

## Effect of plant growth regulators on callus induction and plant regeneration of cucumber (*Cucumis sativus* L. Beith Alpha)

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**Abstract:** The purpose of this study was to develop an efficient protocol for callus induction and regeneration of cucumber cultivar Beith Alpha. Shoot tip, leaf, nodal segment and internode explants of cucumber *Cucumis sativus* L. (Beith Alpha) were cultured on MS medium supplemented with different concentrations of auxin and cytokinins. Formation of calli from leaf, nodal segment and internode were obtained on MS media with NAA 1mg/L and BA 1mg/L while, the best result of calli from shoot tip was obtained on MS with 0.5 mg/L 2,4-D and 0.5 mg/L Kin. Optimum shoot regeneration was observed on MS media containing 1mg/L BA. The obtained results revealed that the highest significant of root number formation (5.33) and length of root (3.63 cm) was recorded with MS supplemented with 2mg/L NAA.

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### 1. Introduction

Vegetables are good sources of carbohydrates, fat, protein and mineral salts. Many human nutritional deficiencies and diseases commonly encountered in the developing countries are preventable by an intelligent and fuller utilization of vegetables. The improvement of vegetable crops through conventional breeding techniques has several limitations (Thomas *et al.*, 2004). Among the flowering plants, the members of cucurbitaceae are duly useful for human being that is a good source of vegetables and also a better resource for phytochemical compounds. Plant tissue culture is an important frontier area in plant biotechnology and to support the production of phytochemical compounds in laboratory conditions (Jeyakumar *et al.*, 2013). Cucumber (*Cucumis sativus* L.), one of the most economically important cucurbit crops, is commercially represented by both pickling and fresh market cultivars all over the world. This species is extremely difficult to propagate vegetatively *in vivo* condition, and therefore, the development of *in vitro* micropropagation methods (regeneration system) would be very useful for its clonal multiplication (Deakin *et al.* 1971).

Callus is defined as an unorganized tissue mass growing on solid substrate. Callus forms naturally on plants in response to wounding, infestations, or at graft unions (Bottino, 1981). Cell division usually occurs in parenchymatous cells by dedifferentiation. During this process adult cells temporarily revert to juvenile state (Rejuvenation) and hence show intense growth and division activity (Bais *et al.*, 2001). Callus formation is central to many

investigative and applied tissue culture procedures. Callus can be multiplied and later used to clone numerous whole plants (Prasad, 2012).

Kim *et al.* (1988) obtained the callus of ten cultivars of cucumber on Murashige and Skoog (MS) medium supplemented with 2,4-D and BA. Ewais (1995) reported that 2,4-D and Kinetin produced markedly higher percentage of callus from cucumber cotyledons than other plant growth regulators. Tantasawat *et al.* (2010) initiated callus from shoot tips and embryonic axes of cucumber in MS medium supplemented with NAA and kinetin in the presence of proline. Usman *et al.* (2011) found that the greater concentration of 2,4-D showed the greater percentage of callus formation from both leaf disc and cotyledon leaf explants. Furthermore, Abu-Romman *et al.* (2013) in their study found that callus induction frequency, callus growth rate and nature of callus in cucumber were significantly affected by type and concentration of the plant growth regulators and were greatly higher when incorporating auxins in the medium compared to cytokinins.

The aim of this study is to evaluate cultivar of cucumber in relation to their ability to produce callus and regeneration plant from different explants under different conditions of cultivation.

### 2. Material and Methods

#### 2.1. Plant material

Seeds of cultivar of *Cucumis sativus* L. (Beith Alpha) were obtained from The Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Seeds of the cultivar of *Cucumis sativus* L. were sterilized by immersion in 70% Ethanol for

30 seconds and then immersed in different concentration of commercial Clorox (5%, 10%, 15% and 20%) for 5,10,15 and 20 minutes.

Seeds were germinated in basal MS medium (Murashige and Skoog, 1962) containing 3% sucrose and solidified using 0.8% agar added prior to autoclaving at 1.2 Kg/cm<sup>2</sup> for 15 min. The pH was adjusted to 5.7 by addition of 0.1N HCl or 0.1N KOH. Cultures were incubated for 4 weeks under controlled conditions in the growth chamber at 26±2°C by power air condition. Day/night schedule controlled 16 hrs light and 8 hrs darkness, controlled automatically. *In vitro* germinated seedlings (2weeks old) were subjected as plant material in this study.

## 2.2. Callus Induction

Different explants including shoot tip, nodal segment, leaf and internode were excised from seedlings and used as source of callus. Four segments of each type of explants (1cm) were cultured on basal MS medium (Control treatment). In addition, different concentrations of 2,4-D and NAA as auxins and BA and Kin as cytokinines were used. Two concentrations (0.5 and 1mg/L) of auxins and cytokinines were added to MS medium. This experiment included 17 treatments (Table 1).

The percentage of callus formation, callus nature and callus fresh weight dry weight and dry matter contents were recorded at the end of the fifth week of cultivation. Three replicates of each treatment were recorded.

## 2.3. Shootlets regeneration

An equal pieces of calli cultures (~250 mg/jar) produced from shoot tip, nodal segment, leaf and internode were recultured on MS medium supplemented with different concentrations of BA at the rate of 0, 0.5, 1.0, 1.5, or 2.0 (mg/L). The number of shootlets, shootlet length (cm), the number of nodes and number of leaves per shootlet were recorded.

## 2.4. Roots formation

Regenerated shoots were separated individually and transferred on to MS containing different concentrations of NAA at the rate of 0.5, 1.0, 1.5, or 2.0 (mg/L). The number and length (cm) of roots formation per shootlet were recorded.

## 2.5. Statistical Analysis

The collected data of 3 replicates of each treatment were subjected to the analysis of variance (ANOVA) and means were separated according to Holm-Sidak method at 0.05 level of probability using SigmaPlot 12.

Table 1. Treatments from different concentrations of auxins and cytokinins

Treatments	Conc. of 2,4-D (mg/L)	Conc. of NAA (mg/L)	Conc. of BA (mg/L)	Conc. of Kin (mg/L)
Control	-	-	-	-
MS1	0.5	-	-	-
MS2	0.5	-	0.5	-
MS3	-	0.5	-	-
MS4	-	0.5	0.5	-
MS5	1	-	-	-
MS6	1	-	1	-
MS7	-	1	-	-
MS8	-	1	1	-
MS9	-	-	0.5	-
MS10	-	-	-	0.5
MS11	0.5	-	-	0.5
MS12	-	0.5	-	0.5
MS13	-	-	1	-
MS14	-	-	-	1
MS15	1	-	-	1
MS16	-	1	-	1

## 3. Results and Discussion

### 3.1. Seeds sterilization and germination.

Seeds of *Cucumis sativus* L. (Beith Alpha) were obtained from The Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Data in table (2) showed the highest percentage

(31.25%) of germination when surface sterilized by 20% Clorox for 10 min. These finding are in agreement with Mohiuddin *et al.* (2005). They treated cucumber seeds with 70% ethanol for 1 min then with 20% of Clorox solution for 15 min and rinsed three times with sterile water.

Table 2. Effect of commercial Clorox on percentage of free contamination and germination seeds of *Cucumis sativus* L. (Beith Alpha) cultured *in vitro*

Germination Percentage (%)				
Treatment	5 min	10 min	15 min	20 min
<b>Beith Alpha cv.</b>				
<b>Clorox 5%</b>	0	18.75	25	<b>18.75</b>
<b>Clorox 10%</b>	0	12.5	18.75	<b>12.5</b>
<b>Clorox 15%</b>	0	18.75	25	<b>25</b>
<b>Clorox 20%</b>	<b>0</b>	<b>31.25</b>	<b>12.5</b>	<b>12.5</b>

### 3.2. Callus production

Data in table (3) and Fig. 1 (A and B) showed that the percentage of callus formation in *Cucumis sativus* L. Beith Alpha cv. from four explants (leaf, shoot tip, node and internode). Callus formed from leaf explants showed higher percentage in most of concentrations used than other explants followed by node then shoot tip explants. The obtained results are in agreement with those obtained by Tantasawat *et al.* (2010) on cucumber they found

that incubation of shoot tips and embryonic axes explants with MS basal medium supplemented with NAA and kinetin achieved of callus formation. Furthermore, Abu-Romman *et al.* (2013) in their study found that callus induction frequency, callus growth rate and nature of callus in cucumber were significantly affected by type and concentration of the plant growth regulators and were greatly higher when incorporating auxins in the medium compared to cytokinins.

Table 3. Percentage of calli development and morphological characters of calli derived from leaf, shoot tip, nodal segment and internode explants of *Cucumis sativus* L. Beith Alpha cultured for four weeks on MS medium supplemented with different combinations of growth regulators. All cultures were incubated under light condition

PGR (mg/L)	Callus formation (%)				Morphological characters (color; texture)			
	Leaf	Shoot tip	Node	Internode	Leaf	Shoot tip	Node	Internode
<b>Free PGR</b>	0	0	0	0	-----	-----	-----	-----
<b>0.5 2,4-D</b>	83.33	50	41.67	16.67	Cr; L	Cr; C	Cr; C	Cr to LG; L
<b>0.5 2,4-D+0.5 BA</b>	100	41.67	75	25	LG; C	Cr to W; C	Cr to W; C	Cr; L
<b>0.5 NAA</b>	50	16.67	33.33	8.33	Cr to W; F	Cr to LG; C	Cr to G; L	Cr; L
<b>0.5 NAA+0.5 BA</b>	100	75	100	83.33	Cr to LG; C	Cr; C	Cr to W; C	Cr to W; C
<b>1 2,4-D</b>	100	25	100	25	Cr; L	Cr; C	Cr; C	Cr; L
<b>1 2,4-D+1 BA</b>	58.33	83.33	83.33	33.33	Cr to LG; C	Cr to W; C	Cr to LG; C	Cr; L
<b>1 NAA</b>	58.33	33.33	58.33	16.67	LG; F	LG; C	LG; L	Cr to W; L
<b>1 NAA+1 BA</b>	100	91.67	100	100	Cr to W; C	Cr; C	Cr; C	Cr to W; C
<b>0.5 BA</b>	75	33.33	25	100	Cr; C	Cr to G; C	Cr; C	Cr; C
<b>0.5 Kin</b>	83.33	0	25	16.67	Cr; C	-----	Cr to W; C	Cr; L
<b>0.5 2,4-D+0.5 Kin</b>	100	100	100	75	Cr to W; C	LG; C	Cr; C	Cr to W; C
<b>0.5 NAA+0.5 Kin</b>	100	91.67	100	16.67	Cr; C	Cr; C	Cr to LG; C	Cr; C
<b>1 BA</b>	91.67	41.67	41.67	91.67	Cr to G; C	Cr to W; C	Cr; C	Cr to LG; L
<b>1 Kin</b>	83.33	0	41.67	25	Cr to G; C	-----	Cr; C	Cr; L
<b>1 2,4-D+1 Kin</b>	100	100	75	75	Cr to G; C	Cr to LG; C	Cr to W; C	Cr to W; C
<b>1 NAA+1 Kin</b>	100	100	100	83.33	Cr to W; C	LG; C	Cr to G; C	Cr; L

G: green, LG: light green, Cr: cream, W: white, C: compact, F: friable, L: loose

Moreover, data presented in tables (4 & 5) show the effect of MS medium supplemented with different combinations of Kin. or BA as a cytokinin and NAA or 2,4-D as auxin on fresh and dry weight (g/ Jar) of calli produced from four explants (leaf, shoot tip, node and internode) after five weeks of cultivation. The result showed that the highest weights of callus (4.67 g/ Jar) were obtained from

internode explants cultured on MS media supplemented with 1 mg/L NAA + 1 mg/L BA. The combinations of auxins and cytokinins show higher responses to form callus than treatments of only auxins or cytokinins. These results are in agreement with the studies of **Ewais (1995)** used 2,4-D and Kinetin for mass production of cucumber callus.

Table 4. Effect of MS medium supplemented with different concentrations of BA or Kin and NAA or 2,4-D on fresh weight (g/jar) of calli produced from leaf, shoot tip, nodal segment and internode explants of *Cucumis sativus* L. Beith Alpha, after 5 weeks of cultivation under light condition

PGR (mg/L)	Callus fresh weight (gm/jar)			
	Leaf	Shoot tip	Node	Internode
Free PGR	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
0.5 2,4-D	1.51 ± 0.221	0.34 ± 0.066	0.41 ± 0.087	0.21 ± 0.074
0.5 2,4-D+0.5 BA	3.41 ± 0.050	0.38 ± 0.015	1.23 ± 0.060	0.41 ± 0.058
0.5 NAA	0.58 ± 0.023	0.05 ± 0.007	0.24 ± 0.049	0.01 ± 0.007
0.5 NAA+0.5 BA	1.29 ± 0.037	1.12 ± 0.515	1.74 ± 0.173	0.50 ± 0.041
1 2,4-D	1.41 ± 0.063	0.09 ± 0.018	0.85 ± 0.145	0.21 ± 0.052
1 2,4-D+1 BA	0.48 ± 0.136	1.54 ± 0.068	1.85 ± 0.155	0.13 ± 0.055
1 NAA	0.51 ± 0.344	0.20 ± 0.023	0.85 ± 0.104	0.08 ± 0.030
1 NAA+1 BA	2.29 ± 0.026	1.91 ± 0.059	2.21 ± 0.121	4.67 ± 0.435
0.5 BA	1.42 ± 0.035	0.58 ± 0.035	0.16 ± 0.081	0.64 ± 0.112
0.5 Kin	1.11 ± 0.025	0 ± 0.00	0.18 ± 0.055	0.04 ± 0.018
0.5 2,4-D+0.5 Kin	2.15 ± 0.952	2.23 ± 0.142	0.52 ± 0.114	0.44 ± 0.062
0.5 NAA+0.5 Kin	2.62 ± 0.201	1.31 ± 0.131	2.71 ± 0.239	0.20 ± 0.059
1 BA	1.90 ± 0.073	0.65 ± 0.058	0.73 ± 0.056	1.31 ± 0.055
1 Kin	1.58 ± 0.035	0 ± 0.00	0.45 ± 0.058	0.10 ± 0.023
1 2,4-D+1 Kin	2.86 ± 0.057	2.44 ± 0.326	1.13 ± 0.091	0.51 ± 0.015
1 NAA+1 Kin	2.86 ± 0.262	1.39 ± 0.150	2.72 ± 0.026	3.93 ± 0.972

All data expressed in Mean ± SE

Table 5. Effect of MS medium supplemented with different concentrations of BA or Kin and NAA or 2,4-D on dry weight (g/jar) and dry matter content (%) of calli produced from leaf, shoot tip, nodal segment and internode explants of *Cucumis sativus* L. Beith Alpha, after 5 weeks of cultivation under light condition

PGR (mg/L)	Callus dry weight (gm/jar)				Dry matter content (%)			
	Leaf	Shoot tip	Node	Internode	Leaf	Shoot tip	Node	Internode
Free PGR	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	-	-	-	-
0.5 2,4-D	0.0937 ± 0.009	0.0266 ± 0.0065	0.0284 ± 0.0082	0.0122 ± 0.0045	6.21	7.89	6.88	5.73
0.5 2,4-D+0.5 BA	0.227 ± 0.0164	0.0285 ± 0.0032	0.102 ± 0.0197	0.0239 ± 0.0019	6.66	7.44	8.31	5.79
0.5 NAA	0.0376 ± 0.0064	0.0034 ± 0.0008	0.0154 ± 0.0038	0.0004 ± 0.0004	6.45	7.28	6.42	6.00
0.5 NAA+0.5 BA	0.0848 ± 0.0071	0.0816 ± 0.0418	0.117 ± 0.0085	0.0337 ± 0.0057	6.56	7.31	6.71	6.70
1 2,4-D	0.106 ± 0.0154	0.0063 ± 0.0011	0.0571 ± 0.0159	0.0132 ± 0.0032	7.50	7.27	6.74	6.38
1 2,4-D+1 BA	0.0377 ± 0.0114	0.104 ± 0.0137	0.153 ± 0.0061	0.0082 ± 0.0035	7.85	6.75	8.26	6.31
1 NAA	0.0304 ± 0.0174	0.0128 ± 0.0014	0.0586 ± 0.0034	0.004 ± 0.0015	5.93	6.50	6.89	5.25
1 NAA+1 BA	0.193 ± 0.0249	0.104 ± 0.0045	0.139 ± 0.0013	0.26 ± 0.0191	8.43	5.44	6.29	5.57
0.5 BA	0.115 ± 0.005	0.0456 ± 0.008	0.0128 ± 0.0074	0.0517 ± 0.0101	8.10	7.90	8.00	8.04
0.5 Kin	0.0801 ± 0.0041	0 ± 0.00	0.0111 ± 0.0029	0.0032 ± 0.0014	7.22	-	6.27	7.46
0.5 2,4-D+0.5 Kin	0.141 ± 0.0765	0.147 ± 0.0101	0.0337 ± 0.0068	0.0264 ± 0.0029	6.57	6.59	6.52	5.96
0.5 NAA+0.5 Kin	0.225 ± 0.005	0.101 ± 0.0112	0.173 ± 0.0262	0.0128 ± 0.0029	8.58	7.71	6.38	6.50
1 BA	0.171 ± 0.0142	0.0438 ± 0.0044	0.0472 ± 0.0035	0.101 ± 0.0121	9.01	6.71	6.44	7.69
1 Kin	0.109 ± 0.0053	0 ± 0.00	0.0314 ± 0.0044	0.0055 ± 0.0017	6.90	-	6.93	5.31
1 2,4-D+1 Kin	0.21 ± 0.0333	0.174 ± 0.0108	0.0789 ± 0.0118	0.0297 ± 0.0031	7.34	7.12	6.98	5.79
1 NAA+1 Kin	0.189 ± 0.0272	0.0912 ± 0.0125	0.182 ± 0.0102	0.297 ± 0.0658	6.60	6.56	6.68	7.55

All data expressed in Mean ± SE

### 3.3. Shootlets formation

The recorded results of cucumber Beith Alpha cv. in Table (6) and Fig. (1 C and D) reveal that the highest number of shootlets (2.667) and longest shootlets (6.733 cm) were obtained using MS medium supplemented with 2 mg/L BA while the highest number of nodes (3.667) and leaves (10.667) were achieved using media containing 1.5 mg/L BA.

These data are in agreement with those Usman *et al.* (2011), which found that high concentration of BA 5 mg/L with calli of cucumber showed increased of adventitious shoots formation. Our findings regarding shoot regeneration on BAP are in conformity with report of Han *et al.* (2004) who concluded BA as an essential factor for shoot regeneration in bottle gourd.



Figure 1 (A-G). Clonal propagation of *Cucumis sativus* L. (Beith Alpha): A, Callus induction; B, Shoot bud formation from callus; C&D, Regeneration and multiple shoots; E, Rooting on multiple shoots; F, flowering of regeneration plant & G, regenerated and hardened plantlet

Table 6. Effect of MS medium supplemented with different concentrations of BA on number of shootlets, length of shootlet (cm), number of leaves/shootlet and number of nodes regenerated from leaf, shoot tip, nodal segment and internode calli cultures of *Cucumis sativus* L. Beith Alpha

MS medium supplemented With	Shootlet Length (cm)	No. of leaves	No. of nodes	No. of shootlets
Free PGR	0±0.00	0±0.00	0±0.00	0±0.00
0.5 BA	2.533±0.448	5±1.155	1.333±0.333	1.667±0.333
1 BA	3.967±1.017	9.667±2.848	3±1.155	2±0.577
1.5 BA	2.9±0.153	10.667±1.764	3.667±0.333	1.333±0.333
2 BA	6.733±0.318	9.333±0.882	2.333±0.333	2.667±0.333

All data expressed in Means ± SE

### 3.4. Rooting formation

Rooting stage is considered as a bottle neck in success of new plantlets production through tissue culture techniques. Data in Table (7) and Fig. (1 E, F and G) showed that effect of different concentrations of NAA on number and length of roots. The result showed the use of NAA with the range of 0.5–2 mg/L gave approximate results of rooting while the use of

MS medium free of plant growth regulators for rooting had a significant difference from NAA concentrations. These results were in line with the report of Rasheed *et al.* (2013) on their studies on watermelon in that the high concentrations of NAA (1 mg/l) formed no roots while maximum number of roots was obtained with 0.1 mg/L.

Table 7. Effect of different concentrations of NAA on number and length of roots originated from Beith Alpha cv. Shootlets

PGRs (mg/L)	No. of roots	Root length (cm)
Free PGR	7.67 ± 0.333	4.23 ± 1.581
0.5 NAA	4.33 ± 0.882	3.63 ± 0.926
1 NAA	5.33 ± 0.333	3.37 ± 0.536
1.5 NAA	3.33 ± 0.667	2.63 ± 0.841
2 NAA	2 ± 1.155	1.3 ± 0.7

All data expressed in Means ± SE

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