., Rêgo, M.M., Rêgo, E.R., Floh, E.I.S. and Otoni, w.c. (2013) Suppression of ethylene levels promotes morphogenesis in pepper (*Capsicum annuum* L.) *In Vitro Cellular & Developmental Biology Plant* 49,759-764.

Brand, M.H and Lineberger, R.D. (1986). *In vitro* propagation of *Halesiacaro lina* L. and the influence of explanations timing on initial shootproliferation. Plant Cell, Tissue and Organ Culture 7:103-113.

Christopher T.; Rajam M. V. *In vitro* clonal propagation of Capsicum spp. Plant Cell Tiss Organ Cult 38: 25–29; 1994.

Deepa, A. V.; Anju, M.and Thomas T. D. (2018). The applications of TDZ in medicinal plant tissue culture. In Thidiazuron: From Urea Derivative to Plant Growth Regulator (pp. 297-316).

FAOSTAT (2016).Food and Agricultural Commodities Production; Available online: http://faostat.fao.org

Gatz A., 2014 Morpho-Histological Aspects Of Adventitious Shoot Formation With Out Plant Growth Regulators In Seed Explants Of *Capsicum annuum* L., And Impact Of Preculture On Regeneration .Acta Sci. Pol., HortorumCultus 13(2) 2014, 135-150

Gayathri.N; Gopalakrishnan.M and Sekar. T (2015) *In vitro* micropropagation of *Capsicum Chinense*Jacq. (naga king chili).Asian Journal of Plant Science and Research, 2015, 5(12):13-18

George, E.F., Michael, A.H and Greek, D.K. (2008).Plant Propagation by Tissue Culture (3 Edition). The rd Background, Springer, pp: 1.

Hailu,T; Abera.B and Daksa. J (2015) In Vitro Direct Organogenesis Protocol for Mass Propagation of an Elite Ethiopian Hot Pepper (*Capsicum annuum* L.) Cultivar: MarekoFana. American Journal of Plant Sciences,2015, 6, 1435-1443

Hartmann, H. T., Kester, D. E., Davies,F. T and Geneve, R. L. (1997). Plant propagation: principles and practices (No. Ed. 6). Prentice-Hall Inc.

Hyde,C. and Phillips.G (1996) Silver Nitrate Promotes Shoot Development and Plant Regeneration of Chile Pepper (*Capsicum annuum* L.) via Organogenesis.In VitroCell.Dev.B ~ ol.—Plant 32 : 72 ~80, April-June 1996

Khan,H; Siddique.I; Anis .M and Pervaiz. R.K (2011): *In vitro* organogenesis from internode derived callus cultures of *Capsicum annuum* L.J. Plant Biochem. Biotechnol. (Jan–June 2011) 20(1):84–89

Majda, M. and Robert, S. (2018). The role of auxin in cell wall expansion. International journal of molecular sciences, 19(4), 951.

Mezghani. N, Jemmali. A, Elloumi.N, Gargouri-Bouzid.R and Kintzios. S (2007) :Morpho-histological study on shoot bud regeneration in cotyledon cultures of pepper (*Capsicum annuum*).Biologia, Bratislava, 62/6: 704—710, 2007

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

Otroshy.M ;Moradi. K and Nekouei.M.K(2011) The effect of different cytokenins in propagation of *Capsicum annuum*L. by *in vitro* nodal cutting. Trakia Journal of Sciences, Vol. 9, No 3, pp 21-30, 2011

Sanatombi . K. and Sharma. G.J. (2007): Micropropagation of *Capsicum annuum* L. Not . Bot. Hort. Agrobot. Cluj. 35:57-64.

Sanatombi K.; Sharma G. J. (2008a) *In vitro* plant regeneration in six cultivars of Capsicum spp. using different explants.Biol Plant 52: 141–145;.

Sanatombi K.; Sharma G. J. (2008b) *In vitro* propagation of *Capsicum chinense* Jacq.Biol Plant 52: 517–520;.

Steel RGD, Torrie JH. (1980) Principles and Procedures of Statistics: A Biometrical Approach,2nd ed.. New York: McGraw-Hill Book Co.,;137-77.

Taiz, L. and E. Zeiger. (2009): Plant physiology. 4rd Ed. Sinauer, Sunderland, MA.

Tapia, M.I. and Read, P.E. (1998).Propagation of grape hybrids by *in vitro* culture of Axillary.Agro.Ciencia., 14(1): 35-41.

Torrey, J. G. (1976). Root hormones and plant growth. Annual Review of Plant Physiology, 27(1), 435-459.

Wilkins, M.B. (1989). Advanced Plant Physiology. The Bath Press, Avon, pp: 13-15.

التكوين العضوى المباشر فى نبات الفلفل

هارون ابو شامه ، ايناس عامر و اسلام مبروك ابو حسين

جامعه مدينه السادات – معهد الهندسه الوراثيه والتكنولوجيا الحيويه – قسم البيوتكنولوجيا النباتيه

تم فى هذا البحث وضع تقنيه لانتاج نباتات فلفل عن طريق التكوين العضوى المباشر من الورقه والورقه الفلقيه .اخذت الاوراق والاوراق الفلقيه من بادرات ناتجه من انبات بذور الفلفل صنف (توب استار) معمليا بعمر 21 يوم وزرعت فى بيئه موراشيج وسكوج مضاف اليها بعض منظمات النمو بتركيزات مختلفه. وكانت منظمات النمو المستخدمه عباره عن الثياديزورن TDZ وبنزيل ادنين BA مع اندول حامض الخليك (IAA). وقد اظهرت النتائج تكوين الافرع مباشره على الورقه فى معظم البيئات المحتويه على اى من منظمات النمو المختبره, وكانت اعلى معدل لانتاج الافرع على الجزء النباتى الواحد (الورقه الفلقيه) (10.5فرع) وكانت اعلى قدره على تكوين الافرع كانت مع استخدام 1.0 ملليجرام / لتر الثياديزورن مع (0,5 ملليجرام / لتر) اندول حامض الخليك . زرعت الاجزاء النباتيه وماعليها من افرع على بيئه موراشيج وسكوج مضافا اليها تركيزات مختلفه من حامض الجبرلين بالاضافه الى تركيز ثابت من الكاينتين (0,25ملليجرام / لتر)وذلك لمعرفه تاثيرتركيزات حامض الجبرليك على استطاله والافرع المتكونه على الاجزاء النباتيه المختلفه. وكانت افضل النتائج تشير الى ان اعلى استطاله للافرع (2,2 سم) كانت عند تركيز 0,5 ملليجرام / لتر من حامض الجبرلين بالاضافه الى التركيز الثابت من الكاينتين (0,25ملليجرام / لتر). فصلت الافرع الناتجه بعد استطالتها وزرعت على بيئه بغرض تجذيرها وكان اعلى عدد جذور(4.33 جذر) على العقل المنزرعه فى بيئه موراشيج وسكوج عند ربع قوه مضاف اليها اندول حامض البيوتيرك عند تركيز 1.0 ملليجرام / لتر حيث كانت الجذور بطول 4,6 سم. نقلت النباتات الى خليط من كميات متساويه حجما من بيتموس و فرموكيوليت ورمل بنسب متساويه حجما وكانت نسبه النباتات الحيه المؤقلمه بعد شهر من النقل الى الاصص 80%.