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MICROTUBERIZATION OF POTATO (*Solanum tuberosum* L.):-

1- PROTOCOL FOR PRODUCTION OF MICROTUBER *IN VITRO.*

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ABSTRACT

This study was conducted in the tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, Mansoura University during the period from 2002 to 2005, aiming to investigate the effect of cytokinins (BAP or Kin) and/or auxin (NAA) on multiplication rate as well as auxins (2,4-D, IBA and NAA) or cytokinin (BAP) on microtuberization of potato as a new and simple protocol for production of potato *in vitro*. The effect of cytokinins and auxin on multiplication rate (shoot length, number of both shoots and leaves per explant and fresh weight of cluster) was recorded in this study. Shoot length was increased by all Kin levels and decreased by BAP levels while was not affected by NAA. Moreover, either BAP or Kin increased number of shoots per explant, especially at 0.2mg/L (4.80 and 2.77, respectively).The highest number of leaves per explant was obtained by 0.2 mg/L BAP (16.41) or 0.5 mg/L Kin (14.48). BAP and Kin had the same effect on increasing cluster fresh weight. The combination between NAA combined with either BAP or Kin increased number of leaves per explant and cluster fresh weight. BAP at (0.2 mg/L BAP + 0.1 mg/L NAA) proved its efficiency than the similar combination of Kin and NAA (at the same concentrations) for all studded characters.

Potato microtuberization can be done by using of any tested auxins alone (2,4-D, IBA or NAA) each at 0.0, 0.5, 1.5 and 1.5 mg/L in MS medium supplemented with 80g/L sucrose and incubated in dark condition. The Best results of microtuber weight, diameter, length and number/shoot were at 1.0 mg/L 2,4-D (151, 5.30, 7.50 and 1.66, respectively) and at 1.5 mg/L IBA (129, 4.70, 7.50 and 1.57, respectively) or NAA (158, 5.10, 8.20 and 1.66, respectively).

In addition, microtuberization also can be done by using BAP alone at 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/L under the same condition. The Best results were at 2.0 mg/L BAP (109, 4.80, 7.70 and 2.88 for microtuber weight, diameter, length and number/shoot, respectively).

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae. It is one of the most important vegetable crops for local consumption and exportation. Its production ranks fifth among major food crops behind wheat, rice, maize and barley (Hooker, 1981). There are many problems limited the production of potato as fungus and virus diseases. Disease-free plants are high yielding and produce tubers of better marketable quality and resulting higher price. The loss of potato yield through diseases has been estimated to be 30% and the seed costs represent about 50 % of total costs of potato production. (Wang and Hu, 1982).

The use of tissue culture techniques is the possible alternative for the production of minitubers and using them as seed tubers. Potato can be micropropagated rapidly and on a large scale by meristem and shoot tip culture (Escalada and Garcia, 1982). Proliferation can be done by axiliary shoot development from *in vitro* cultured nodal cuttings (Hussey and Stacey, 1984). Nodal segment is mainly used for rapid *in vitro* shoot multiplication (Nozeran *et al.*, 1977), especially which obtained plantlets which are usually true to type (Hu and Wang, 1983). On the other hand, plants regenerated from callus often differ genetically from the original plants (Van Harten *et al.*, 1981). *In vitro*-grown shoots and nodal explants proved superior to shoot tips for shoot formation, shoot length, number of roots, root length, number of microtubers and microtubers weight (Fatima *et al.*, 2005). There are many advantages for potato seed production by using culture as reduction of imported potato seeds, *in vitro* microtubers is easy for storage and transportation, rapid multiplication, reduction of potato seed costs and production of virus free seeds. In this study, the effect of either auxin or cytokinin was used to develop a system for *in vitro* tuber induction with applicability to a wide range of commercial laboratory as well as easy and low cost. Therefore, this work aimed to develop protocol for production of microtubers by auxin or cytokinin only through tissue culture technique.

MATERIALS AND METHODS

This study was carried out in the tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, Mansoura University during the period from December 2002 to December 2005 to study proliferation of shoots (multiplication) and the effect of auxins type (2,4-D, IBA, NAA) or cytokinin (BAP) on production of microtubers *in vitro* of *Solanum tuberosum*, L. cv. spunta.

Plant materials

Sprouts of spunta cultivar were used as a source of shoot tips in this study. Sprouts about 1-1.5 cm were cut carefully from tubers and washed with running tap water for 30 min. Surface disinfection of explants was achieved by immersion with 20% commercial clorox solution (v/v) (0.5% sodium hypochlorite) for 15 min. followed by four rinses in sterile distilled water to remove all traces of the disinfectant. Aseptic shoot tips were excised at 3-4 mm long and cultured on MS (Murashige and skoog, 1962) medium supplemented with 30 g/L sucrose, 2.0 mg/L calcium panthothinate and solidified with 7.0 g/L agar. The pH medium was adjusted to 5.7 prior to the addition of agar. Medium was distributed into culture jars (250 ml), each jar contained 30 ml of the medium. The jars were capped with polypropene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 20 min. Three shoot tips were cultured in jars. All cultures were incubated at 22±2°C under 16 hours using cool white fluorescent light (about 2000 lux). Three subcultures (one every 4 weeks) to obtain sufficient shoots. These shoots were a source of single nodes which were used in all further experiments in this study.

1- Multiplication stage

The aim of these experiments was to determine the optimal combination of cytokinins and auxin levels on multiplication from single node culture of potato. Aseptic single nodes were excised from *in vitro* shoots as mentioned above. These single nodes were cultured on MS medium supplemented with 30 g/L sucrose, 2.0 mg/L calcium panthothinate and solidified with 7.0 g/L agar.

Plant growth regulators were tested including two types of cytokinins; BAP or Kin at 0.0, 0.1, 0.2, 0.5 and 1.0 mg/L singly or combined with one auxin (NAA) at 0.0 or 0.1 mg/ L. The medium pH was adjusted to 5.8 prior to the addition of agar. The medium was distributed into culture jars (250 ml) each contained 30 ml. The jars were capped with polypropyen closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 20 min. Three single nodes were cultured in jar. All cultures were incubated at 22 °C ± 2 and photoperiod of 16 hours light using florescent lamps (about 2000 lux) and 8 hours darkness. The following data were recorded after 4 weeks from culture initiation:

- Shoot length (cm).
- Number of Shoots/ explant.
- Number of Leaves/explant.
- Fresh weight of cluster (mg).

Microtuberization.

A- Effect of auxins.

The aim of this experiment was to study the effect of types of auxins at different concentrations on forming microtuber *in vitro* of potato. Aseptic stem cuttings (each one included 4 nodes) were excised from the best treatment (0.2 mg/L BAP + 0.1 mg/L NAA) of multiplication stage. These stem cuttings were cultured on MS medium supplemented with 80 g/L sucrose and different types of auxins (2,4-D ,IBA and NAA) at 0.0, 0.5, 1.0 and 1.5 mg/L. The medium was gelled with 7.0 g/L agar and pH was adjusted to 5.7 prior to addition of agar. Medium was distributed into culture jars (325 ml), each contained 40 ml. The jars were capped with polypropene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 15 min. Three stem cuttings were cultured in one jar. All cultures were incubated at 22±2°C under dark conditions.

B-Effect of cytokinin (BAP).

The aim of this experiment was to study the effect of BAP alone at different concentrations on forming microtuber *in vitro* of potato. Aseptic stem cuttings (each one included 4 nodes) were excised from the best treatment (0.2 mg/L BAP + 0.1 mg/L NAA) of multiplication stage. These stem cuttings were cultured on MS medium supplemented with 80 g/L sucrose and BAP at 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg/L). The medium was gelled with 7.0 g/L agar and pH was adjusted to 5.7 prior to addition of agar. Medium was distributed into culture jars (325 ml), each contained 40 ml. The jars were capped with polypropene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 15 min. Three stem cuttings were cultured in one jar. All

cultures were incubated at 22±2°C under dark conditions. Data on microtuberization in both experiments were recorded after two months from culture initiation:

- Number of Microtubers/shoot.
- Microtuber diameter (mm).
- Microtuber length (mm).
- Microtuber fresh weight (mg).

Statistical Analysis

Experiments were set up in completely randomized block design with five replicates; each replicate consisted of one jar containing three explants. All experiments were repeated two times. The results were analyzed by analysis of variance (ANOVA) according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Shoot proliferation

Effect of BAP and NAA concentrations:-

Table (1) and Fig (1) show the effect of growth regulators on shoot length, number of both shoots and leaves per explant and fresh weight of potato clusters. Data of the main effect of BAP on the previous parameters clear that shoot length was gradually decreased with increasing BAP up to 1.0mg/L. However, the increase in BAP concentrations resulted in a significant increase in the number of both shoots and leaves per explant, especially at 0.2 mg/L (4.80 and 16.41, respectively).Similar result was obtained by Hoque *et al.*, (1996) who reported that on potato, the maximum number of shoots was regenerated when 1.0 mg/L BAP was added to the culture medium. In addition, fresh weight of potato cluster was gradually increased with increasing BAP level and the heaviest weight (484) was obtained at 1.0mg/L. The same data show that there was insignificant effect of NAA on shoot length, while the number of both shoots and leaves per explant and fresh weight of cluster were significantly increased with NAA at 1.0mg/L. Concerning the interaction effect between BAP and NAA on these parameters, data in the same Table indicate that all BAP levels significantly decreased shoot length at all NAA concentrations. The highest length (8.90) was obtained with the medium lacking both BAP and NAA, while the lowest one (4.44) was obtained at 1.0 mg/L BAP alone. Moreover, all the interaction treatments had a significant increment in the number of both shoots and leaves. The highest values (5.89 and 18.11, respectively) were obtained at 0.2 mg/L BAP combined with 0.1 mg/LNAA, whereas the lowest values (1.85 and 10.18, respectively) were obtained in the absence of BAP and NAA. In this concern, Yousef *et al.*, (1997) reported that BAP at 2.0 mg/L combined with 0.1 mg/L NAA gave the largest number of axillary shoots per shoot in spunta. In addition, in the absence of NAA, fresh weight of potato cluster was not significantly enhanced at all BAP levels, while at 0.1 mg/L NAA, this character was significantly enhanced at all BAP levels, particularly at 1.0 mg/L where the highest record was obtained (603).

Table (1): Effect of BA P and NAA concentrations on shoot proliferation from single node culture of *potato* after 4 weeks *in vitro*.

Treatments mg/l	Shoot length cm		Mean A	Number of shoots/explant		Mean A	No. of leaves/explant		Mean A	Fresh weight of cluster mg		Mean A
NAA (B) / BAP(A)	0.0	0.1		0.0	0.1		0.0	0.1		0.0	0.1	
0.0	8.90	8.37	8.63	1.85	2.88	2.36	10.18	12.21	11.19	215	353	284
0.1	6.76	6.05	6.40	3.43	3.81	3.62	13.18	14.94	14.06	253	503	378
0.2	5.25	5.86	5.55	3.72	5.89	4.80	14.72	18.11	16.41	280	560	420
0.5	5.41	5.75	5.58	3.75	5.12	4.43	14.05	17.33	15.69	336	566	451
1.0	4.44	5.77	5.10	3.36	5.06	4.21	13.38	16.77	15.07	365	603	484
Mean B	6.15	6.36		3.22	4.55		13.10	15.87		289	517	
LSD 5%	A = 0.95			A = 0.62			A = 1.79			A = 115		
	AxB = 1.34			AxB = 0.87			AxB = 2.53			AxB=163		
NAA F test	NS			*			*			*		

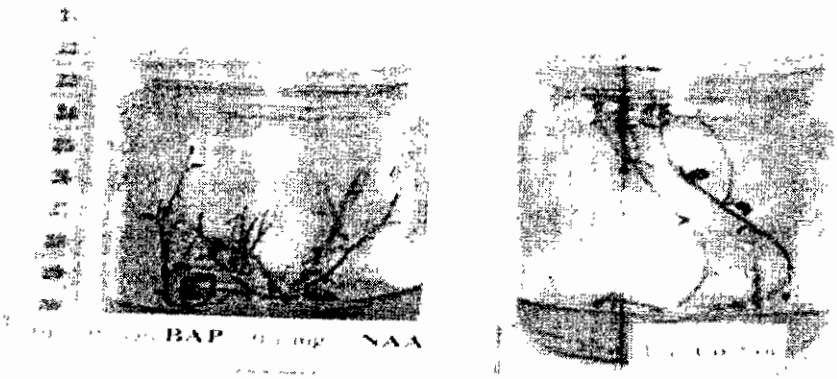

Fig (1): Effect of BAP and NAA on shoot proliferation of potato single node cultured on MS medium after 4 weeks.

Effect of Kin and NAA concentrations.

Data presented in Table (2) clearly show the effect of different levels of Kin and NAA on shoot proliferation of potato after 4 weeks from culture. Concerning the main effect of Kin, the results show that there was a significant increment in shoot length due to the increase in Kin level up to 0.5 mg/L. The increase in the number of shoots per explant was significant only at 0.2mg/L Kin. The data also clear that similar trend took place for shoot number /explant. However, Abo-Bakr *et al*., (1994) indicated that kinetin at rates of 0.5 or 1.0 mg/L resulted in an increase in the shoot number of potato *in vitro*. The number of leaves per explant and cluster fresh weight was significantly increased with increasing Kin levels. The highest records (14.48 and 475, respectively) were obtained at 0.5 and 1.0 mg/L, respectively. This result was in agreement with that of Welander (1985) who indicated that, KIN or BAP gave better results for shoot proliferation of raspberry from the explants than 2ip or Zeatin.

Data in Table (2) reveal that NAA had an insignificant effect on all parameters under study, except of fresh weight of cluster which was significantly increased at 0.1 mg/L compared with the control. Regarding the interaction effect between Kin and NAA on shoot proliferation, Table (2) and Fig (2) show that Kin and NAA combinations increased shoot length significantly. The number of shoots per explant was significantly enhanced due to all Kin and NAA combinations, except at 0.1 mg/L Kin without or with 0.1 mg/L NAA and at 1.0 mg/L Kin combined with 0.1 mg/L NAA. in this respect, Badawi *et al.*, (1996) reported that shoots of potato cv cara were produced in media containing 0.1 mg/L NAA + 0.1 or 2.0 mg/L kinetin and 0.1 mg/L IBA + 0.1 mg/L BA. In addition, adding Kin with NAA to the media resulted in increasing number of leaves per explant significantly, except 0.1mg/L NAA only which had an insignificant effect on this character. Fresh weight of cluster was also significantly increased due to all Kin and NAA combinations, except the treatments of either 0.1 or 0.2 mg/L Kin alone which did not increase this parameter significantly.

Table (2): Effect of Kin and NAA concentrations on shoot proliferation from single nude culture of *potato* after 4 weeks *in vitro*.

Treatments (mg/l)	Shoot length cm		Mean (A)	Number of shoots/explant		Mean (A)	No. of leaves/explant		Mean (A)	Fresh weight of cluster mg		Mean (A)
NAA (B) / Kin(A)	0.0	0.1		0.0	0.1		0.0	0.1		0.0	0.1	
0.0	8.86	8.18	8.57	1.96	2.70	2.33	9.96	12.11	11.03	211	347	279
0.1	11.33	12.32	11.82	2.28	2.44	2.36	13.33	14.05	13.69	287	451	369
0.2	12.77	11.77	12.27	2.55	3.00	2.77	13.88	14.05	13.96	298	504	401
0.5	11.50	10.54	11.02	2.83	2.62	2.72	14.81	14.16	14.48	346	551	448
1.0	10.44	9.65	10.04	2.88	2.00	2.44	12.61	13.00	12.80	370	580	475
Mean B	10.98	10.50		2.50	2.55		12.92	13.47		302	486	
LSD 5%	A = 1.17 AxB = 1.65			A = 0.39 AxB = 0.55			A = 1.53 AxB = 2.16			A = 91 AxB = 130		
NAA F test	NS			NS			NS			*		

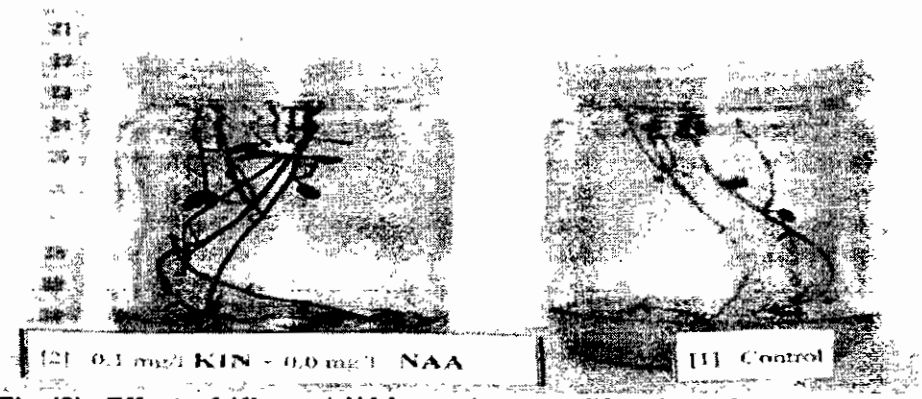

Fig (2): Effect of Kin and NAA on shoot proliferation of potato single node cultured on MS medium after 4 weeks.

Comparing the previous results of Tables 1 and 2 , its could be concluded that BAP at (0.2 mg/L BAP + 0.1 mg/L NAA) proved its efficiency than the similar combination of Kin and NAA(at the same concentrations) for all studded characters.

Microtuberization

The hormonal control of potato tuberization is a highly complex developmental process, and many ways exist to alter the hormonal balance of the plant and thus induce tuberization. Some researchers have added anti-gibberllins such as chlorocholinechloride (CCC) or using any growth retardant as paclobutrazol, uniconazol...etc.(in order to inhibit growth and stimulate microtuber formation) with high cytokinin in culture medium, all of these methods resulted in the formation of *in vitro* tubers.

For this purpose, stem cuttings (each one contained four nodes) were cultured on MS medium supplemented with 80 g/L sucrose. Three types of auxins (2,4-D, IBA or NAA) each at 0.0, 0.5, 1.0 and 1.5 mg/L. were tested. One cytokinin (BAP) at 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0mg/L was also tested for microtuberization. All cultures were incubated under dark conditions at 22 ± 2°C. Data were recorded after two months from culturing.

Effect of types of auxin on microtuberization.

Table (3) and Fig. (3) reveal the effect of auxins; 2,4-D, IAB and NAA on potato microtubers after two months from culture. Data show that the increase in the number, weight, length and diameter of microtubers did not reach to the significance level at 0.5 and 1.5 mg/L 2,4-D, while 1.5 mg/L 2,4-D increased microtubers numbers significantly (1.57). Moreover, 2,4-D at 1.0 mg/L gave the highest values of all characters under study (1.66, 151, 7.5 and 5.3, respectively). The response of potato microtubers to IBA is shown in the same Table which reveal that a gradual increase in number, weight, length and diameter of microtubers was observed with the gradual increase in IBA from 0.0 to 1.5 mg/L. However, the significant increase was observed only in microtuber weight, length and diameter at 1.5 mg/L IBA (129, 7.50, and 4.70, respectively).

Table "(3) effect of auxins (2,4-D,IBA and NAA) on microtuber characteristics of potato after two months from culture.

Con. mg/L	2,4-D				IBA				NAA			
	No. pershoot	weight mg	length mm	diameter mm	No. pershoot	weight mg	length mm	diameter mm	No. pershoot	weight mg	length mm	diameter mm
0.0	1.00	46	4.90	3.30	1.00	46	4.90	3.30	1.00	46	4.90	3.30
0.5	1.16	105	6.80	4.50	1.28	87	5.40	3.90	1.42	117	7.40	5.10
1.0	1.66	151	7.50	5.30	1.33	113	7.00	4.20	1.47	147	7.50	4.60
1.5	1.57	113	7.10	4.20	1.57	129	7.50	4.70	1.66	158	8.20	5.10
LSD at 5%	**0.54**	**79**	**2.60**	**1.20**	**0.57**	**79**	**2.50**	**1.40**	**0.55**	**75**	**2.50**	**1.40**

Also, data of the same Table indicate that all previous parameters were gradually increased with increasing NAA level. The significant increase in microtuber data was obtained at 1.0 or 1.5 mg/L NAA. The highest records of microtuber number, weight, length and diameter were obtained at 1.5 mg/L NAA (1.66, 158, 8.2 and 5.1, respectively). These results might be due to that

auxins increased the weight of microtubers as a result of the induction of tuber cell enlargement, the increase of cell number, the increase of cell volume and subsequent increase in tuber weight. These results are also in agreement with those obtained by Romanov *et al.*, (2000).

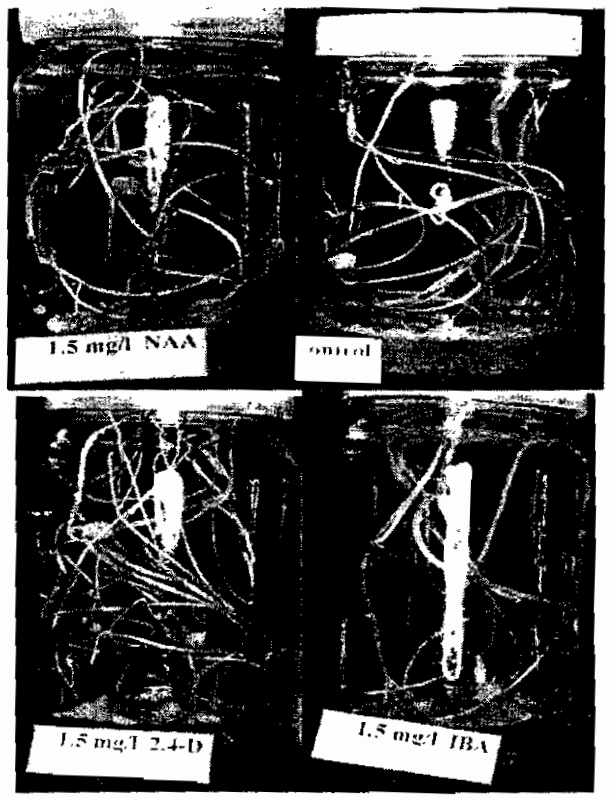

Fig (3): Effect of auxin types on forming microtuber of *in vitro* potato after two months.

Effect of different BAP concentrations on microtuberization of potato.

Results presented in Table (4) prove that microtuberization can be induced *in vitro* in the absence of BAP, when the medium supplemented with 80g/L sucrose. However, the addition of BAP increased number of microtubers per shoot significantly, especially at 2.0, 3.0, 4.0, and 5.0 mg/L (2.88, 2.00, 1.87, and 2.17, respectively). In addition, data of the same Table reveal that all BAP concentrations increased weight, length and diameter of microtuber, except length and diameter of microtuber at 6.0 mg/L which a slight decrease was obtained compared with the control.

Table (4): Effect of different concentrations of BAP on microtubers characteristics of potato after two months from culture.

Characters / BAP mg/L	No. of micro-tubers/ shoot	weight mg	Length f mm	Diameter mm
0.0	1.00	48	5.00	3.30
1.0	1.66	67	5.60	4.10
2.0	2.88	109	7.70	4.80
3.0	2.00	89	6.10	4.20
4.0	1.87	84	6.60	3.90
5.0	2.17	95	6.60	4.50
6.0	1.55	72	4.80	3.00
L.S.D at 5%	0.68	47	1.60	1.40

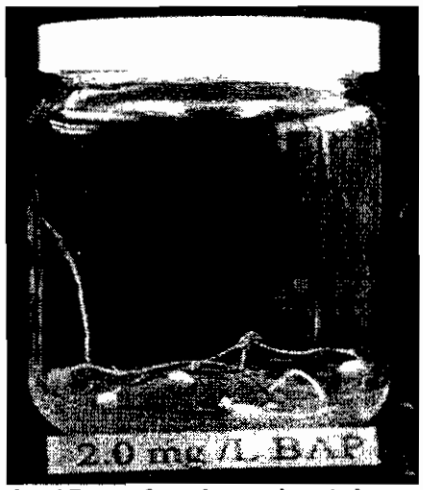

Fig (4): Effect of BAP on forming microtuber of *in vitro* potato after two months under dark condition.

It is quite evident from the same Table that the concentration of 2.0 mg/L BAP resulted in the highest values of number, weight, length and diameter of potato microtuber (2.88, 109, 7.70 and 4.80, respectively) (Fig 4). This result was agreement with that Hussey and Stacey 1984 who found that BAP at 2.0 mg/L gave grater promotion of tuberization in short photoperiod and increased mean tuber per ten nodes after 6 weeks. However, the lowest values (1.55, 72, 4.80 and 3.00, respectively) were obtained at 6.0 mg/L BAP. Similar results were obtained by Guoe (1992), Balletti *et al.* (1994) and Anjum and Villiers (1997).

Comparing the previous results of Tables 3 and 4, its could be concluded that BAP at 2.0 mg/L gave the highest values of number of microtuber efficiency than all auxins tested at all concentrations. This enhancing effect may be due to the role of cytokinins in cell division and the formation of axillary shoots which involved to proliferation of microtubers from stem cuttings (Gamborg et al., 1976). Abbott and Belcher (1986), pointed out that cytokinins stimulate the tuberization process by transforming the up right leafy shoots into horizontal stolons, that later grown down and form tubers.

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تكوين درينات البطاطس معمليا.

١- بروتكول لانتاج درينات البطاطس معمليا.

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****جامعه المنصوره - كليه الزراعه- قسم الخضر والزينه**

أجريت هذه الدراسة فى معمل زراعة الأنسجة النباتية بكلية الزراعة جامعة المنصورة خلال الفترة من ديسمبر ٢٠٠٢ حتى ديسمبر ٢٠٠٥ ، بهدف إكثار(تضاعف) نباتات البطاطس صنف اسبونتا بواسطه عقده ساقيه واحده(كجزء نباتى) ومدى تاثرمعدل النمو والتضاعف عند استخدام نوعان من السيتوكينين هما (بنزيل امينو بيورين و الكاينتين) بتركيزات (صفر، ٠,١ ، ٠,٢ ، ٠,٥ و ١,٠ ملليجرام/لتر) وحدها او متحده مع نوع واحد من الاكسينات (نفثالين حامض الخليك) بتركيزات (صفر او ٠,١ ملليجرام/لتر) وذلك باستخدام تكنيك زراعة الأنسجة. و كذلك دراسه تاثير انواع وتركيزات الاكسينات (٢,٤ داى كلوروفونكسى حمض الخليك ، اندول حمض بيوتريك ، نفثالين حمض الخليك) (وحدها) او السيتوكين وحده متمثلا فى بنزيل امينو بيورين على تكوين الدرينات بالمعمل(دون استخدام معوقات النمو فى بيئه الزراعه) بغرض تطوير الاسلوب المتبع فى تكوين الدرينات بالمعمل وجعله اكثر سهوله واقل تكلفه.

و كانت اهم النتائج لمرحله التضاعف ما يلى:-

ادت جميع تركيزات الكينتين الى زياده طول الافرع ،على العكس من ذلك ادت المعامله بالبنزيل امينو بيورين الى نقص هذا الطول، بينما لم يؤثر نفثالين حامض الخليك معنويا على هذه الصفه. كما ادت المعامله بكل من البنزيل امينو بيورين والكاينتين الى زياده عدد الافرع المتكونه وكانت افضل معامله هى ٠,٢ ملليجرام/لتر حيث اعطت ٤,٨٠ و ٢,٧٧ فرع على التوالى. اما عدد الاوراق فوصلت لاعلى قيمه (١٦,٤١ ورقه) باستخدام ٠,٢ ملليجرام /لتر بنزيل امينو بيورين. تساوى تاثير كل من البنزيل امينو بيورين والكاينتين على الوزن الطازج للكلاستر المتكون. ادى تفاعل كل من نفثالين حامض الخليك والبنزيل امينو بيورين او الكاينتين الى زياده عدد الاوراق والوزن الطازج للافرع المتكونه.

و كانت اهم النتائج الخاصه بتكوين الدرينات بالمعمل ما يلى:-

اثبتت النتائج ان استخدام الاكسين وحده مؤثر وفعال فى تكوين الدرينات بالمعمل عندما اضيف لبيئه موراشيج وسكوج و ٨٠ جم/لتر سكروز وفى ظروف اظلام تامه. حيث كانت افضل النتائج (١,٦٦ درينه/فرع) عندما استخدم ٠,١ ملليجرام/لتر من ٢,٤ داى كلوروفونكسى حمض الخليك ، اما عند استخدام اندول حمض بيوتريك فكان افضل نتائج (١,٥٧ درينه/فرع) عند استخدام تركيز ١,٥ ملليجرام/لتر. بينما عند استخدام نفثالين حمض الخليك فكان افضل نتائج (١,٦٦ درينه/فرع) عند تركيز ١,٥ ملليجرام/لتر . كذلك اثبتت النتائج ان وجود البنزيل امينو بيورين فى بيئه الزراعه يؤدى الى تكوين الدرينات عند اى من التركيزات المختبره. بينما كان افضل تركيز لتكوين الدرينات عندما استخدم بتركيز ٢,٠ ملليجرام / لتر فى موراشيج وسكوج و ٨٠ جم/لتر سكر وفى ظروف اظلام حيث اعطى عند هذا التركيز اعلى معدل من الدرينات(٢,٨٨ درينه/فرع).