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RESEARCH ARTICLE

Plant growth regulators affecting *in vitro* cultivation of *Stevia rebaudiana*

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Abstract In order to maximize efficiency of plant propagation *via* direct organogenesis, the influence of plant growth regulators on the growth and development of *Stevia rebaudiana* grown *in vitro* was studied. Results indicated that 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 BA recorded the highest number of shoots, but these shoots were very thin and vitrified and not suitable for multiplication through several subcultures .The nutrient medium (MS basal medium) supplemented with 10 mg /l kinetin recorded 45 shoots / explant as compared to MS nutrient medium which recorded 6.63 shoots / explant but growth parameters for *Stevia rebaudiana* plantlets grown in MS medium without kinetic is better than MS medium containing kinetin at high concentrations. Nutrient medium (MS basal medium) supplemented with IBA at low concentrations (0.01mg/l) or without auxins achieved the best *in vitro* growth of *Stevia rebaudiana* plantlets (100 % root formation). IBA was better than NAA and IAA for shoot and root formation. Increasing NAA concentrations decreased gradually number of shoots . Regarding the effect of NAA on root formation, data indicated that the per cent of shoots formed roots was 87% on MS basal medium without plant growth regulators as compared to MS basal medium supplemented with NAA at low concentrations 0.001, 0.01 and 0.1 mg/l NAA where root per cent was 80% , 73 % and 53 % respectively.On the other hand, NAA at 1.0 and 1.5 mg/l did not help shoots to form roots. Statistical analysis of variance showed no significant differences amongst IAA treatments for *in vitro* growth of plantlets**.** *Stevia rebaudiana* plants were adapted and grown well in planting media containing peat moss, sand and vermiculite at equal volume.

Key words : *Stevia, in vitro* cultivation, plant growth regulations

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Introduction

Stevia*, Stevia rebaudiana,* Bertoni is a small shrub belong to family Asteraceae (Compositae). Estimates of total number of species in this genus ranges from 150 to 300, all distributed in the new world, from the southwestern United States to the northern Argentina and became known by the Europeans due to its discovery by Moises Bertoni in 1899. Attempts to use cultivate the plant in Paraguay soon after its discovery by Bertoni were abandent because it could not be grown easily from cuttings and the seeds were generally infertile (Soejarto *et al.,* 1982). It is now cultivated extensively in the Far East (in particular Japan, Singapore, Taiwan, Malaysia, South Korea and China), as well as in parts of South America (including Brazil and Paraguay) for the sweet diterpene glycosides which occur maininly in its leaves and which are known collectively as either ent-kaurene glycosides, diterpene glycosides, or more popularly as, stevioside, or *Stevia*. (Soejarto *et al.,* 1982 ; David 1996; Singh and Rao, 2005; Ahmed *et al*., 2007). The plant is perennial with a productive life of about 5 to 7 years and is propagated mainly by rooted cuttings. The leaves of this plant contain a natural material called stevioside, which was used for long time as a natural sweetener in its origin. The sweetener

of this material was reported to be over 200 times that of sucrose sugar. The material does not induce tooth decay, could safely be used by diabetic patients and could be used in the low calorie diets to reduce human body weight without side effects. For these reasons, many of the developed countries are now using this plant to produce a large portion of their sugar consumption. The sugar is concentrated in the leaves of plant. The processing of the leaves is quite simple and it could be used as dried leaves or after extracting the sweetener in pure form. The dried leaves could be mixed within the tea packages to reduce the consumption of sugar. It could also be used for the production of candy, chocolates, marmalades, biscuits, ice cream, sweets, juices and beverages. The native occurrence of *Stevia rebaudiana* is between 22-24°S and 53-56 °W in Paraguay and Brazil. The plant growth requires mild temperature between 15 and 38° and relative humidity of about 80 %. The cultivation of such a plant could be an alternative for the new land reclamation projects to meet the demands of the Egyptian markets and generate income for the growers (Amat 1982; Soejarto *et al* 1983 , Wally, 1998 ; El-Zefzafi, 2003; Uddin *et al.,* 2006).

The present study aimed to establish new protocol for *Stevia rebaudiana,* Bertoni propagation *via* tissue culture techniques to observe the effect of plant growth regulators especially cytokinins (benzyl adenine and kinetine) and auxins (indole buturic acid , naphthalene acetic acid and indole acetic acid) on *in vitro* growth of *Stevia rebaudiana* during micropropagation to introduce these new plants to Egyptian Agriculture as a new sweetener material instead of sugarcane or sugar beet to diabetes militias diseases and for diet and pharmaceutical industries.

Materials and Methods

The present work was carried out to study in details the whole protocol for *Stevia rebaudiana,* Bertoni propagation through tissue culture techniques to produce and introduce *Stevia rebaudiana* plants as a new sweetener crop to Egyptian agriculture. The herb of *Stevia rebaudiana* is a potential source of low caloric-sweeteners in order to efficiently maximizing of plant propagation via direct organogenesis. Mother stock plants (*Stevia rebaudiana* Bertoni) were brought from Agriculture Research Center and kept in controlled green house at 25±2ºC and used as start material. In principle, there are four sources of infection, the plant material (internal as well as external), the nutrient medium (insufficient sterilized),the air and manipulation work. The most important of these is the plant itself. The plant material should be well sterilized before being isolated *in vitro*. So, before being sterilized, any remaining soil or dead parts and the outer leaves were removed. The preliminary studies were conducted to select the best anti-oxidant solution and surface sterilization agent and levels. The explants were washed by water, then they were rinsed with a small amount of soap for 10 minutes for assuring the removal of most external contamination. *Stevia rebaudiana* shoot tips were rinsed again under running tap water for 30 minutes to remove all the remaining detergent after that the explants were dipped in anti-oxidant solution (150 mg /l ascorbic acid +100 mg / citric acid) for 2 hours after that surface sterilization began under aseptic conditions in Laminar flow cabinet. Explants were rinsed for 20 minutes in 12 .5 % Clorex (0.625% NaOCl), then rinsed in sterilized distilled water (three times) to remove all traces of the disinfect . All the remaining parts and outer small leaves which surround the shoot tip of *Stevia rebaudiana* under study were removed by dissecting with sterile scalpel and forceps to expose the growing point especially the meristematic dome with two leaf primordia of shoot tips.The explants were dissected to 2 mm in length , then explant was subsequently placed down vertically in the culture tube (150 ×25 mm) in 20 replicates containing 15 ml of MS (Murashige and Skoog,1962) nutrient medium (Table 1) supplemented with 0.5 mg /l benzyl adenine (BA) and anti-oxidant (150 mg /l ascorbic acid +100 mg /l citric acid)and solidified with 5 g/l agar.The culture tubes were directly plugged with polypropylene closure caps . Cultures were incubated in growth room at 27 ±2 ºC for 6 weeks and repeated for five subcultures. Established of micropropagated *S. rebaudiana* plantlets, which produced from this step (starting stage) were used in all the following experiments.

In this experiment, explants were cultured on 15 jars (350 ml containing 40 ml of nutrient medium).Each jar contained one explant .Cultures were incubated in growth room at 27±2 ºC for 6 weeks under 2500 lux derived from 2 x120 ordinary white fluorescent lamps for 16- hours daily photoperiod. Shoot number , shoot length , number of leaves , shoot growth vigour, rooting percentage, root number and root length were recorded every six weeks for four subcultures and the experiment repeated three times. Mainly these experiments were conducted to study the effect of cytokinin (BA or kinetin) at different concentrations on *in vitro* growth of *Stevia rebaudiana* during multiplication stage. BA was used from 0.25 mg/l to 2.5 mg/l , while kinetin was used from 1.0 to 11.0 mg/l. Auxins (IBA , NAA and IAA) were used at different concentrations (0.001 ,0.01, 0.1, 0.5 ,1.0 and 1.5 mg/l) on the *in vitro* growth of *Stevia rebaudiana* during rooting stage as shown in Tables 1 and 2.The pH of all nutrient media were adjusted at 5.7 ±0.1 with 0.1 N HCl or KOH. All the previous nutrient media were solidified with 5 g/l agar and sterilized under 1.2 Kg/cm² and 121º C for 20 minutes .Explants were cultured on the previous media and incubated at 27±2 ºC under 2500 Lux derived from 2 x120 cm ordinary white fluorescent lamp for 16 hours daily photoperiod

Acclimatization was done by allowing the *in vitro* plants gradually get used to a lower relative humidity , which is the case *in vivo*. Rooted shoots were washed from agar in running water and soaked in 2 g/l solution of fungicide (Benalate). Plants were transported to plastic containers (6 cm diameter and 6 cm height) which contain peat moss, sand and

vermiculite at equal volume . Plants were covered with plastic bags and maintained in greenhouse at 30 ±2 ºC. Twenty five replicates were made for each treatment and date (survival , shoot number , shoot length, leaf number, root number and root length) were recorded after 6 weeks from culturing. Shoot growth was estimated (as scores) and presented as follow according the method described by Pottino (1981).

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

Statistical analysis

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

Results and Discussion

Hormones are organic compounds naturally synthesized in higher plants , which influence growth and development ; they are usually active at a different site in the plant from that where they are produced and are only present and active in very small quantities. In *in vitro* culture of higher plant regulators, especially auxins and cytokinins, are very significant. It can be said that *in vitro* culture is often impossible without regulators (Pierik , 1987).

Effect of cytokinins

Cytokinins are now fully recognized as one of the major groups of endogenous plant hormones , Several cytokinins are adenine derivatives occur in plants as a nucleosides and nucleotides.

Effect of benzyl adenine (BA)

The effect of benzyl adenine on the *in vitro* growth of *Stevia rebaudiana* Bertoni estimated in the terms of shoot number, shoot length, leaf number and shoot growth (parameters of shoot formation) and root percentage, root number and root length (parameters of root formation) was shown in Fig 1 and Table 1. Shoot number was affected by (BA) concentrations. Increasing (BA) concentration increased gradually number of shoots and which was significant at 5 % level. Multiplication rate ranged from 6.63 to 73.65 shoots / explant due to BA which increased multiplication rate. The best result (73.65 shoots / explant) was achieved with BA at 2 mg/l compared to BA at 1.75 and 2.25 mg/l which recorded 52.13 and 61.21 shoots / explant with significant differences ($p<0.05$) between two treatments as shown in Table 4. On the other hand, shoot length decreased by increasing BA concentration. Shoot length ranged from 0.46 to 6.92 cm/shoot , and it depends on BA concentration. At high concentration (2.25 mg/l) of BA , data recorded the lowest length of shoots (0.46 cm/shoots) as compared to 4.08 an 6.92 cm/shoot at low concentration of BA (0.25 mg / l) and control (without BA) respectively on *Stevia rebaudiana* . MS basal medium without plant growth regulators was found best medium to improve the *in vitro* growth of *Stevia rebaudiana,* Bertoni estimated as shoot length, (6.92 cm), leaf number (7.81), shoot growth (4.42) and root percentage (93.3%).

Our finding suggested that BA increased the shoot number and decreased shoot growth as compared to control medium (MS without plant growth regulators) with significant differences amongst the treatments . MS basal medium supplemented with BA at 2 mg/l recorded the highest number of shoots, but these shoots were very thin and vitrified and not suitable for micropropagation through several subculturing (Table 1).

Average number of leaves ranged from 2.19 to 7.81 at control without BA, were significant differences effective in increasing the average number of leaves. The highest number of leaves (7.81) was achieved with (control without BA) compared to the lowest one (5.56) which recorded with (0.25 mg/l BA) with significant differences($P<0.05$) between two treatments as shown in (Table 1). Results showed significant differences amongst the treatments for root formation estimated as root percentage, root number and root length .

Effect of kinetin

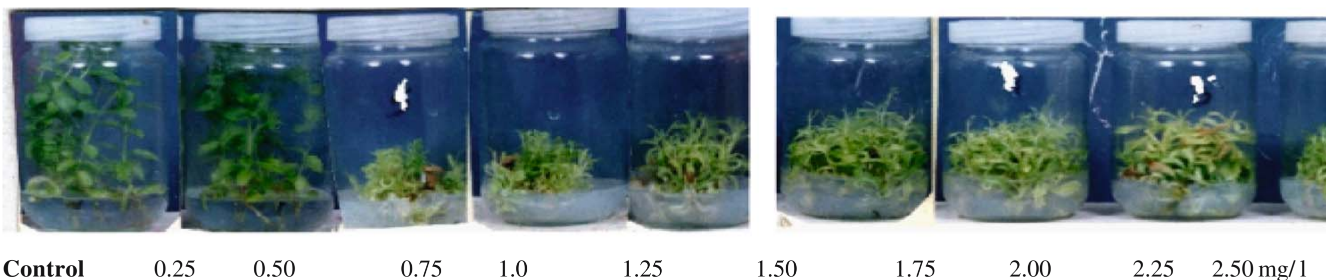
Data presented in Fig 2 and Table 1 showed the effect of kinetin (Kin) on shoot (shoot number, shoot length ,shoot growth and number of leaves) and (root percentage) of *Stevia rebaudiana,* Bertoni cultured *in vitro.* Data indicated that kinetin at high concentration (10 mg/l) recorded 45.08 shoots/ explant, while it was 6.63 shoots / explant at MS basal medium without plant growth regulators but growth parameters at MS medium without kinetine is better than MS media containing all kinetin concentration tested.

Results under discussion are in agreement with Kornilova and Kalashnikova (1996) who mentioned that use of MS medium without plant growth regulators gave results comparable to those obtained with growth regulators. Accordingly, plain MS medium is recommended as being cheap and effective without somaclonal variation.

In plant tissue culture , the cytokinins act as (a) stimulators of cell division, (b) retardant of senescence, and (c) stimulators of seed germination. Moreover, cytokinins counteract the role of auxins in the control of apical dominance, in certain combinations with auxins, are necessary for the commitment of cultured cells to organogenesis (Evans *et a*l .,1983; Pierik, 1987; George, 1993; El-zefzafi,2003; Ahmed *et al.,* 2007). Hyperhydricity or vitrification is a physiological disorder

Table 1. Effect of Benzyleadenine (BA) and Kinetin (Kin) on growth and development of *Stevia rebaudiana,* Bertoni cultured *in vitro* after 6 weeks from culturing.

Treatment (BA) mg / l	Average Shoot number	Average Shoot lenght	Average Leaf number	Average Shoot growth	Root %	Hyperhy dricitty %
Control	6.63 h	6.92 a	7.81 A	4.42 a	93.3a	0
Benzyleadenine (BA) mg / l						
0.25	15.36 g	4.08 b	5.56 B	3.44 b	0.0 b	0
0.50	18.96 fg	3.54 b	4.77 C	2.90 bc	0.0 b	0
0.75	22.46 ef	2.63 c	3.90 D	2.63 cd	0.0 b	5
1.00	26.56 de	2.30 c	3.31 DE	2.25 de	0.0 b	13
1.25	31.40 cd	1.92 d	3.23 DE	1.92 ef	0.0 b	25
1.50	36.56 c	1.00 e	2.63 EF	1.81 efg	0.0 b	55
1.75	52.13 b	0.73 e	2.38 F	1.61 efgh	0.0 b	78
2.00	73.65 a	0.54 e	2.19 F	1.25 gh	0.0 b	89
2.25	61.21 b	0.46 e	2.24 F	1.18 h	0.0 b	96
2.50	58.58 b	0.65 e	2.28 F	1.19 fgh	0.0 b	100
LSD at 0.05	6.827	0.6393	0.7532	0.5758	0.3567	-
Kintin (Kin) mg / l						
1.0	8.73 ij	5.98 b	7.17 ab	4.11 ab	0.0 b	0
2.0	10.36 hi	5.83 bc	6.77 b	3.92 ab	0.0 b	0
3.0	12.73 gh	5.56 bc	6.04 c	3.67 bc	0.0 b	4
4.0	14.94 g	4.94 cd	5.02 d	3.15 cd	0.0 b	12
5.0	18.69 f	4.36 de	4.13 e	3.02 cde	0.0 b	27
6.0	23.65 e	3.73 ef	3.65 ef	2.74 de	0.0 b	41
7.0	27.29 d	3.11 f	3.21 fg	2.42 ef	0.0 b	56
8.0	32.11 c	2.21 g	2.94 gh	2.00 fg	0.0 b	69
9.0	40.31 b	1.75 gh	2.44 hi	1.65 gh	0.0 b	87
10.0	45.08 a	1.29 h	2.19 i	1.25 h	0.0 b	97
11.0	4133 b	1.06 h	2.00 i	1.19 h	0.0 b	100
LSD at 0.05	3.223	0.8973	0.7004	0.6944	0.3404	-



Control 0.25 0.50 0.75 1.0 1.25 1.50 1.75 2.00 2.25 2.50 mg/ l

Fig. 1. Effect of Benzyl adenine (BA) on growth and development of *Stevia rebaudiana,* cultured *in vitro* after 6 weeks from culturing.

Fig. 2. Effect of Kinetin (Kin) on growth and development of *Stevia rebaudiana,* cultured *in vitro* after 6 weeks from culturing.

Fig. 3. *Stevia rebaudiana,* produced via tissue culture grown in the field under desert conditions (Sadat area, Egypt)

affecting *Stevia* micropropagation and this phenomena is increased by increasing cytokinins especially BA. So, multiplication of *Stevia* without BA is better to produce normal plants suitable to root formation and acclimatization stages.

Effect of auxins

Data presented in Table 2 clearly showed the effect of auxins (IBA , NAA and IBA) on the *in vitro* growth of *Stevia rebaudiana* Bertoni.

Table 2. Effect of Indole – 3 - butyric acid (IBA), Nahpthalin acetic acid (NAA) and Indole-3-acetic acid (IAA) on growth and development of *Stevia rebaudiana,* cultured *in vitro* after 6 weeks from culturing.

Treatment	Average Shoot number	Average Shoot lenght	Average Leaf number	Average Plant strenth	Root %	Average Root number	Average Root length
Control	6.21 a	5.44 d	7.11	3.61	87.6 c	3.17 cd	2.54 bc
Indole-3-butyric acid (IBA) mg/L							
0.001	5.12 b	5.42 d	7.04	3.89	93.3 b	3.44 bc	2.58 bc
0.01	4.79 bc	5.94 cd	6.63	4.17	100 a	4.19 b	3.06 b
0.1	4.54 bc	6.39 bcd	6.58	4.25	100 a	5.42 a	4.21 a
0.5	4.15 c	7.00 abc	6.69	4.33	100 a	4.11 bc	2.92 b
1.0	3.98 cd	7.71 ab	7.13	4.42	80 d	2.38 de	1.75 cd
1.5	3.19 d	7.96 a	7.23	4.25	60 e	1.42 e	1.13 d
MEAN	4.57	6.52	6.91	4.13	76.19	3.44	2.60
LSD at 0.05	0.9206	1.403	N.S	N.S	3.620	1.009	0.9877
Nahpthaline acetic acid (NAA) mg/L							
0.001	4.94 b	6.27 b	6.52	4.13	80 b	2.36 b	2.02 b
0.01	4.63 bc	6.33 bc	6.63	4.19	73 c	1.36 c	1.02 c
0.1	4.42 bc	6.59 b	6.83	4.42	53 d	1.31 c	0.98 c
0.5	4.38 bc	7.11 ab	6.98	4.31	20 e	0.92 c	0.52 cd
1.0	4.36 bc	7.65 a	7.06	4.38	0.0 f	0.0 d	0.0 d
1.5	3.71 c	7.94 a	7.23	4.61	0.0 f	0.0 d	0.0 d
MEAN	4.66	6.76	6.91	4.23	32.37	1.30	1.02
LSD at 0.05	0.9696	0.8586	N.S	N.S	3.052	0.4481	0.5578
Indole-3-acetic acid (IAA) mg/L							
0.001	5.58 b	6.06 cd	6.94 c	3.96	80 b	3.00 a	2.23 ab
0.01	5.25 b	6.50 bcd	6.98 c	4.61	73 c	2.94 a	2.04 abc
0.1	5.08 b	6.73 bc	7.06 c	4.38	60 d	2.19 b	1.79 bcd
0.5	4.33 c	7.11 bc	7.31 abc	4.31	53 e	1.75 bc	1.46 cde
1.0	3.86 cd	8.76 a	8.17 a	4.11	33 f	1.29 cd	1.19 de
1.5	3.33 d	7.61 ab	7.98 ab	4.29	20 g	1.04 d	1.00 e
MEAN	4.81	6.89	7.36	4.29	58.0	2.19	1.76
LSD at 0.05	0.6232	1.211	0.8938	N.S	3.610	0.54586	0.6268

Effect of indole butyric acid (IBA)

The effect of indole butyric acid on *in vitro* growth of *Stevia rebaudiana,* Bert estimated as shoot and root formation parameters was shown in Table 2. Shoot number ranged from 3.19 to 6.21 shoots / explant .Shoot number decreased by increasing IBA concentration .The best value of shoot number was achieved with MS basal medium without plant growth regulators followed by IBA at very low concentrations (.001, 0.01 and 0.1 mg/l IBA). The adverse effect was found with shoot length with significant differences amongst the treatments . Regarding leaf number and and shoot growth , data clearly indicated that there is no significant differences amongst the treatments at 5 % level .

The effect of IBA on root formation was significant where root percentage was 100 % at low concentration of IBA (0 .001 , 0.01 and 0.1 mg/l)and at the same time recorded the best results for root number and length .

Data presented in Table (2) show the effect of naphthalene acetic acid on shoot and root formation of *Stevia rebaudiana,* Bert cultured *in vitro* . Shoot number was affected by NAA concentrations. Increasing NAA concentration decreased gradually number of shoots and this increasing was significant at 5% level. Shoot number ranged from 3.71 to 6.21 shoots / explant. The best results (6.21 shoots / explant) was achieved at MS basal medium without NAA as compared to plantlets of *Stevia rebaudiana* grown at different concentrations of NAA with significant differences ($p<0.05$) between two treatments as shown in Table 2. On the other hand, shoot length increased by increasing NAA concentration. Shoot length ranged from 5.44 to 7.94 cm/shoots, and it depends on (NAA) concentration .MS basal medium without plant growth regulators recorded the lowest number of shoots (5.44 cm/ shoots) compared to 7.94 cm/shoot at 1.5 mg/l NAA on *Stevia rebadiana.*

Data also showed no significant differences amongst the treatments for the effect of NAA at different concentrations on average number of leaves and shoot growth as shown in Table 2. Regarding the effect of NAA on root formation , data indicated that the percentage of shoots formed roots was 87 % on MS basal medium without plant growth regulators as compared to MS basal medium supplemented with NAA at low concentrations 0.001, 0.01 and 0.1 mg/l NAA where root percentage was 80 . , 73 and 53 % respectively . On the other hand NAA at 1.0 and 1.5 mg/l did not help shoots to form roots .

Effect of indole-3- acetic acid (IAA)

The effect of Indole-3-acetic acid on *in vitro* shoot formation of *Stevia rebaudiana* (estimated as shoot number, shoot length, leaf number and shoot growth) and root formation estimated as root percentage, root number and length)were shown in Table 2. The best treatment for *in vitro*

growth was MS without IAA which recorded the highest values for shoot (6.21 shoots / explant) and root formation (87 % root percentage, 3.17 root number and 2.54 cm root length). On the other hand, shoot length increased by increasing (IAA) concentration. shoot length ranged from 5.44 to 8.76 cm cm/shoots, and it depends on (IAA) concentration. At high concentration, 0.001mg/l (IAA) recorded the lowest at shoot length (5.44 cm/shoots) compared to (8.76 and 7.61) cm/shoot 1.0 and 1.5 mg/l (IAA) on *Stevia rebaudiana.* Average number of leaves ranged from 6.94 to 8.17 number of leaves. at 1.0 mg/l were significant differences effective in increasing the average number of leaves. The highest number of leaves (8.17) was achieved with (IAA) concentration compared to the lowest one (6.94) which recorded with 0.001 (IAA) concentration with significant differences($p<0.05$) between two treatments as shown in Table 2. Data also showed no significant differences amongst the treatments for the effect of IAA at different concentrations on average shoot growth as shown in Table 2.

Analysis of the effects and mode of action of auxins is difficult, as conflicting results of auxins action have been reported for different tissues. One can generalize that some effect of auxins can be found in any living plant tissue, but it is very often impossible to prove whether this effect is a direct or a secondary consequence of auxins application. Auxins are thought to control the following procedures: (a) apical dominance, (b) cell elongation in roots and shoots, (c) permeability changes of the plasmalemma, deformation of ethylene, induction of adventitious root formation, (f) enhancement of the respiration rate, (g) induction of disorganized growth at higher concentrations, (h) inhibition of embryo formation in cell suspension cultures, (i) formation of parthynocarpic fruits in some species , and (j) mitotic regulation in long term tissue cultures. All auxin affects are mediated by a single mechanism (Evans *et al*., 1983). Results under discussion are in line with Kornilova and Kalashnikova (1996) and El-Zefzafi (2003) who studied clonal micropropagation of *Stevia rebaudiana* Bert. using stem segments with 2 axillary buds. Use of MS medium without growth regulators gave results comparable to those obtained with growth regulators (BA + NAA and kinetic + NAA) .They mentioned that plain MS medium is recommended as being cheap and effective. IAA at 0.5 mg/l gave good results in activating rhizgenesis. No reduction in growth was obtained with increasing number of subcultures *in vitro.*

Our results suggests that *Stevia rebaudiana* can be produced via tissue culture techniques without plant growth regulators during multiplication or using low concentrations of BA or Kin. and at rooting stages by using low concentrations from IBA or NAA.

The *ex vitro* plantlets were acclimatized well in planting medium containing peatmoss, sand and vermiculite at equal volume and transferred to the field under Egyptian desert conditions in Sadat area as illustrated in Fig 3. Here *Stevia in vitro* propagation has been demonstrated with its overall potentiality. *In vitro* propagation can become an important alternative to conventional propagation and breeding procedures for wide range of plant species (Uddin *et al*., 2006).

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