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SOME FACTORS AFFECTING *IN VITRO* CLONAL PROPAGATION OF A NEWLY INTRODUCED SWEETENER PLANT (*STEVIA REBAUDIANA* BERTONI) IN EGYPT

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ABSTRACT: ***Stevia rebaudiana Bertoni, is an important non-caloric natural sweetener plant used for the treatment of diabetes which is estimated to be 300 times sweeter than sugar cane. This work carried out in Plant Biotechnology Department Laboratories, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Egypt. An advanced protocol for tissue culture was established.***

Some factors affecting in vitro growth and development were studied (STS- BAP- Calcium chloride, light and total darkness as well as type of culture container during multiplication stage and IBA and NAA during rooting stage). Shoot number was maximized with increasing of BAP concentrations through subcultures. Shoot number was not clearly affected by increasing STS concentrations; it ranged from 30 to 36 shoots/jar, but growth vigor was highly affected by increasing STS concentration. Shoot number and shoot fresh weight were enhanced by liquid medium supplemented with different $CaCl_2$ concentrations. Also, culture containers; conical flasks; in both dark and light conditions increased shoots number compare with jar containers (88 and 80shoots/conical flasks and 48 and 68shoots/jar, in dark and light conditions respectively). Root percent possessed the highest values (100%) on MS medium supplemented with 0.25, 0.50 and/or 1.00mg/l IBA

Rooted shoots were transferred to plastic pots (6 cm diameter) containing planting medium (peatmoss, perlite and sand at equal volume). Stevia plantlets grow well in greenhouse during acclimatization stage.

Key words: ***Stevia rebaudiana – In vitro - Growth regulators- Multiplication- Rooting- Acclimatization- STS- CaCl2 -Illumination-NaOCl***

INTRODUCTION

Stevia rebaudiana **Bertoni is an important non-caloric sweetening perennial herb belongs to the Asteraceae family. It is a natural sweetener plant known as "Sweet Weed", "Sweet Leaf", "Sweet Herbs" and "Honey Leaf", which is estimated to be 300 times sweeter than**

sugar cane (Chalapathi and Thimmegowda, 1997, Liu and Li, 1995 and Uddin *et al*., 2006).

The property of the species that called attention to the plant was the intense sweet taste of the leaves and aqueous extracts. From the leaves of stevia, stevioside, sweet crystalline diterpene glycosides are extracted. Pure extract stevioside is non-caloric and 30 times sweeter than sugar. Stevioside, a natural noncaloric sweetener isolated from *Stevia rebaudiana* Bert., possesses anti-inflammatory and antitumor promoting properties; however, no information is available to explain its activity (Boonkaewwan *et al*., 2006 and Uddin *et al*.,2006).

Seeds of stevia show a very low germination percentage (Felippe and Lucas, 1971 and Toffler and Orio, 1981) and vegetative propagation is limited by lower number of individuals (Sakaguchi and Kan, 1982). Due to the above-mentioned difficulties, tissue culture is the only alternative for rapid mass propagation of stevia plants. Plant tissue culture technology may help to conserve rare and endangered medicine plants. Many important medicinal herbs have been successfully propagated *in vitro*, either by organogenesis or by somatic embryogenesis (Debnath *et al.,* 2006).

Tissue culture is the only rapid process for the mass propagation of stevia and there have been few reports of *in vitro* growth of stevia (Miyagawa and Fujioka, 1986; El Zifzafi *et al,* 2003 and Ibrahim *et al.* 2008 a and b). *In vitro* micropropagation can become an important alternative to conventional propagation and breeding procedures for wide range of plant species (Akita *et al,* 1994).

Benzyl adenine increased multiplication rate, vitrification and somaclonal variation. However, the best results were recorded with MS nutrient medium without plant growth regulators during in vitro growth and development of *Stevia rebaudiana*. MS basal medium supplemented with 2 mg/l BAP recorded the highest number of shoots, but these shoots were very thin and vitrified and not suitable for multiplication through several subcultures (Ibrahim *et. al.,* 2008 b).

Ascough and Fennell (2004) stated that very few reports are found about liquid culture system for stevia shoot multiplication. The establishment of culture in liquid media has several advantages, such as faster growth and multiplication rate. Liquid medium allows the close contact with the tissue which stimulates and facilitates the uptake of nutrients and phytohormones, leading to better shoot and root growth. Continuous shaking promotes lesser expression of apical dominance which generally leads to induction and proliferation of numerous axillary buds. Further, with in the shake culture conditions, the growth and multiplication rate of shoots is enhanced by forced aeration, since continuous shaking of medium provides ample oxygen supply to the tissue which ultimately leads to their faster growth (Mehrotra *et al*., 2007).

The recent work aims to study some factors (STS, BAP , CaCl2, light and total darkness and culture containers) affecting *in vitro* growth and development of *Stevia rebaubdiana* as a newly sweetner plant in Egypt.

MATERIALS AND METHODS

***Stevia rebaudiana* plants were harvested from the nursery of Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofiya University, Sadat City, Egypt. *In vitro* work was initiated using terminal and middle nodes ranging in size from 2.5 to 3 cm which were collected from young growing plants. The mother plants were maintained in the nursery. After excision, Stevia explants were rinsed in running tap water for one min. and immersed in Tween 20 solution for another one min. After three washes with double-distilled water, further sterilization was carried out in the laminar airflow chamber using different concentrations of NaOCl (0.125, 0.250, 0.375 and 0.50% (v/v) for 5 min. The explants were then rinsed three times with sterile distilled water. Stevia explants were cultivated on MS basal medium (Murashige and Skoog, 1962), supplemented with different concentrations of 6-benzylamino purine (0.0, 0.4, 0.8 and 1.2mg/l BAP). Sucrose was added (30 g /l) and 0.6% Difco Bacto-agar was added for solidification. The pH of the nutrient medium was adjusted to 5.7 with 0.1N KOH and/or 0.1N HCl before autoclaving at 1.2 kg/ cm^2 and 121°C for 20 min. *S. rebaudiana* explants were prepared aseptically and were vertically planted on sterilized MS media. The cultures were incubated at a temperature of 25±2°C and 16h photoperiod and light intensity 2000lux. Data were recorded after four weeks; two subcultures were followed up on the same conditions.**

Effect of silver thiosulphate (STS) on *in vitro* growth of *Stevia rebaudiana*

1. **Preparation of silver thiosulfate (STS):**
   In order to prepare 0.02 M STS stock solution, 1.58g of Sodium thiosulfate was dissolved into 100ml of water.
2. **To prepare a 0.1M Silver Nitrate stock solution, 1.7g of Silver Nitrate was dissolved into 100ml water (store the stocks solution in the dark until needed to prepare STS).**

In general, the STS solution was prepared with a molar ratio between Silver and thiosulfate of 1:4 respectively. Nearly all of the Silver present in the solution is in the form of $[Ag(S_2O_3)_2]^{-3}$, the active complex for ethylene effect inhibition. Prepare a 0.02M STS by slowly pouring 20ml of 0.1M Silver Nitrate stock solution in to 80ml of 0.1M Sodium thiosulfate stock solution (Biochemicals plant cell and tissue culture Duchefa Catalogue, 2000-2001).

After three subcultures, shoots seemed thin and were not healthy, so, shoots were transplanted on MS nutrient medium supplemented with different concentrations of STS solution (0.0- 0.5- 1.0- 1.5- 2.0- 4.0- 8.0- 12.0- 16.0- 20 and 24 mM). STS was added to MS nutrient medium by volume as

the follows: 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 ml/l) prepared from 0.02M STS

Effect of $CaCl_2$,culture containers and lighting conditions on *in vitro* growth of *S. rebaudiana* during multiplication stage: Shoots which resulted from 2nd subculture were divided into clusters (about 0.5±0.1g) and replanted on liquid MS medium supplemented with different concentrations of $CaCl_2$ (440, 550, 660 and 770mg/l). Two clusters were planted in 250ml-Jars (contain50ml media) or 500ml conical flasks (CF) (contain 100ml media). The experiment was divided into two parts, one of them incubated in total dark and the other incubated under 2000lux light intensity. Data were recorded after four weeks. Shoot growth vigor was estimated as scores and presented as follow according to the methods described by Pottino (1981)

a- Negative growth results = 1
b- Below average growth= 2
c- Average growth =3
d- Above average= 4
e- Excellent=5

Effect of auxin types on root formation of *Stevia rebaudiana* Bert: *In vitro* shoots were cultured on MS solid medium supplemented with various concentrations (0.0, 0.25, 0.50, 1.00, 1.50 and 2.00mg/l) of different auxin types (IBA and NAA). Then Cultures were incubated under 3000lux for 16hours light period. Data were recorded after 30days.

Rooted shoots were transferred to plastic pots (6 cm diameter) containing planting medium (peatmoss, perlite and sand at equal volume) and covered with white plastic bags and left in the uncontrolled greenhouse for adaptation. After one month survival plants were transferred to plastic pots (30cm) diameter containing planting medium (peat moss, sand at equal volume) for more growth and development. Stevia rebaudiana plants were photographed after six months in the greenhouse.

Statistical analysis: Data of results were statistically analyzed by one or two factorial randomized complete design using SAS (1988) package. The least significant difference among levels of each treatment was compared using L.S.D. test at 5%level according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

***In vitro* growth and development of *Stevia rebaudiana* is determined by a number of complex factors namely, genetic make - up of the plant; nutrients (major and minor elements), sugars, plant growth regulators and physical growth factors (light, temperature, pH and aeration). Here some of these factors will be discussed (BAP, IAA and NAA as plant growth regulators, STS as ethylene inhibitors, $CaCl_2$, and physical growth factors (illumination and plant tissue culture vessels) and its effect on *in vitro* growth during multiplication and rooting stages.**

Results in Table (1) cleared negative relationship between NaOCl concentrations and number of contaminated explants of *S. rebaudiana*. Concerning, type of explant, terminal nodes explants possessed lower number of contaminated explant than middle nodes (2.0 and 3.75, respectively). Regarding the inter action, terminal nodes and NaOCl at 0.375% recording the lowest contaminated explants number. On the other side, contamination percentage adversely related with survival percentage. Survival percent was positively affected by increasing of NaOCl concentration. The highest non contaminated terminal nodes (100%) obtained when nodes were sterilized with 0.375% NaOCl, on the other side middle nodes needed higher NaOCl concentration (0.5%) to possess the same result.

Table (1): Effect of NaOCl concentrations on contamination and survival percentage of *S.rebaudiana Bert.*

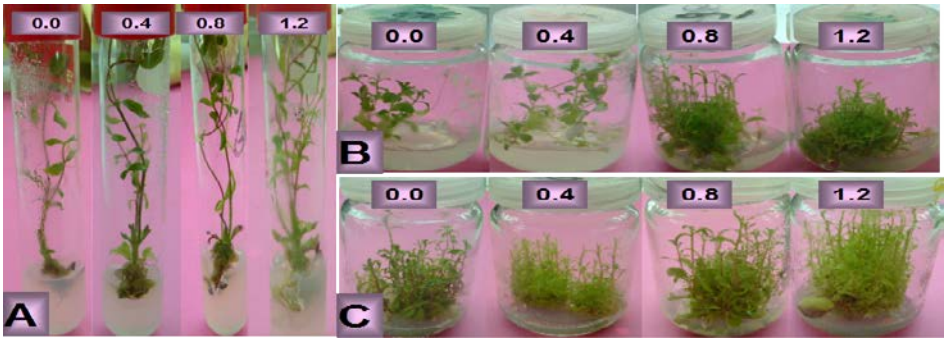
NaOCl % (A)	Number of explans	Number of contaminated explant			Contamination %			Survival %		
		Terminal node	Middle node	Mean	Terminal node	Middle node	Mean	Terminal node	Middle node	Mean
0.125	24	6	8	7	25	33	29	100	100	100
0.250	24	2	4	3	8	16	12	100	100	100
0.375	24	0	2	1	0	8	4	71	80	75.5
0.500	24	0	1	0.5	0	4	2	25	75	50
Mean (B)		2.00	3.75		8.25	15.25		75.97	89.80	
LSD: 0.05%		A	B	AxB						
		0.46	0.36	0.56						

Data in Table (2) and Figure (1, a, b and c) show the effect of different BAP concentrations and subcultures number on clonal propagation of *S. rebaudiana*. After surface sterilization, explants with two nodes were cultured in culture tubes contained 15ml nutrient medium supplemented with different BAP concentrations (0.0, 0.4, 0.8 and 1.2mg/l) after four weeks data were recorded. Data revealed that shoots number were significantly maximized with increasing BAP concentrations (11.18, 16.80, 26.25 and 35.72shoots/jar, respectively). Regarding subcultures, shoot number possessed the highest value in second subculture (73.15shoots/jar). Interaction cleared high shoot number (83.4shoots/jar) at 1.2 mg/l BAP and second subculture. Shoot length seemed to be negatively affected by increasing BAP concentration (9.8, 11.6, 10.77 and 9.77cm, respectively). Also, increasing subculture number adversely affected shoot length. Regarding interaction, shoot length was not clearly affected by BAP concentrations in establishment stage and first subculture, but, obviously decreased through second subculture, which may

due to high potential of multiplication through this subculture. Also, results revealed that growth vigor was affected by subculture number more than BAP concentrations, any way; interaction showed no significant differences were observed. Results came in agreement with those obtained by Ahmed *et al.,* 2007 and Debnath, 2008 who reported that MS medium supplemented with BAP was most effective in inducing bud break and growth, and in initiating multiple shoot proliferation.

Table (2): Effect of different BAP concentrations and subculture number on *in vitro* growth of *Stevia rebaudiana* Bert during multiplication stage.

BAP con.(mg/l) (A)	Shoot number			
Culture stage (B)	Starting stage	1st sub culture	2nd sub culture	Maen
0.0	1.83	6.3	25.4	11.18
0.4	2.21	8.1	40.1	16.80
0.8	3.16	15.3	60.3	26.25
1.2	3.66	20.1	83.4	35.72
Mean	2.71	12.48	73.15	
LSD:0.05%	BAP con. (A)	Subculture (B)	AxB	
	1.34	1.16	2.33	
	Shoot length (cm)			
0.0	15.2	7.1	7.1	9.8
0.4	15.5	10.3	7.4	11.06
0.8	15.1	9.1	8.1	10.77
1.2	15.5	6.3	7.5	9.77
Mean	15.3	8.20	7.53	
LSD:0.05%	BAP con. (A)	Subculture (B)	AxB	
	0.72	0.61	1.25	
	Growth vigor			
0.0	5	3	5	4.33
0.4	5	3	3	3.67
0.8	5	5	4	4.67
1.2	5	4	4	4.33
Mean	5.00	3.75	4.00	
LSD:0.05%	BAP con. (A)	Subculture(B)	AxB	
	0.33	0.29	0.57	



»A:establishment stage **»B: 1st subculture** **»C: 2nd subculture**

Figure (1): Effect of different BAP concentrations and subculture number on *in vitro* growth of *S. rebaudiana* Bert during multiplication stage

Results in Table (3) and Figure (2) show the effect of different concentrations of STS (0.0- 0.5- 1.0- 1.5- 2.0- 4.0- 8.0- 12.0- 16.0- 20 and 24 Mm 24 mM) on *in vitro* growth of *Stevia rebaudiana* after four subcultures during multiplication stage. STS was added to MS nutrient medium by volume as the follows: 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 ml/l) prepared from 0.02M STS

Table (3): Effect of different concentrations of STS on shoots characteristics of *S. rebaudiana* Bert after four subcultures

STS con. (ml/l)	Shoot number	Shoot length (cm)	Growth vigor	Node number
0	30	6.85	2.0	5.25
0.25 (0.5mM)	32	8.50	2.5	7.25
0.5 (1 mM)	35	8.30	2.5	6.30
0.75 (1.5 mM)	36	6.88	2.5	5.40
1 (2 mM)	35	7.15	3.0	5.50
2 (4 mM)	36	7.37	5.0	6.62
4 (8 mM)	36	8.34	5.0	7.80
6 (12 mM)	35	7.06	5.0	5.83
8 (16 mM)	35	7.55	5.0	6.20
10 (20 mM)	35	6.63	5.0	6.33
12 (24 mM)	35	7.15	4.0	6.00
LSD:0.05%	NS	NS	0.60	1.63

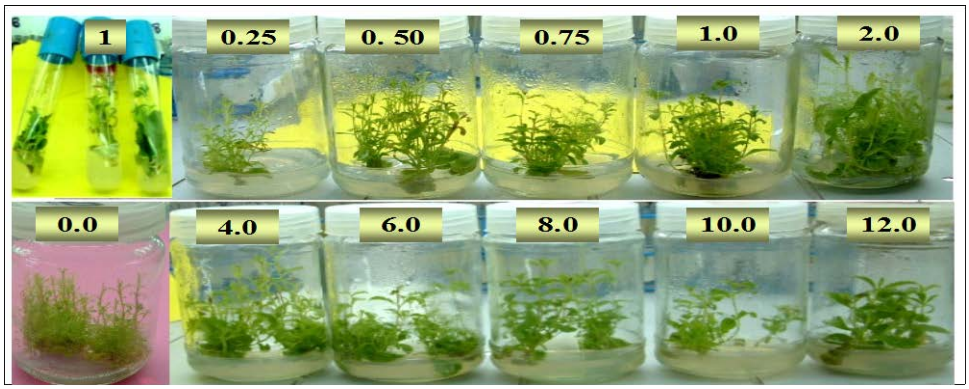


Figure (2): Effect of different concentrations of STS on *in vitro* growth of *S. rebaudiana* Bert. during multiplication stage

Shoot number was not significantly affected by increasing STS concentrations; it ranged from 30to36 shoots/jar. Similar observation was detected in the case of shoot length which varied from 6.63 to 8.50cm. On the other hand growth vigor was highly significant enhanced by increasing STS concentrations, 2ml/l (4 mM) was possessed the highest growth vigor among all treatments. Nodes number had the same trend of growth vigor and ranged from 5.25to 7.80nodes/shoot. Results may be due to the inhibitory effects of STS on ethylene gas which has negative effects on growth parameters.

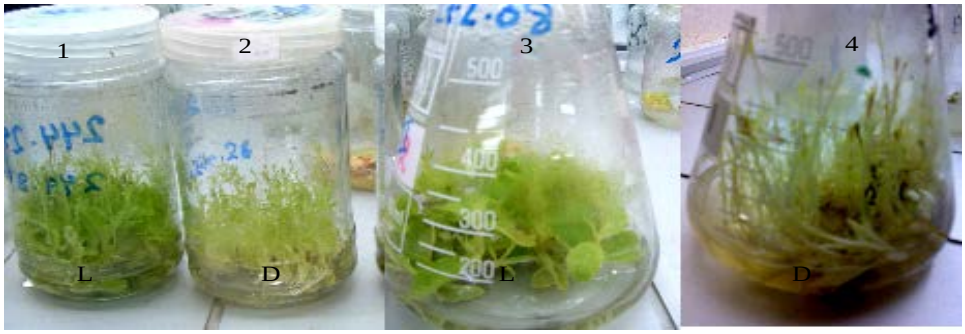
Concerning effect of different $CaCl_2$ concentrations and culture vessels incubated in light or dark conditions on clonal propagation of *S. rebaudiana*, data in Table (4) and Figure (3) revealed that shoots fresh weight was highly affected by $CaCl_2$ concentrations, the highest final fresh weight was obtained at 660mg/l for both dark and light incubation conditions (5.85g and 5.57g/culture vessel, respectively). Concerning tissue culture vessel, shoot fresh weight was strongly affected by tissue culture vessel used. Conical flask (500ml) significantly increased final fresh weight (7.19g/conical flask) compared with jar (250ml) (2.69g/jar). Interaction data revealed that 770mg/l $CaCl_2$ and conical flask; as culture vessel, incubated in light condition possessed the highest final shoot fresh weight (8.22g/conical flask) followed by 660mg/l $CaCl_2$ and conical flask as culture vessel incubated in light or dark condition, respectively (8.16 and 7.42g/ conical flask). While, 440mg/l CaCl2 and jar; as culture vessel, incubated in dark condition possessed the lowest value (1.51 g/jar). Regarding shoot number, $CaCl_2$ concentrations significantly positive enhanced shoot number. $CaCl_2$ at concentration 660mg/l and incubated in light condition showed the highest shoot number (74.80 shoots/vessel) followed by CaCl2 at concentration 550mg/l incubated

in light condition (71.50 shoots/vessel). On the other hand, $CaCl_2$ at concentration 440mg/l incubated in dark condition showed the lowest shoot number (59.0 shoots/vessel). Also, culture vessel (500ml conical flasks and 250ml jars) significantly affected shoot number. Conical flask significantly enhanced shoot number (80.5shoots/conical flask) compared with shoot number of jars as a culture vessel (53.0shoots/jar). Concerning interaction, the highest shoot number was obtained on 660mg/l $CaCl_2$ and conical flask as culture vessel incubated in dark condition (88shoots/conical flask).

Table (4): Effect of different $CaCl_2$ concentrations, culture containers and lighting conditions on *in vitro* growth of *S. rebaudiana* Bert during multiplication

$CaCl_2$ Con.(mg/l)(A)	Tissue Culture Vessel (B)		Mean	Tissue Culture Vessel (B)		Mean	Tissue Culture Vessel (B)		Mean
	Final shoot weight (g)			Shoot number			Growth vigor		
	CF.	Jar		CF.	Jar		CF.	Jar	
	Light condition								
440	5..43	1.42	3.42	75	62	68.50	3	3	3.0
550	6.98	2.55	4.76	79	64	71.50	5	4	4.5
660	8.16	2.98	5.57	80	68	74.80	5	4	4.5
770	8.22	2.71	5.46	74	60	67.00	5	4	4.5
Dark condition									
440	4.13	1.51	2.82	80	38	59.00	3	3	3
550	6.95	3.12	5.03	82	39	60.50	4	3	3.5
660	7.42	3.75	5.85	88	45	66.50	5	4	4.5
770	7.32	3.51	5.41	86	48	67.00	5	4	4.5
Mean	6.82	2.69		80.5	53		4.37	3.62	
L.S.D.:	A	B	AxB	A	B	AxB	A	B	AxB
at 0.05%	0.27	0.14	0.38	5.73	2.87	8.11	NS	NS	NS

»CF: conical flask (500ml)



»1: Jar culture container incubated in light conditions
»2:. Jar.culture container incubated in dark conditions
»3: . Conical flask culture container incubated in light conditions
»4: Conical flask culture container incubated in dark conditions

Figure (3): Effect of culture containers and lighting conditions on *in vitro* growth of *S. rebaudiana* Bert during multiplication stage

Also, growth vigor of *S. rebaudiana* was enhanced by $CaCl_2$ concentrations. Conical flasks culture containers possessed high positive effect on growth vigor compared with jar culture containers incubated in light or dark conditions, with no significant differences among all treatments.

Results may be due to effect of $CaCl_2$ in strengthen cell walls or may be due to liquid medium allows the close contact with the tissue which stimulates and facilitates the uptake of nutrients and phytohormones, leading to better shoot and root growth. Continuous shaking promotes lesser expression of apical dominance which generally leads to induction and proliferation of numerous axillary buds. Further, with in the shake culture conditions, the growth and multiplication rate of shoots is enhanced by forced aeration, since continuous shaking of medium provides ample oxygen supply to the tissue which ultimately leads to their faster growth (Mehrotra *et al*., 2007).

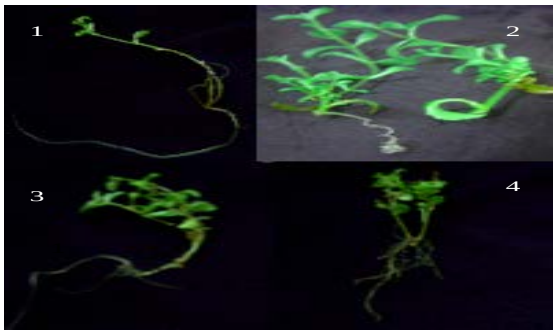
Data in Table (5) and Figure (4) cleared effect of auxins type and concentrations on growth of *Stevia rebaudiana* Bert. during rooting stage The highest plantlet length was observed on MS medium supplemented with 0.25mg/l IBA and followed by 2.0 mg/l NAA (4.91 and 4.70cm, respectively). While the lowest plantlet length obtained on MS medium supplemented with 1.00 mg/l IBA (4.20cm). Node number was maximized on MS supplemented with 0.50mg/l NAA (4 node/plantlet). While, the lowest node number (3.00 node/plantlet) was observed on free auxin MS medium. The highest leaf number was observed on MS medium supplemented with 0.50mg/l NAA followed by 1.00mg/l NAA and/or 0.25mg/l NAA (7.80, 7.80 and 7.40 leaf/plantlet, respectively). Root percent possessed the highest values (100%) on MS medium supplemented with 0.25, 0.50 and/or 1.00mg/l IBA and on MS

medium supplemented with 0.25 and 0.50mg/l NAA. While, the lowest value (37.50%) possessed on MS medium supplemented with 2.00mg/l IBA. MS medium supplemented with 0.50 and/or 1.00mg/l IBA and/or 0.50mg/l NAA gave the highest root number (4.66, 4.00 and/or 3.33 root/plantlet, respectively) and the highest root length (3.33 and 3.00cm, respectively). While, the lowest root length observed on MS medium supplemented with 2.00mg/l NAA (1.60cm). Data agree with Ahmed *et al.,* (2007) who stated that different concentrations of IBA, NAA and IAA were used for rooting and the highest rooting percentage (97.66%) was recorded on MS medium with 0.1 mg /l IAA.

Rooted shoots were transferred to plastic pots (6 cm diameter) containing planting medium (peatmoss, perlite and sand at equal volume). Stevia plantlets grow well in greenhouse during acclimatization stage. Development of acclimatized plants was observed after one and six months. Plants development is shown in figure (5). Stevia plants grew well and its length ranged from 100 to 120cm after six months.

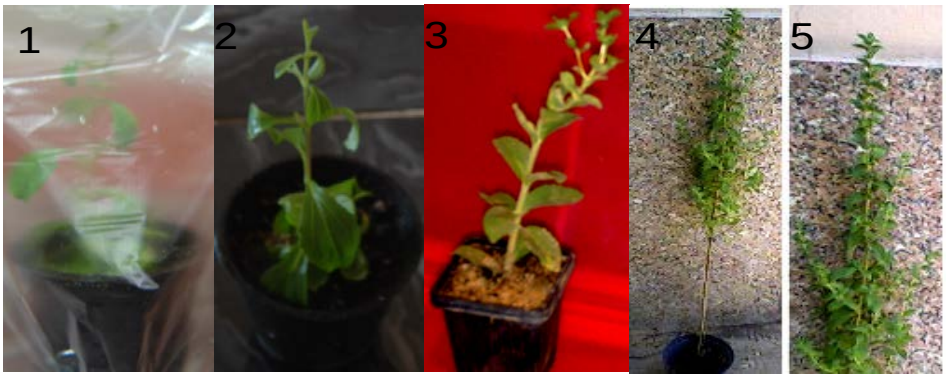
Table (5): Effect of concentrations of different auxin types on growth parameter and rooting of *Stevia rebaudiana* Bert.

Auxin type	concentration	Shoot length	Node No.	Leaf No.	Root %	Root No.	Root length (cm)
Control		4.50	3.00	6.66	87.50	3.50	2.41
IBA	0.25	4.91	3.50	6.33	100	3.66	2.50
	0.50	4.50	3.50	7.00	100	4.66	3.33
	1.00	4.20	3.33	6.16	100	4.00	3.00
	1.50	4.33	3.83	6.00	87.50	3.16	2.83
	2.00	4.33	3.50	6.50	87.50	3.00	2.58
NAA	0.25	4.40	3.80	7.40	100	3.20	3.10
	0.50	4.60	4.00	7.80	100	3.00	3.30
	1.00	4.50	4.00	7.80	87.50	2.60	2.90
	1.50	4.60	3.80	7.40	50.00	2.40	2.60
	2.00	4.70	3.00	7.40	37.50	2.25	1.60
	L.S.D:at 5%	0.31	0.31	0.42		0.30	0.21



»1: Free MS medium. »2: MS medium supplemented with 0.5mg/l IBA.
»3: MS medium supplemented with 1.00mg/l IBA. »4: MS medium supplemented with 0.5mg/l NAA.

Figure (4): Effect of auxins type and concentrations on growth of *Stevia rebaudiana* Bert. during rooting stage



»1: Transplanting of *s. rebaudiana* plantlet in to 6cm pots filled planting medium with equal volume of peatmoss, perlite and sand
»2: *S. rebaudiana* plantlet in to 6cm pots at the end of acclimatization stage.
»3: *S. rebaudiana* plantlet in to 16cm pots after one month of the end of acclimatization.
»4:and 5: *S. rebaudiana* plantlet in to 25cm pots after sex month of the end of acclimatization.

Figure (5): Acclimatization and growth development of *Stevia rebaudiana* Bert after one and six month of plantlet acclimatization

Conclusion

***In vitro* growth and development of *Stevia rebaudiana* is affected by BAP, STS, $CaCl_2$, illumination and type of culture vessel. BAP at low concentration**

is very important for *Stevia rebaudiana* propagation through tissue culture techniques but hyperhydricity as a physiological disorder increased by increasing BAP concentration. shoots number were maximized with increasing of BAP concentrations through subcultures, while, shoot length and growth vigor were negatively affected by both increasing BAP concentration and subculture number. Although, Shoots number were not affected by increasing STS concentrations growth vigor was highly enhancement by increasing STS concentration. Increasing concentrations of CaCl2 in liquid medium and using high volume culture vessel as culture containers and dark incubation conditions strongly affect multiplication and final shoots weight /culture vessel of *Stevia rebaudiana*. Increasing CaCl2 concentration in MS liquid medium resulted in decreasing vetrification phenomena and culture could be incubated in total darkness without decreasing of shoot number, anyway, subculture number which Stevia culture could maintain be incubated in total darkness with out descending in growth parameters need more studies. Root percent possessed the highest values (100%) on MS medium supplemented with 0.25, 0.50 and/or 1.00mg/l IBA. Rooted plants were well acclimatized and highly vigor.

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بعض العوامل المؤثرة علي إكثار نباتات الاستيفيا ريبديانا كنبات تحلية جديد بمصر

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الملخص العربى

نبات ستيفيا ريبديانا هو محلى طبيعي لا يعطى سعرات حرارية ويستخدم لمعالجة مرضى السكر. وتبلغ درجة تحلية نبات الستيفيا ٣٠٠ مرة مثل قصب السكر.أجرى هذا البحث في معامل قسم البيوتكنولوجيا النباتية معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية–مدينة السادات–مصر. تم تأسيس بروتوكول لزراعة الأنسجة حيث تم دراسة بعض العوامل المؤثرة علي إكثار الاستيفيا مثل تأثير ثيوسلفات الفضة STS والبنزيل ادينين وكلوريد الكالسيوم أثناء مرحلة التضاعف وكذلك تأثير اندول حامض البيوتيريك ونفثالين حامض الخليك أثناء مرحلة التجذير وكذلك تم دراسة الإضاءة والإظلام ونوع أوعية الزراعة وتركيز الكالسيوم كلورايد علي نمو النباتات أثناء مرحلة التضاعف. خلال هذه الدراسة لوحظ زيادة عدد الأفرع مع زيادة تركيز البنزيل أدينين خلال النقلات المتتابعة. لم يتأثر عدد الأفرع الناتجة بزيادة تركيز محلول STS والتي تراوحت بين ٣٠و٣٦ فرع للبرطمان، و لكن قوة النمو زادت بصورة واضحة بزيادة التركيز. كما تم زيادة عدد الأفرع والوزن الطازج للافرع عند استخدام البيئة السائلة المزودة بكلوريد الكالسيوم وقد تأثرت تلك الزيادة بنوع اوانى الزراعة. حققت الفلاسكات سعة ٥٠٠ملل والمحضنة فى الاضاءة او الاظلام افضل عدد للفروع مقارنة بالجارات. كما تحققت أعلى نسبة للتجذير على بيئة موراشيجى وسكوج المضاف اليها٠.٢٥و ٠.٥٠ ملليجرام/لترحمض اندول بيوتريك. تم أقلمة النباتات بنجاح في صوب الأقلمة حيث تم نقل النبيتات من مرحلة التحذير إلي مرحلة الأقلمة في أصص قطرها ٦سم بها خليط من بيت موس وبيرليت ورمل بنسب متساوية.

Tables and figures